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EUROPEAN UNION - LATIN AMERICA

SCIENTIFIC COOPERATION IN THE 90' s

*Vol II: International Scientific
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EUROPEAN UNION - LATIN AMERICA

SCIENTIFIC COOPERATION IN THE 90' s

UNION EUROPEA - AMERICA LATINA

COOPERACION CIENTIFICA EN LOS AÑOS 90

Vol II: International Scientific Cooperation (ISC)

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Preface

European Union - Latin America Scientific Cooperation in the 90's

It gives me great satisfaction to present this overview of the results of almost a decade of continuous support from the European Community to cooperation between our scientists and their Latin American counterparts. In addition, this publication provides researchers with a valuable source of information on the projects supported, their scope, objectives, and results, and gives full details of the teams involved and how to contact them.

The reader will find in the pages that follow the practical results of the Community's policy on scientific cooperation with the Latin American region. As in the case of other developing regions, Community policy has sought to harmonise a contribution to the region's socio-economic progress with our own scientific interests.

Implementation of this policy has allowed Community scientists to gain access to localities displaying particular environmental, agricultural, ecological and public health characteristics, and to undertake their research in these areas. As a counterbalance, we believe that Latin American researchers have derived great benefit from interaction with their European peers. Given their own scientific quality, this sharing of experience places local teams in a privileged position from which to contribute to finding science-based solutions to problems faced by their communities.

It is precisely with the aim of tackling these problems effectively that, after extensive dialogue with the scientific authorities and communities of the region, the Commission selected areas on which to target cooperation. Agriculture and agroindustry, health and environmental issues were considered the most important priorities, as the reader will be able to see in the body of this publication. However, in order to capitalize on the human potential available, research in other relevant fields such as earth sciences, materials and different branches of engineering was also supported when resources permitted.

We firmly believe that our cooperation has led to the creation of a permanent network of scientific interaction, embracing a vast number of Latin American and European scientists, and which is even broader and more far-reaching than the sum of the results of the projects presented here.

The importance of Latin America for the European Community has recently been brought to the forefront by the Summit of Heads of State of Latin America and the Caribbean, and the European Union, which took place last June in Rio de Janeiro. The dialogue that has taken place over the years in different fora has been reinforced by the Heads of State of the two regions with their decision to establish a Working Group of Representatives. This institutionalised Working Group should provide a renewed impetus to our cooperation: whether this will be achieved through the enlargement of the specific programme for cooperation, by further facilitating access to the specific thematic programmes of the framework programmes, by the conclusion of cooperation agreements, or by the combination of some of these options, is still an open question.

The Working Group of Representatives will be the forum for reflection and advice on the most appropriate way to develop the full potential of our cooperation in the future. The Rio Summit underscored the will of both regions to deepen that cooperation, and the European Commission will apply its best efforts and full capacity to the successful achievement of that aim.

Brussels, October 1999

J. Gabolde
Director

Introduction


During the 1990s, the European Community pursued scientific cooperation with Latin America through a series of different programmes.

For the period 1990-1994 two complementary schemes were in operation. First, the Life Sciences and Technologies for Developing Countries (STDIII) programme, which formed part of the EC's Third Framework Programme for Research and Technological Development aimed at mobilizing EC and Developing Country scientists to work on pressing problems of all developing countries, including Latin American countries, in the areas of human health and agriculture. Second, the International Scientific Cooperation (ISC) scheme, which aimed at developing long-lasting working relationships between EC and Latin American scientists, covered a wider range of subjects and set priorities by mutual agreement with the national authorities of individual countries. Through these two schemes a wide-ranging development effort was complemented by a country-specific initiative. The ISC scheme also granted fellowships for Latin American scientists to do research in European laboratories and develop contacts with the European scientific community.

In 1994, a new scheme combining these ideas was introduced. This was the INCO-DC programme (Scientific and Technological Cooperation with Developing Countries), which formed part of the EC's Fourth RTD Framework Programme and which ran until 1998. It focussed specifically on three sectors of widespread importance (sustainable management of renewable natural resources, sustainable improvement of agricultural and agroindustrial production, and health) and used a regional basis, in this case the region being Latin America, on which to set research priorities and build projects.

The newest programme, which started in 1999 and runs for a further four years, is the Research for Development (INCO-DEV) component of the Fifth RTD Framework Programme. This programme targets research of a problem-orientated nature, maintains the regional approach and subject-matter coverage of the earlier INCO-DC programme but adds to it a section on policy research for sustainable development.

This volume contains summaries of joint research projects involving partners in Latin America. It covers all STDIII and INCO-DC projects, and ISC projects which started in the 1992-1994 period. A table summarizing the number of activities carried out and EC financial contribution is given below.



Jaak Sinnaeve
Head of Unit XII-E-4
Research for Development

EC-Latin America S + T cooperation activities			
	Number of activities	Number of institutional partners	EC financial contribution (million ECU)
Joint Research projects			
STD III (1990-1994)	96	388*	31.76
ISC (1990-1994)	363	933	57.88
INCO-DC (1994-1998)	121	818*	58.50
Fellowships (1990-1994)	319	638	10.44
TOTAL	899	2777	158.58

** Includes some partners from non-Latin American developing countries*

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ISC

Agricultural Sciences

PRODUCTION AND NUTRITIONAL VALUE OF COFFEE PULP SILAGE

Co-ordinator : University of Surrey, Guildford, United Kingdom (Martin R Adams)

Objectives

Coffee pulp is an under-utilised by-product of the wet processing of coffee that poses a serious disposal problem in coffee producing countries. The project examined its potential for utilisation as animal feed by investigating the production and nutritional value of coffee pulp silage.

Activities

- * A number of visits and short courses were used to transfer and share the expertise of the partners. In the case of the UK partner, this was in the chemical and microbiological analysis of coffee pulp while the Belgian partner contributed expertise on the ensilage process. The project work was centred on Latin America, the source of the raw material.
- * During the project, pilot trials were conducted to produce silage in mini silos to determine optimum ensilage conditions. Larger scale trials were then conducted using the optimum procedure. The silage produced was analysed chemically and used in feeding trials in a variety of farm animals.

Results

- ⇒ The work found substantial differences between pulp obtained by hand and that produced from commercial depulping operations. This was attributed to the leaching of solubles during the commercial process.
- ⇒ To ensure a satisfactory ensilage process it was necessary to reduce the moisture content of the pulp by sun drying or pressing to give a dry matter content of 25-30%.
- ⇒ The various ensilage protocols tried did not differ in their effect on the tannin and caffeine content of the silage. In terms of chemical analysis and their behaviour in feeding trials, no clear difference was discernible between "natural" coffee pulp silage and that produced using a starter culture. Microbiological results obtained in Ecuador and Venezuela supported this, showing the pulp to contain an indigenous microflora capable of producing substantial quantities of lactic acid.
- ⇒ The use of coffee pulp silage in broiler and lamb rations proved not to be economically viable. Rabbits, pigs and fish tolerated the presence of coffee pulp silage in their rations well.

Follow-up

Since the end of the project feeding trials have been continued in Venezuela and contacts have been made with a number of other coffee producing countries in the region. During the

project, progress was made in the quantification and characterization of the coffee pulp proanthocyanidins. These were shown to be very complex and work on their structure is continuing.

Selected publications

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ENVIRONMENT AND PEST LOCUSTS AND GRASSHOPPERS OF BRAZIL

Co-ordinator : Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France (Michel Lecoq)

Objectives

To study *Rhammatocerus schistocercoides* (Rehn, 1906), an important pest grasshopper of Mato Grosso state (Brazil), where outbreaks have, up to now, been explained as being a consequence of the deforestation and accelerated agricultural development that has occurred since the early 1980s.

Activities

Numerous field trips have been made to Mato Grosso to study the *in situ* ecology, biology and behaviour of the grasshopper and to predict outbreaks. In Campinas, vegetation and biotope maps have been made using satellite data (LANDSAT TM) to allow a spatialisation of the problem and to study the relationship between grasshoppers and agriculture.

Results

Many results obtained in the project refute previous hypotheses, especially concerning the assumption that grasshopper outbreaks are a recent phenomenon in Mato Grosso, that agricultural development has had an important impact on the grasshopper situation, and that these grasshoppers are a potential threat to neighbouring states.

Some of the main results are:

- ⇒ The evidence that *R. schistocercoides* outbreaks are a long-standing phenomenon in Mato Grosso; they are in no way a new phenomenon prompted by the recent agricultural development in this region.
- ⇒ The understanding of the relationships between land use (agricultural, pastoral and traditional) and grasshopper outbreaks.
- ⇒ The findings concerning factors that determine grasshopper outbreaks, which seem to be mainly associated with the rainfall regime, especially from August to October, a critical period for the grasshopper cycle.
- ⇒ The clarification of many points concerning the biology and ecology of this grasshopper; especially the finding that swarm movements are much more limited than previously assumed; considerable data has been collected on imaginal diapause, sexual maturation, development times for various biological stages, the number of instar stages, the absence of phasal polymorphism, etc.
- ⇒ The mapping of grasshopper biotopes (breeding biotopes and dry-season refuge biotopes), essential for understanding the consequences of agricultural development in outbreak zones and basic documents required for organising efficient grasshopper survey and control operations as part of a renovated control strategy.

Overall, the results of this project have been the topic of one book, twelve scientific publications, six conference papers, fourteen maps (covering an area of about 145.000 km²) and nineteen unpublished reports. Several publications are currently in progress.

Follow-up

The results of this project will have a critical operational impact. Strategies to control *R. schistocercoides* should now be completely reconsidered, while promoting local preventive spot treatments. There would be considerable research potential in continuing the current project, i.e. to perfect grasshopper outbreak survey, early-detection (high temporal resolution satellites) and grasshopper control (mycopenesticides) techniques.

Selected publications

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Lecoq M. & Pierozzi Jr I., 1995a. *Rhammatocerus schistocercoides* locust outbreaks in Mato Grosso (Brazil) : a long-standing phenomenon. *The International Journal of Sustainable Development and World Ecology* **2**: 45-53.

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Period: March 1993 to March 1996

**GRAZING ASSOCIATED FACTORS AND CHARACTERISATION OF
MYCOLOGICAL SPECIES IN THE COORDINATED EPIDEMIOLOGICAL
COMPARISON OF EQUINE DYSAUTONOMIA IN SCOTLAND AND PATAGONIA**

Coordinator : University of Edinburgh, Edinburgh, United Kingdom (D.L. Doxey)

Objectives

The discovery in 1989-91 by F.A. Uzal that the equine disease known by Patagonians as 'mal seco', was in fact closely related to dysautonomia, prompted this investigation. The objective was to compare and contrast the two diseases and the pastures and fungal population of those pastures in Scotland and Patagonia with a view to identifying common features.

Activities

- * Study of the plant population in areas where dysautonomia and mal seco occurred.
- * Analysis of the fungal population obtained from plants and soil in these areas when culture was undertaken at 8°C.
- * Assessment of clinical signs and histological confirmation of the lesions seen in the two disorders. This included immunohistochemical studies.
- * Feeding trials using *Festuca argentina* and fungal extracts.

Results

- ⇒ The study, which allowed the researchers involved to see the disease in their opposite numbers' country, showed that equine dysautonomia and mal seco are the same disease, that no common plant could be found (out of over 850 examined) which induced disease, and that *F. argentina*, which was considered the most likely plant to cause toxic damage, did not produce mal seco. Both diseases are weather and season related.
- ⇒ Numerous fungi were isolated but only *Fusaria cladosporum* and *trichoderma* species occurred in both areas. Of these, only *Fusaria* is a known toxin producer and it occurred as between 10 and 100% of the fungi isolated. Its exact role in mal seco and equine dysautonomia is still unproved. The role of intestinal bacteria in dysautonomia was studied in relation to fungal involvement.

Follow-up

Work by the partners is continuing on *clostridial* problems in cattle. *Clostridial* and fungal work is being undertaken in horses by other researchers in Edinburgh at the present time.

Selected publications

Doxey D.L., Milne E.M., Woodman M.P., Gilmour J.S. and Chisholm H.K. 1995. Small intestine and small colon neuropathy in equine dysautonomia (grass sickness). *Veterinary Research Communications*. **19** : 529-543.

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Contract number: CI1*CT920072

Period: October 1993 to September 1995

**ALGAL PATHOLOGY: INFECTIOUS DISEASES AND FACTORS DETERMINING
SUSCEPTIBILITY IN ECONOMICALLY IMPORTANT SEAWEEDS.**

Co-ordinator: Universidad de Las Palmas, Las Palmas, Spain (Guillermo Garcia-Reina).

Selected publications

Bouarab K, Potin P., Correa J. and Kloareg B. Sulfated oligosaccharides mediate cross-talk between a marine red alga and its green algal pathogenic endophyte. Submitted.

Sánchez P., Correa J.A. and García Reina G. 1996. Host-specificity of *endophyton ramosum* (Chlorophyta), the causing agent of the green patch disease in *Mazzaella laminarioides* (Rhodophyta). *European Journal of Phycology*. **31**: 173-179.

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AROMATIC POTENTIAL OF MUSCAT GRAPE VARIETIES FROM CHILE

Co-ordinator: Catholic University of Chile, Santiago, Chile (E. Agosin)

Objectives

This work aimed to study, identify and quantify the flavour compounds and their precursors present in the berries of Muscat grape varieties cultivated in Chile, in order to evaluate their aromatic potential. Since the effects of native yeasts during grape transformation into wine are not well known, and varying aromatic products are to be obtained, the work attempted to acquire a better knowledge of the enzymatic capabilities of autochthonous yeasts, and particularly their hydrolytic efficiency against glycosidically bound terpenols, in order to make new technological developments for enhancing flavour of grape juice and wine. Finally, the mechanism of action of these implied hydrolases was to be determined and compared with the sequential schema of hydrolysis already described for other biological systems.

Activities

The Chilean group was in charge of the selection and preparation of samples from the raw material. It was also responsible for the preparation of extracts, purification of the enzymes and of the resolution of the problem of microbiological selection (selection, culture of strains). The French group was responsible for the resolution of analytical problems to enable the detection and structural determination of aroma compounds. It has also played a role in the determination, characterization and identification of selected enzymes. Training of young researchers was undertaken by the French laboratory.

Results

⇒ Selection of muscat varieties grown in Chile.
⇒ Determination of their aroma potential.
⇒ Microvinification of the various grape varieties.
⇒ Isolation of strains of indigenous yeasts in various vineyards and spontaneous vinification. Ten varieties were chosen in the INIA agricultural stations in IV region (Vicuna, Limari) and in VII region (Cauquenes). Grapes were sampled randomly at maturity in the selected vineyards. Two different extraction procedures were used to obtain the aroma. The extract obtained by crushing the berries had the highest level of volatile and glycosylated terpenols. This extraction method was then selected. Comparisons of the aroma potential between varieties show significant differences according to their terpene level. Varieties such as Moscatel Amarilla and Muscat Orange displayed high level of volatile and glycosylated terpenols. In addition, Muscats of Alexandria and Frontignan, grown in the Vicuna region, were found to be richer in terpenols than their French homologues. The microvinification of the various muscats was performed in the Chilean laboratory. Fermentation was done with indigenous yeasts and sampling was carried out both in fermentation media and in fresh berries before fermentation. A large number of yeast strains (610) were isolated in on agar impregnated with synthetic glycosides having a fluorescent aglycon. The strains displaying

high p-glucosidase activities were detected by fluorescence. Among these, 22 strains were selected for their hydrolysis ability. In addition, further studies were conducted on the 4 most active strains. The hydrolysis of terpenols glycosides, whether from synthetic and natural origin, by these yeast strains was investigated. Furthermore, the hydrolysis ability of their enzymatic systems was examined, in vivo and in vitro, in presence of glucose.

Follow-up

- Several vinifications of small volumes of grapes were made and could be followed-up in larger amounts. Local industries producing wines or fruit juices should benefit from the work through better control of juice production, vinification and wine treatments.
- Prospects for co-operation: The project continues as a Chilean FONDEF contract to develop a commercial yeast strain, from native origin, with hydrolytic enzymic capabilities.

Publications

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**INTERACTION BETWEEN SOIL SAPROPHYTE FUNGI AND
VESICULAR-ARBUSCULAR MYCORRHIZAE TO RECOVER FERTILITY OF
DEGRADED SOILS IN THE PROVINCE OF BUENOS AIRES (ARGENTINA)**

Co-ordinator: Estación Experimental del Zaidin, Granada, Spain (Juan A. Ocampo)

Objectives

- ◆ Selection of the most common effective strain of vesicular-arbuscular mycorrhiza (VAM) fungi from (Pergamino) Argentine soil.
- ◆ Study of the effect of saprophyte fungi on establishment, competition and efficiency of mycorrhization.
- ◆ Study of the mechanisms of beneficial interactions between saprophyte fungi and VA mycorrhizal fungi.
- ◆ Selection of the most effective saprophyte fungi-VA mycorrhiza-host plant combination in plant competition with the non-VA hosts weeds.

Activities

- ★ Saprophytic and arbuscular mycorrhizal fungi were isolated by Buenos Aires.
- ★ The effect of the saprophytic fungi on germination of the selected VAM spores, on VAM penetration in plant roots and on the total mycorrhization of the plants were studied by Granada.
- ★ Buenos Aires and Granada studied the establishment of the antagonistic and/or synergistic relationships between the saprophytic and VAM fungi, and the level at which these relationships were done.

Results

The saprophytic fungi : *A. niger*, *T. koningii*, *F. solani*, *F. equiseti* and *A. alternata* were selected. From these fungi : *F. solani* did not inhibit germination of *G. mosseae* spores, and endophyte hyphal development was markedly stimulated. *F. equiseti* inhibit spore germination but hyphal growth of *G. mosseae* spores was markedly stimulated. *F. solani* and *F. equiseti* did not affect negatively AM colonization of maize roots. Among the *Fusarium* strains, *F. solani* (51), and *F. stilboide* (2169) were the most effective in their increase of the percentage of spore germination and the number of vegetative spores of *G. mosseae*, the percentage of AM root length colonization of soybean root and shoot dry wt. of soybean plants.

Selected publications

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EVALUATION AND SELECTION OF COFFEE GERMPLASM FOR RESISTANCE TO PREVAILING NEMATODES IN CENTRAL AMERICA

Co-ordinator : CIRAD, Montpellier, France (Bernard Decazy)

Objectives

Given the narrow genetic base of cultivated coffee and its sensitivity to nematodes, the main objective of the project was to characterize the main nematodes that parasitize coffee in Central America and to search for new sources of resistance that might be used in commercial varieties and resistant root-stocks.

Activities

- * Different nematode populations of the two main genera parasitising coffee (*Meloidogyne* and *Pratylenchus*) were collected in Guatemala, Costa Rica, Salvador, Honduras, and Nicaragua and characterized for morphology, enzyme profile and virulence.
- * A large quantity of coffee germplasm from the CATIE collection was evaluated for resistance to different species/pathotypes of *Meloidogyne*.
- * Taking advantage of the wide genetic variation in *Coffea canephora*, many lines were evaluated and selected. Resistant lines were also tested with *Pratylenchus*.
- * Different Catimor lines (*C. arabica*) and wild Ethiopian lines were also evaluated.

Results

- ⇒ The project has revealed the complexity and diversity, particularly in terms of pathogenicity, of the nematode fauna parasites on coffee in Central America. Hence the necessity to evaluate germplasm in the different countries according to local nematode populations. New pathotypes have been described, the economic importance of which for coffee culture is still underestimated.
- ⇒ The evaluation of *C. canephora* has led to the identification of two Robusta clones the descendants of which are resistant to nematodes, in particular to some that are very pathogenic in Guatemala and Salvador. Their large-scale culture *in vitro* has been achieved by micropropagation and somatic embryogenesis in temporary immersion. A system of acclimatisation of *in vitro* plants has been developed so that they can be used in the different countries of the region. Crossing the two clones gave the new resistant rootstock Nemaya. Some resistance to *Meloidogyne* was observed in the Catimor and Ethiopian lines but not to all populations collected. Use of this material to create new F1 hybrids will thus probably not be possible for all countries, but its distribution should be made according to the occurrence of nematode pathotypes.

Follow-up

- Study of the nematofauna in Central America coffee will be pursued with PROMECAFE. 2,500 *in vitro*-plants of the parent clones of the Nemaya rootstock are currently acclimatizing in Guatemala and Salvador in order to be installed in a seed-field this year.

A further 2,500 plants in February 1998 and 10,000 in May 1998 were dispatched to these countries. Seeds could be distributed to producers as from the year 2000.

- The Ethiopian lines resistant to certain nematodes are now being used in a regional F1 hybrid arabica breeding programme.

Publications

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**CRYOPRESERVATION OF GAMETES, EMBRYOS AND LARVAE OF MARINE
ORGANISMS OF COMMERCIAL IMPORTANCE TO MANKIND**

Co-ordinator: Medical Research council, London, United Kingdom (D.G. Whittingham)

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**THERMAL REQUIREMENTS AND HOST RANGE OF *NACOBBUS ABERRANS*
AND IMMUNOASSAYS FOR ITS DETECTION AND QUANTIFICATION**

Co-ordinator: Institute of Arable Crops Research, Harpenden, United Kingdom
(Kenneth Evans)

Objectives

- ◆ Assemble collection of *Nacobbus aberrans* populations;
- ◆ Study the thermal requirements of candidate populations;
- ◆ Compare morphology and protein profiles of all populations;
- ◆ Assess host crop ranges;
- ◆ Investigate population dynamics and methods of control in the field;
- ◆ Test the potential of an immunoassay for *N. aberrans*.

Activities

Nacobbus aberrans populations were collected in Mexico and from North America, Peru, Bolivia and Argentina. All were established in culture at Rothamsted in the UK and the Colegio de Postgraduados in Mexico. Field work (on population dynamics, host range and methods of control) was conducted in Mexico, along with certain taxonomic studies. Some host range work, thermal studies, taxonomic work and immunoassay investigations were carried out at Rothamsted in the UK. The taxonomic studies encompassed morphological description based on both light and scanning electron microscopy, protein profiling and DNA profiling. Information has been disseminated during training exchanges, at scientific meetings (both national and international), and by publishing scientific papers and a thesis.

Results

Thermal characteristics have been determined for populations from Mexico and Bolivia and can be related to the different climates. Morphological studies were made after first culturing all populations on a standard host under standard conditions in order to remove any effect of environment on morphology. Measurements and observations were compared with those made on specimens representative of other species within the genus *Nacobbus*. Certain characters previously believed reliable for the separation of species of this genus were shown to be variable and therefore unreliable. Canonical variate analyses grouped Peruvian and Bolivian populations away from others but populations from Argentina tended to be grouped with those from Mexico. One Mexican population (Chapingo) was generally isolated from the rest of the Mexican populations, a relationship borne out by the protein and DNA profiles. The host range work identified non-host crops which might be useful in population management and suggested that different populations may have different host ranges, but did not provide firm evidence for the existence of host races. The work on population dynamics emphasised the potential importance of weeds in nematode management and indicated that most of the life cycle of *N. aberrans* occurs inside roots rather than in the soil. None of the

control treatments improved yield significantly, at least partly due to the intermittent non-availability of irrigation. Immunoassay tests suggested that such technology may provide the much needed reliability of population determination necessary for efficient management of this pest.

Follow-up

Further work is being done on methods of control and publication of project results continues.

Selected publications

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ECOPHYSIOLOGY AND SENSITIVITY TO CERCOSPORIOSES IN PLANTAIN AT HIGH ALTITUDE IN COLOMBIA

Co-ordinator : Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Montpellier, France (Hugues Tezenas du Montcel)

Objectives

- ◆ Develop co-operative research programmes to evaluate the components of inter-specific competitiveness between the two fungal phytopathogens (*Mycosphaerella fijiensis* and *M. musicola*) causing cercosporiosis,
- ◆ Study the interaction between ecophysiology and sensitivity in order to design strategies for stabilization and improvement in quality and quantity of plantain production in Colombia's central coffee zone.

Activities

- * Study of the role of climatic factors (especially temperature) on epidemiological components (incubation period, length of infection period, sexual and asexual reproduction in the two pathogens);
- * Study of the importance of the host range (genome) in the epidemiology of the two pathogens;
- * Study of the role of secondary metabolites (polyphenols) on cercosporiosis-susceptibility mechanisms, and the role of altitude;
- * Physico-chemical characterization of soils (andosols principally) in the coffee zone;
- * Identification of the role of endomycorrhizae (VAM) in banana trees;
- * Development of sustainable alternative cultural techniques to improve disease management and production;
- * Evaluation and mapping of the risk of black-streak disease in the whole of the central coffee zone.

Results so far

- ⇒ Confirmation of a high level of natural colonization of endomycorrhizae on the secondary roots of plantain, and their positive effects on growth;
- ⇒ Confirmation of the role of secondary metabolites, especially flavanes, on cercosporiosis-resistance mechanisms, and variation with altitude (temperature);
- ⇒ Large variation in epidemiological behaviour between varieties towards the two pathogens in transition zones (800-1100 m) due to different resistance mechanisms;
- ⇒ Identification of the important role of nutrition (physico-chemical characteristics and fertilization) and cultural techniques in the integrated control of cercosporioses. A precise fertilizer recommendation has been established by soil type and micro-zone.

Expected results

- Mapping the risk of black-streak cercosporiosis in the whole of the central coffee zone;
- Studying cation dynamics in the soil water of andosols planted with plantain in the coffee zone in relation to exchange properties, rainfall and fertilization

Selected publications

- Henaó M.C., Delvaux B., Suárez S. 1997. Comparación de los métodos de análisis granulométricos aplicados en andosoles de la zona cafetera central de Colombia. *Cenicafé*, Vol. 49 n°1.
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A STUDY OF THE DISEASE IN PIGS CAUSED BY THE PARAMYXOVIRUS LPMV

Co-ordinator : The Queen's University of Belfast, Stormont, Belfast BT4 3SD,
United Kingdom (B.M. Adair)

Objectives

- ◆ Establish the basic characteristics of the pathogenesis of the disease in pigs.
- ◆ Define methods for the diagnosis and control of LPMV infection.
- ◆ Elucidate the RNA-editing process of the virus 'in vivo'.

Activities

- * The project was aimed at providing basic information on the pathogenesis, diagnosis, control and molecular biology of the virus LPMV that causes an economically important disease of pigs in Mexico. The disease is characterised by nervous disorders, respiratory disease, reproductive problems and deaths.
- * The project was co-ordinated from Belfast, N. Ireland (UK) who were responsible for the pathogenesis work and experiments on diagnostic methods. Puebla (Mexico) also contributed pathogenesis studies and work on reproductive problems, while Uppsala (Sweden) was responsible for studies on molecular biology and also for production of monoclonal antibodies.

Results

- ⇒ Studies on the pathogenesis confirmed field observations that clinical signs following LPMV infection are dependent on the age of the pigs. Virus replication in the respiratory tract and tonsil from 1 day after inoculation, was a consistent finding, indicating that the primary site of replication of LPMV may be located in these tissues. The primary site of virus replication was previously not known, and was one of the targets for this part of the work. Virus replication appears to occur initially in the nasal mucosa and spread to the CNS via the trigeminal and olfactory nerves, and subsequently remain localised in the olfactory bulbs and midbrain. Dissemination of virus to other sites in the CNS is dependent on the age of the pigs at infection. The virus excretion studies demonstrated that excretion via the respiratory tract and in urine are important methods of spread of LPMV, probably more so than by the faeces.
- ⇒ Studies on diagnosis showed that tonsil, lung, and olfactory bulb were the tissues of choice for virus isolation, and these tissues should preferentially be chosen by virus isolation laboratories faced with diagnosis of the disease. (the editing process of the viral RNA has been determined). These studies have shown that transcripts of the V gene of LPMV inhibit viral RNA synthesis. In cells persistently infected with LPMV, the percentage of V gene-to-P gene transcripts has been shown to be higher than in productively infected cell cultures or in acute experimental infection. This is an important

finding, since it provides a marker for the determination of sites of viral persistence in the pig.

Follow-up

The project has successfully achieved its aims, and has also been successful in pinpointing areas where future study should be concentrated, if the disease in Central America is to be contained and eventually eliminated. The work has permitted to promote links and collaboration between scientists in Mexico and Europe, and several Mexican scientists have spent periods of time in the 2 European centres involved and have also registered for degrees at European Universities. The work has stimulated additional funding for associated projects covering several aspects of the biology of this important virus.

Selected publications

Allan G.M., McNeilly F., Walker I., Linne T., Moreno López J., Hernández P., Kennedy S., Carroll B.P., Herron B., Foster J.C., and Adair B.M. 1996. A sequential study of experimental porcine paramyxovirus (LPMV) infection of pigs; immunostaining of cryostat sections and virus isolation. *J. Vet. Diag. Invest.* **8**:405-413.

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COMPARISON OF GENETIC VARIABILITY IN EUROPEAN AND SOUTH AMERICAN POPULATIONS OF POTATO CYST NEMATODES MEASURED BY VARIATION IN DNA AND VIRULENCE TOWARDS PLANT RESISTANCE GENES

Co-ordinator: Institute of Arable Crops Research, Harpenden, United Kingdom
(Kenneth Evans)

Objectives

- ◆ Assemble a collection of potato cyst nematode (PCN) populations from diverse areas of the UK and Peru and characterise the virulence of these populations towards a standard assortment of resistant potato clones.
- ◆ Use the same populations with a selection of random primers in RAPD-PCR to search for bands or banding patterns able to discriminate between populations and which might be related to virulence. Should appropriate bands be found, attempt to develop DNA probes for virulence.

Activities

PCN population collections were made both in Europe (by IACR-Rothamsted) and in Peru (by the University of La Molina) and virulence assays on a common assortment of potato clones and cultivars were performed at both centres. Banding profiles from a large number of random primers were produced using the UK and European populations collected at Rothamsted, and using the South American populations. Various methods were used to produce *Globodera pallida* populations with enhanced levels of virulence towards partially resistant potato cultivars in order to target potential DNA markers for virulence more specifically. These included deliberate searching out of naturally virulent populations, selection of populations over a number of generations for enhanced virulence, and collection of maturing females (virulent by definition) directly from the roots of resistant potato plants. Dissemination of results and information was by means of teaching courses, presentations at scientific meetings (both national and international), preparation of scientific papers for publication and preparation of a thesis.

Results

120 random primers were screened against some 57 PCN populations. Differences in DNA profiles were identified between the two species of PCN, populations of species from different localities and populations of the same species but with different degrees of virulence. These differences were correlated with the results of the virulence assays and cluster analysis was used to display the relatedness of populations based on DNA profiles and virulence characteristics.

Follow-up

Beyond the end of the project, two DNA bands, identified with random primers, that seemed to have potential for use as virulence markers have been cloned into *Escherichia coli*. They will be assessed for possible use as DNA probes, as a basis for the synthesis of more specific primers for virulence assessment, and for the likelihood of their direct link to virulence genes.

Selected publications

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Bendezu I.F., 1997. Comparison of genetic variability in European and South American populations of potato cyst nematodes measured by variation in DNA and virulence towards plant resistance genes. PhD Thesis, University of Nottingham, 221p.

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Contract number: CI1*CT930058

Period: February 1994 to February 1997

**ORGANIZATION AND EXPRESSION OF THE POTATO MITOCHONDRIAL
GENOME**

**Co-ordinator: Centre National de la Recherche Scientifique (CNRS), Bordeaux, France
(Simon Litvak)**

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Period: July 1994 to June 1997

FACTORS AFFECTING SENSORY QUALITY OF LACTIC-ACID PRESERVED MEAT

Coordinator: University of Nottingham, Sutton Bonington, United Kingdom,
(Andy J. Taylor)

Objectives

In many parts of the developing world, meat needs to be preserved using cheap, appropriate technology. Lactic acid treatment using either addition of the acid, or fermentation by lactic-acid producing organisms, was proposed and experiments were carried out in the UK and in Mexico to study the effectiveness of the treatment in terms of microbiological safety and sensory quality.

Activities

Overall, the effects of acid treatment on the spoilage and pathogenic organisms of meat were determined at temperatures typically found in developing countries. The production of malodorous compounds and volatiles indicative of spoilage processes like lipid oxidation were also monitored. The analytical facilities at Nottingham were used to develop assays for the volatiles, while UAM Mexico investigated the efficacy of lactic acid under field conditions.

Results

⇒ Malodorous compounds

The effect of temperature (15-25°C) on the production of malodorous volatiles in lactic acid treated meat was determined. Around 15-20°C, volatiles were derived chemically from lipid oxidation or from spoilage organisms like *Brochothrix thermosphacta*. At 25°C or above, there was the potential for biogenic amines like cadaverine and putrescine to be produced. Treatment with lactic acid, or with inocula of *L. pentosus* or *S. carnosus* significantly reduced the formation of these compounds. Using the analytical data, selected samples were prepared and assessed in Mexico by a trained sensory panel who rated the relative flavour of each treatment.

⇒ Microbiological safety

This quality parameter was of primary importance and considerable effort was placed on this area. Using lactic acid solutions, chunks of meat could be adequately preserved in plastic bags stored at ambient (15 to 25°C) temperature, using meat obtained from UK slaughterhouses, which are subject to strict hygiene procedures. When the same study was conducted in Mexico, similar results were obtained, demonstrating the effectiveness of lactic acid. Other acids were more effective (e.g. acetic) but imparted unacceptable flavour to the meat and were therefore not studied further. The survival of specific pathogens was monitored; *Listeria* by inoculation of test samples, followed by acid treatment and *Enterobacteriaceae* by reduction in initial numbers.

⇒ *Sensory Quality*

The R-index method of O'Mahoney was adopted so that training of the panel was minimised, an important aspect given the need to carry out analyses on two sites. Acid treatment reduced rancidity scores, a finding that correlated with the instrumental analyses. QDA of cooked meat samples allowed the key sensory attributes to be identified. Overall, lactic acid treatment of the meat prior to cooking, had a significant difference on only one of the sensory attributes whereas the use of lactic producing starter cultures had significant effects on between 7 and 10 attributes, depending on the culture used.

Follow up

Work is continuing at UAM (Mexico) on mixed starter cultures that might provide a cheap, effective way to preserve meat on a local basis throughout developing countries.

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- Prado Barragan, LA, Guerrero Legarreta, I., Taylor, A.J. 1997 Efecto del hierro (orgánico e inorgánico) sobre la oxidación de lipidos de carne de cerdo. In "Productos Naturales" **3**: 103-115 ISBN 970-654-120-9 UAM Press, Mexico

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DETERMINATION OF SOME FACTORS AFFECTING THE NUTRITIONAL AND BIOTOXICOLOGICAL VALUE OF FISHMEAL FOR USE IN FEEDS FOR SHRIMP CULTURE AND THE ESTABLISHMENT OF QUALITY-CONTROL NORMS

Co-ordinator: International Fishmeal & Oil Manufacturers Association, St. Albans, Hertfordshire, United Kingdom (Ian H. Pike)

Objectives

The main source of protein for marine carnivorous species of shrimp (*Penaeus spp.*) is fish meal. The methods by which this is produced affect the productivity of shrimp to which it is fed. The objective of this project was to study how these aspects of the production process affect the quality of fish meal for shrimp, and then to optimize them. In so doing, quality control norms were to be established, to provide fish meal that can improve shrimp productivity.

Activities

The projects has investigated the following aspects of fish-meal processing:

- * Freshness of raw material
- * Processing temperature exposure
- * Effects of toxins produced in some fish meals, which cause gizzard erosion in poultry (e.g. gizzerosine) which are known to be toxic to shrimp
- * Oxidation effects of lipids from fish (in both fish meal and fish oil)
- * Standardization of an *in vivo* digestibility procedure and development of an *in vitro* procedure using the protease enzyme trypsin, and relating this to results of the *in vitro* test.

The project linked expertise from the University of Nuevo León (Mexico) and the French Group IFREMER at their AQUACOP Station in Tahiti, working with tank facilities. In the final stages, the findings were applied to shrimp in ponds where productivity was monitored. As well as improving productivity through more efficient feed use, effluent output was monitored, with a view to reducing nitrogen and phosphorus output to decrease environmental pollution.

Results

Spoilage of fishmeal for fish meal affects feed consumption, growth and survival of young *Penaeus* shrimp; biogenic amines, breakdown products of protein, were not found to be the cause. Their content in fish meal does indicate a degree of spoilage and the reduced productivity that may result when this is fed. The digestibility of fishmeal protein in *Penaeus* shrimp is improved by processing it at a lower temperature.

Both salmon and mink may be good indicator-species for predicting digestibility changes. So far, chemical procedures (enzyme digestion) have met with limited success.

Fish meals causing gizzard erosion in poultry have deleterious effects on shrimp growth; in small *Penaeus* shrimp (less than 0.2 g), survival is reduced. The compound gizzerosine produced in these fish meals was shown to be toxic to small shrimp when present at more than 1ppm in the diet. Oxidation of lipids in fish meals and oil can affect shrimp growth adversely, particularly if the diet contains inadequate levels of vitamin E.

Follow-up

Pond studies to test the findings of the laboratory work on commercial shrimp farms in Mexico, with a view to using better-quality fish meals to improve productivity were carried out following delays due to disease problems.

Selected publications

Ricque-Marie D., Abdo-DelaParra Ma. I., Cruz-Suárez L.E., Cuzón G., Cousin M. and Pike I.H. 1998. Raw material freshness, a quality criterion for fish meal fed to shrimp. *Aquaculture* (Elsevier, Amsterdam), accepted for publication, Dec. 8. 1997.

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Cruz-Suárez L.E., Ricque-Marie D., Abdo-DelaParra Ma. I., Tapia-Salazar M., Nieto López M.G., Pike I.H., Galleguillos M. and Castro-Campos E. 1996. Indices de calidad de harinas de pescado y su efecto en la producción de camarón (Fishmeal quality indices and their effects on shrimp production). *Aguacultura del Ecuador*, 12: 12-30.

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**ANALYSIS OF THE INTERACTIONS BETWEEN THE COMPLEMENT SYSTEM AND
ECHINOCOCCUS GRANULOSUS, AND ASSESSMENT OF IMMUNOPROPHYLACTIC
POTENTIAL OF PARASITE MOLECULES IDENTIFIED AS INVOLVED IN
COMPLEMENT EVASION**

**Co-ordinator: University of Oxford, Oxford, United Kingdom
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Period: January 1994 to January 1998

STUDIES ON THE USE OF PALM FATS AND MIXTURES OF FATS AND OILS IN POULTRY NUTRITION

Co-ordinator : Instituut voor Dierhouderij en Diergezondheid (ID-DLO), Lelystad,
The Netherlands (Cor Scheele)

Objectives

Evaluate the local potential sources of fats and oils for animal feeding, especially for poultry in Costa Rica and other Central American countries.

Activities

- ★ Survey of the potential suppliers of different fat products.
- ★ Evaluation of different oil products and blends in broilers and laying hens.
- ★ Compilation of the results of further studies with broilers and laying hens applying the concepts of practical feed formulation as performed by most poultry feed manufacturers in Costa Rica and other Central American countries.

Results

- ⇒ The results of the project show that the main source of fat products for poultry feeding in Costa Rica is palm oil. Processing of crude palm oil in three different plants located in the southern region of the country gives palm free fatty acids with a lower price than the crude oil. However, it is clear from the results of this research project that palm products have a lower digestibility than other oil products and by-products. Therefore, it is recommended to produce blends with soybean free fatty acids and/or restaurant greases.
- ⇒ Also, from palm processing the full-fat kernel is obtained. This product has a high oil content and high digestibility due to the predominant mono-unsaturated fatty acids. The kernel contains a metabolizable energy level of up to 20% above that of corn, which currently is the main cereal included in diets in Central America.
- ⇒ Currently, based on the results of this project, a blend of 50% each crude palm oil and palm free fatty acids is marketed in Costa Rica (palm acid oil) and used by several poultry companies. This product can be improved if a third, more unsaturated fat product is added to favour the fatty acid profile of the blend toward a more unsaturated composition. This will increase the digestibility and metabolizable energy content of the fat blend.
- ⇒ Probably, the second most important fat is beef tallow, which is in a similar situation to palm fats due to its high-saturated fatty acid level. In this case, it is also recommended to blend tallow with restaurant greases and/or soybean free fatty acids in a proportion to get a composition similar to the imported yellow grease. Yellow grease should contain not less than 65% unsaturated fatty acids and 30% linoleic acid.

- ⇒ Considering that locally-produced fat products can replace imported corn and fats, the information obtained from this research project will greatly benefit the poultry industry in Costa Rica and other Central American countries in correctly applied and particularly if the industry works towards producing high-quality fat blends.
- ⇒ A second project on this topic should aim to produce blends of fats at an industrial level. Those blends should be produced following high-quality standards to comply with the requirements of international markets.

Selected publications

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ROLE OF ANTIGEN-PRESENTING CELLS IN DETERMINING THE TH1/TH2 BALANCE *IN VIVO*

Co-ordinator : Université Libre de Bruxelles, Bruxelles, Belgium (Jacques Urbain)

Objectives

The purpose of this project is to define the parameters required for optimal vaccination of *Brucella abortus*.

Activities

We evaluated the role of antigen-presenting cells in the induction of primary responses *in vivo*, in mice models (Brussels) and in bovine models (Bogota). Brussels was involved in the study of the fundamental aspects related with the Th1/Th2 amplitude balance of the response induced by various cell populations, and studied the phenomenon of carrier-induced hapten-specific suppression which may have major implications in the choice of carrier for recombinant vaccines in mice models. Bogota was involved in clinical essays on guinea pig models and bovine models. In particular, they compared the protection induced by the conventional vaccine *B. abortus* strain 19, by *B. abortus* strain RB51, and by purified structural antigen OMP and OMP-O chain; and they characterized the immune response induced in different groups of animals.

Results

⇒ *Links and interchanges between both participants*

During the last year of the project, exchanges between both groups took place in order to evaluate the methodologies and approaches to be used in the experiments in bovines. As a result of the meeting, a technical visit to the Brussels laboratory and training in dendritic cell-handling and the production of monoclonal antibodies against OMP-II purified proteins were planned.

⇒ *Joint scientific activity and scientific co-operation*

Fusion experiments were performed in the Brussels laboratory in order to obtain C-cell hybridoma secreting monoclonal antibodies specific to OMP-II. The hybridoma cloned in Brussels would be used in the Bogota laboratory to discriminate the animals infected out of the animals vaccinated. Two methods were developed to isolate dendritic cells from bovine peripheral blood. These were used to research the interactions between antigen-presenting cells and antigens of *Brucella abortus*.

⇒ *Evaluation of three vaccines for Brucella abortus*

For the purpose of comparatively evaluating the type of immune response induced by purified structural *Brucella abortus* antigens as well as live vaccines, five groups were defined for safety and efficiency in vaccination-challenge experimental calves. The experimental results showed that utilization of the *Brucella abortus* mutant vaccine RB51 gave protection levels equivalent to the conventional Strain 19 vaccine, with the advantage that it is possible to differentiate between vaccinated animals and infected

animals. In particular, the characterization of the efficient immune response shows that the protection obtained results from the stimulation of T CD4+ Th1 and T CD8+ lymphocytes by the peptides of the outer-membrane proteins.

Selected publications

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Renjifo X., Howard C., Kerkhops P., Denis M., Urbain J., Mosr M. and Pastoret PP Purification and characterisation of bovine dendritic cells from peripheral blood. Manuscript accepted in Vet. Immunol. Immunopathol.

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Period: June 1994 to June 1996

**REGULATION OF TRIACYLGLYCEROL SYNTHESIS AND ACCUMULATION IN
DEVELOPING CEREAL EMBRYOS BY ABSCISIC ACID AND WATER
POTENTIAL**

Co-ordinator: King's College London, London, United Kingdom (M. Black)

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PHENOLOGICAL FORECASTING TECHNIQUES FOR TIMING OF INSECT CONTROL MEASURES AS PART OF INTEGRATED PEST MANAGEMENT SYSTEMS

Co-ordinator: Netherlands Organization for Applied Scientific Research, Delft, The Netherlands (Jan J. de Vlieger)

Objectives

The strategic objective of the proposal was to build up in Uruguay the knowledge and expertise to develop environmentally safe methods for insect pest control based on pheromones that can compete both technically and economically with the available existing methods. Tactically, the research has aimed to make available the pheromones of *Eulia salubricola*, and of *Argyrotaenia spheropa* through isolation, identification and synthesis. The proposed method of monitoring is fully compatible with sustainable agricultural development and integrated and biological pest control strategies. The research had furthermore the objective to evaluate new, very versatile controlled release dispensers for monitoring with sex pheromones the pest insects *Argyrotaenia spheropa* and *Eulia salubricola* in various fruit orchards such as apple, grapes, peaches, plums and pears in South America.

Activities

- * The research focused on the isolation, identification and synthesis of the sex pheromone of *Eulia salubricola* and *Argyrotaenia spheropa*, and the development of forecasting techniques to control both leafrollers species.
- * The identification, synthesis and preparation of long lasting pheromone dispensers were carried out at TNO in the Netherlands. The insect mass rearing and pheromone field evaluation were carried out at INIA in Uruguay.

Results

In the course of this project the sex pheromones of *A. spheropa* and *E. salubricola* have been identified. The pheromone of *A. spheropa* consists of Z11-tetradecen-1-ol aldehyde (Z11-14 Ald), Z11,13-tetradecadien-1-ol aldehyde (Z11,13-14 Ald), Z11-tetradecen-1-ol acetate (Z11-14 Ac) and Z11,13-tetradecadien-1-ol-acetate (Z11,13-14 Ac) in the ratio 1:4:10:40. The pheromone of *E. salubricola* consists of E3,Z5-dodecadien-1-ol-acetate (E3,Z5-12 Ac), Z5-dodecen-1-ol-acetate (Z5-12 Ac) and Z9-hexadecen-1-ol-acetate (Z9-16 Ac). Field experiments with pheromone compounds of *A. spheropa*, showed that Z11,13-14 Ald is attractive at its own, but addition of Z11-14 Ald in the correct ratio increases its activity. Field experiments with pheromone compounds of *E. salubricola*, showed that E3,Z5-12 Ac is attractive at its own, but addition of Z9-16 Ac in the correct ratio increases its activity.

Follow-up

During the last year of the project the longevity of TNO prototype dispensers will be investigated and compared with rubber septa. The efficacy of the dispensers in the still ongoing field trials will be evaluated in February 1998. It is foreseen that in the near future reliable forecasting methods for the population density of *A. sphaleropa* and *E. salubricola*, based on the use of pheromones as a monitoring tool that fits into existing Integrated Pest Management systems, will be established.

Selected publications

Nuñez S., Persoons C. and Rodríguez J.J., 1995. Identificación de feromonas de *Eulia salubricola* y *Argyrotaenia sphaleropa*. (Pheromone identification of *Eulia salubricola* and *Argyrotaenia sphaleropa*). IN: Resultados Experimentales en Protección Vegetal en Frutales. INIA : Serie Actividades de Difusión **70**: 23-24.

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OCCURRENCE AND SIGNIFICANCE OF VIRUS INFECTIONS IN BENTHIC MARINE ALGAE

Co-ordinator: Universität Konstanz, Konstanz, Germany (Dieter G. Müller)

Objectives

Marine algae (kelps and smaller brown algae) are important components of coastal ecosystems. Recent studies have shown that some cosmopolitan species are affected by temperate aquatic viruses in a pandemic fashion. Our study was planned to provide for the first time basic data on infection dynamics of the *Ectocarpus* virus in natural habitats. Parallel laboratory studies were designed to evaluate the effect of virus infection on the reproduction and growth potential of the host.

Activities

- * Epidemiological field study: samples of the algal hosts (the filamentous brown alga *Ectocarpus*) are collected in bi-monthly intervals at two habitats (North Atlantic : Gran Canaria, Spain, and South Pacific : Chile). The samples are processed and analysed for the presence of viral DNA by using the PCR amplification method (Polymerase Chain Reaction).
- * Experimental laboratory studies : genetically defined clonal cultures of *Ectocarpus siliculosus* are used to study the effect of virus infections on basic survival characteristics of the host : reproduction and photosynthesis.

Results so far

- ⇒ Over two years our field survey revealed a persistently high percentage (up to 100 %) of infected host plants in both the Atlantic and Pacific study sites. In addition, in the Canarian *Ectocarpus* population we found two different co-existing virus genotypes.
- ⇒ In *Ectocarpus siliculosus* virus infection does not reduce the somatic fitness of the host plants : photosynthetic capacity and growth rates are not reduced. In contrast, the reproductive potential of the host is completely destroyed by the virus.
- ⇒ In virus-infected *Ectocarpus* of the Chilean study site we found a hyper-infection by a fungus. This pathogen belongs to the Plasmo-diophoromycota and is new to Science.

Follow-up

Analyses of the field samples and remaining parts of the experimental work will be completed and the results published. The detailed study of the new fungus parasite extends beyond the present dimensions of the project and will require additional time for completion.

Selected publications

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Contract number: CI1*CT940032

Period: February 1995 to October 1998

**DEFINITION OF THE BIOLOGICAL BASES FOR THE CONSERVATION,
RESTORATION AND CULTURE OF HIGHLY VALUABLE CHARACIDS IN THE
MAGDALENA RIVER BASIN, COLOMBIA**

Co-ordinator: Université de Liège, Liège, Belgium (Ch. Mélard /E. Baras)

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Period: May 1995 to December 1997

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF CHAYOTE MOSAIC VIRUS: A NEW TYMOVIRUS INDUCING AN IMPORTANT DISEASE IN CULTIVATED CHAYOTES (*SECHIAM EDULE* SWARTZ) IN CENTRAL AMERICA.

**Co-ordinator: CSIC - Centro Nacional de Biotecnología, Madrid, Spain
(Emilio Rodríguez Cerezo)**

Objectives:

- ◆ Learn about the biology and epidemiology of Chayote Mosaic virus, a new pathogen of a crop (chayote) that is an alternative to traditional commodities exported by Central American countries
- ◆ Develop an improved diagnosis for the pathogen and methods for controlling it.

Activities

The project studies aspects of the epidemiology of the pathogen, such as; identifying insect vectors and their transmission efficiency, determining the host range of the virus, determining vector population dynamics, virus incidence, virus spatial distribution and disease progress curves. These activities are carried out by the partner in Costa Rica. This partner also studies the cytopathology of the infected plants at the electron microscopy level and the susceptibility to the diseases of the commonest chayote cultivars. The partner in Spain and co-ordinator concentrates on the characterization of the genome of the pathogen, characterizing the nucleic acid and cloning and sequencing the genome of the virus. The partner in Spain prepares clones from different portions of the viral genome for use as diagnostic probes. Training of technicians from Costa Rica in the Spanish lab in techniques of molecular biology has also been included as an activity of the project

Results

- ⇒ The host range of the virus has been determined and seems restricted to the family *Cucurbitaceae*. Evidence of seed transmission of the virus in chayote crops has been obtained. To date, 5 species of insects belonging to the *Coleoptera* have been shown to be able to transmit the virus experimentally.
- ⇒ In epidemiological studies in Costa Rica experimental fields, disease progress was adequately described by the logistic model ($R^2=0,87$) with an apparent infection rate (r) of 0,012. The spatial distribution appears to be aggregated, with the highest incidence of disease occurring nearest the inoculum source. The cytopathology of infected chayotes is similar to that described for other tymoviruses. About 90% of the viral genome (RNA single-stranded of approx. 6500 nucleotides) has been cloned and sequenced, showing that this virus is not a strain of other well characterized tymoviruses but a new member of this virus group that, in common with other members, is restricted to one botanical family.
- ⇒ Clones have been selected from the library to cover conserved and variable regions of the virus, with the aim to synthesize probes that could identify several strains of the virus if

they were present in the field or to distinguish between strains. Radioactive cRNA probes were generated and used in calibration experiments with purified ChMV RNA and extracts from infected plants. The sensitivity of ChMV RNA detection by hybridization is currently being compared with serological methods. Other experiments in which the probes were generated with digoxigenin (non-radioactive method) gave less satisfactory results than those in which radioactivity was employed.

Follow up

In the last year of the project the efforts are in completing the sequence of the virus and assembling a full-length clone. Also there are continuing studies on insect transmission and virus detection with cloned probes and by PCR to rapidly check germplasm.

Publications

Hord M., Villalobos W., Macaya Lizano A. V. and Rivera C. 1997. Chayote mosaic, a new disease in *Sechium edule* caused by a tymovirus. *Plant Disease* **81**:374-378.

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**BIOLOGICAL CONTROL OF PLANT PARASITIC NEMATODES OF FOOD
CROPS IN PERU**

Co-ordinator: Consiglio Nazionale delle Ricerche, Istituto di Nematologia Agraria Applicata
ai Vegetali, Bari, Italy (Aurelio Ciancio)

Objectives

Collection, identification and study of soil microbial antagonists of parasitic nematodes of food crops occurring in Peru and their application in biological control.

Activities

The project aims to seek, identify and isolate antagonistic microorganisms of plant parasitic nematodes occurring in the highlands and coast regions of Peru. Training of local scientists in the biological control of nematodes at La Molina University (UNALM) and Bari (NEMACNR), through the development of cooperative research and greenhouse and field studies, was also included. The work applied epidemiological concepts and models to evaluate the incidence of antagonistic microflora on host nematode density. The research was integrated, for biological control applications, by the development of non-linear models of the host-parasite dynamics developed at NEMACNR. Both laboratories were involved in the conservation of the isolates discovered and in the study of their biology and pathogenicity.

Results so far

An intensive survey was carried out in the central and highland regions of Peru, with the collection and the identification of several populations of phytoparasitic nematodes of the genera *Globodera*, *Pratylenchus* and *Xiphinema*. Training of the local UNALM personnel focused on the techniques of extraction, identification and study of nematode antagonists of the genus *Pasteuria* (Actinomycetes). Surveys in the regions of Juliaca (Puno) and central Peru allowed the discovery of new *Pasteuria* forms, parasitic in populations of *Hoplolaimus galeatus*, *Pratylenchus andinus*, *Xiphinema krugi* and *Globodera pallida*. Some of these isolates were studied at NEMACNR in cooperation with UNALM staff personnel, using Light and Scanning Electron Microscopy. They represent new host or geographic records. The discovery of a *Pasteuria* form parasitic in the potato cyst nematode (PCN), *Globodera pallida*, represented one of the most important goals achieved by the project. This nematode is one of the main limiting factors of potato crops in the region and no efficient or low-cost products are actually available for its control. Several *Pasteuria* isolates were found parasitising PCN populations at Colcabamba, in the Central Andean department of Huancavelica (Huancayo). This form appeared endemic in the area and was found in almost 21% of the fields infested by PCN. Data from periodic soil samplings were collected in order to identify the host population dynamics in presence of the antagonist. Further studies on the host-parasite relationship and parasitism efficiency were performed in the greenhouse at UNALM. Data obtained reinforce previous hypotheses on nematode density dependent parasitism.

Follow up

The *Pasteuria* isolates discovered and the knowledge related to their natural occurrence in the Andean regions represent a first step in the development of low-cost management strategies for PCN. The density-dependent relationship discovered represent the starting point for the bio-management of this pest through farm-scale production and application of *Pasteuria* in nematode-infested fields.

Selected publications

Carbonell Torres E. and Ciancio A., 1996. Occurrence of new *Pasteuria penetrans* group members on nematodes in Peru. *Nematropica* **26** : 248.

Ciancio A., Vega Farfan V. and Carbonell Torres E., 1997. A new *Pasteuria* form parasitizing juveniles of the potato cyst nematode, *Globodera pallida*, in Peru. *Nematropica* **27** : in print.

Ciancio A., Tapia Ramírez M., Carbonell Torres E. and Lamberti F., 1997. New records of *Xiphinema* spp. and associated *Pasteuria* parasites. *Nematropica* **27** : in print.

Ciancio A., Tapia Ramírez M., Carbonell Torres E. and Grasso G., 1998. Observations on a *Pasteuria* isolate parasitic on *Hoplolaimus galeatus* in Peru. *Journal of Nematology* **30** : in print.

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Period: February 1995 to July 1997

THE *RHIZOBIUM TROPICI* RESPIRATORY CHAIN : ISOLATION OF MUTANTS WITH ENHANCED RESPIRATION AND SYMBIOTIC NITROGEN FIXATION

Co-ordinator: John Innes Centre, Colney, Norwich, United Kingdom (K. J. Allan Downie)

Objectives

The goal of this work was to characterize genetically the cytochromes in a strain of *Rhizobium tropici* that is used commercially as a inoculant for legumes in Latin America. The long term aim was to try to isolate strains of *R. tropici* and *R. etli* with enhanced symbiotic nitrogen fixation as a result of altering their respiratory chain and hence their respiration in legume nodules.

Activities

This project focussed on *Rhizobium* strains that are altered in respiration via cytochromes *c* and *aa₃*. Analysis of mutants with decreased respiration led to the identification of several genes involved in the formation of *c*-type and *a*-type cytochromes. Several of these mutants were defective in symbiotic nitrogen fixation. Among the strains with increased respiration were mutants with increased symbiotic nitrogen fixation. Increased expression of thiamine biosynthetic genes or mutation of genes encoding glycogen synthase or purine biosynthesis enhanced both respiration and symbiotic nitrogen fixation.

Results

Analysis of respiration-defective mutants of *R. tropici* led to the characterisation of the phenotypes of mutants affected in *fbcBC* (cytochrome *bc₁*), *cycHJKL* (cytochrome *c* assembly), *coxA* and other genes involved in cytochrome *aa₃* formation. These mutants were all severely affected in symbiotic nitrogen fixation (Seville and Norwich).

At the outset of this project, two mutants of *R. etli* had been identified that had enhanced respiration via cytochromes *aa₃* and one had enhanced symbiotic performance. Analysis of one of these mutants revealed that the mutation was in *purF* (encoding the first step in purine biosynthesis) and that this mutant has increased expression of the *fixNOQP* genes which encode a cytochrome *cbb₃*-type oxidase that is normally only expressed in nodules. Analysis of other mutants affected in purine biosynthesis led to the conclusion that one of the late intermediates in purine biosynthesis (5-aminoimidazole-4-carboxamide ribonucleotide AICAR) represses the cytochrome *chb₃* oxidase, and that the enhanced respiration arose because of a decreased level of this intermediate (Cuernavaca). The second mutant of *R. etli* arose as a result of increased expression of the thiamine biosynthetic gene cluster (*thiCOGE*) due to insertion of Tn5 in the promoter region. The increased expression of *thiCOGE* is thought to decrease AICAR concentration by promoting utilisation of intermediates involved in its biosynthesis and converting them to thiamin. The resulting decrease in AICAR concentration then allows enhanced symbiotic performance as a result of increased expression of the cytochrome *cbb₃* oxidase (Cuernavaca).

It had been anticipated that analogous mutants of *R. tropici* might be identified following mutagenesis. However different classes of mutants were identified that had enhanced respiration. One group of mutants was affected in the NADH dehydrogenase and presumably compensate for decreased energy conservation by enhanced respiration via cytochromes *aa₃* (these mutants were not enhanced for symbiotic nitrogen fixation). A second class of mutant was shown to be affected in glycogen synthesis and it showed markedly increased symbiotic performance, although the reason for this has not been established (Seville and Norwich).

Follow up

We are working on constructing strains that can be used in field tests for enhancement of symbiotic performance.

Selected publications

Miranda-Ríos J., Morera C., Taboada H., et al., 1997. Expression of Thiamin Biosynthetic Genes (*thiCOGE*) and Production of Symbiotic Terminal Oxidase *cbb₃* in *Rhizobium etli*. *J. Bact.* **179** : 6887-6893

Soberón M., López O., Miranda J., et al., 1997. Genetic evidence for 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) as a negative effector of cytochrome terminal oxidase *cbb₃* production in *Rhizobium etli*. *Mol. Gen. Genet.* **254** : 665-673.

Marroqui S., Zorreguieta A., Soberón M., et al., 1997. Enhanced respiration and symbiotic nitrogen fixation in a *Rhizobium tropici* glycogen synthase mutant. In : *Biological Nitrogen Fixation for the 21st Century. Proceedings of the 11th International Congress on Nitrogen Fixation.* (Eds : Elmerich C., Kondorosi A., Newton W.E.) Kluwer Academic Publishers **159**.

Soberón M., Miranda J., López O., et al., 1998. Characterization of *R. etli* mutants in the purine-thiamin metabolism suggest that 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) is a negative effector of symbiotic cytochrome terminal oxidase *CBB₃* production. In : *Biological Nitrogen Fixation for the 21st Century. Proceedings of the 11th International Congress on Nitrogen Fixation.* (Eds : Elmerich C., Kondorosi A., Newton W.E.) Kluwer Academic Publishers, **156**.

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**STRUCTURE AND FUNCTION OF THE TRANSCRIPTIONAL ACTIVATOR
PROTEIN NIFA FROM NITROGEN FIXING BACTERIA**

Co-ordinator: Imperial College of Science, Technology and Medicine, London, UK
(Martin Buck)

Objectives

The general long term goal is to support a rational manipulation of the process of nitrogen fixation to improve agriculture. The targets for manipulation are the nitrogen fixing bacteria, and the regulatory molecules which control the expression of the nitrogen fixation genes were chosen for study.

Activities

The project focuses on the molecular interactions that lead to activation of a specialised form of RNA polymerase that transcribes nitrogen fixation genes, Two key molecules were analysed: The NIFA positive control protein found in many diazotrophs, and the Sigma-N or Sigma-54 subunit of the RNA polymerase. An interaction between the sigma protein and NIFA is postulated to occur for activation of transcription to occur. The functions of domains of the regulatory proteins was analysed, work on NIFA was at Cuernavaca, Mexico and work on sigma in London.

Results

- ⇒ A model for the domain of NIFA responsible for transcription activation was developed using molecular modelling and structure prediction. Potential surface exposed residues postulated to contact sigma were mutated and defective trans dominant NIFA mutants obtained. The C-terminal DNA-binding domain of NIFA was purified, and its structure probed using spectroscopic methods including NMR and Circular Dichroism. It appears largely alpha helical in agreement with structure prediction.
- ⇒ Two domains in sigma-N were shown to co-operate for DNA-binding of sigma-N. A random pool of sigma mutants was generated, and loss of function mutants isolated to determine critical residues needed for sigma function and potential activator contact. Assays for partial sigma-N functions in crude extracts were set up to measure DNA-binding, core RNA polymerase binding and activation by NIFA. A mutant sigma-N by passing the usual requirement for NIFA activity was constructed.

Follow-up

Work continued in Mexico at the end of the granting period on characterising the defective sigma-N mutants in terms of functions and amino acid substitutions and will also search for suppressors of the NIFA mutants that map in sigma-N in order to define an interaction patch.

Selected publications

J Osuna, X Soberon, and E Morett (1997). A proposed architecture for the Central Domain of the bacterial enhancer binding proteins based on secondary structure prediction and fold recognition, *Protein Science* **6**: 543-555.

W Cannon, M Chaney, X-Y Wang and M Buck (1997). Two domains within sigma-N sigma54 cooperate for DNA binding, *Proc Natl Acad Sci USA* **94**: 5006-5011

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THE USE OF PLANT CYSTATINS AS INHIBITORS OF VIRAL INFECTIONS IN TRANSGENIC TOBACCO AND POTATO

Co-ordinator: Rijksuniversiteit Gent, Gent, Belgium (Marc Van Montagu)

Objectives

The general objective was to investigate the properties of plant cystein proteinase inhibitors, so-called phytocystatins, in order to develop an alternative approach to engineer virus resistance in plants.

Activities

- * Sequencing of the Andean Potato Mottle Virus (APMoV) B-RNA in the region corresponding to the 24 K viral proteinase.
- * Analysis of the *in vitro* cleavage of the APMoV encoded polyproteins.
- * Isolation and sequencing of cystatin cDNAs from different plant species.
- * Cloning the cystatin cDNAs into transcription, expression and plant transforming vectors.
- * Analysis of the effects of different proteinase inhibitors, including the plant cystatins, on the *in vitro* activity of the APMoV proteinase.
- * Analysis of the effects of cystatins on the APMoV replication in protoplasts.
- * Transformation of tobacco and potato with cystatins representing an *in vitro* inhibition of APMoV polyprotein processing and screening for high level of cystatin expression.
- * Resistance tests of transgenic tobaccos and potatoes with the APMoV.

Results

Recombinant rice and maize cystatins were *in vitro* and *in vivo* (in *E. coli* cells) tested on their inhibitory activity against resp. papain and the viral protein precursor. After confirmation of the functionality of the gene constructs, both cystatins were introduced in tobacco. The F0 plants were screened by testing the inhibitory activity of total leaf extracts against papain. The F1 generation is currently under analysis in order to produce homozygous F2 plants in which the viral resistance will be evaluated.

Selected publications

Reis M.R., Couñago R. and Margis R., 1996. Cloning and expression of rice and maize phytocystatins in a *E. coli* heterologous system. XXVI Reuniao anual da sociedade brasileira de bioquímica e biologia molecular, Caxambu, MG, Brasil, 04-07 May 1996, P 39.

Reis E.M., Couñago R., de Oliveira D.E. and Margis R., 1997. Cloning the maize and rice cystatins on binary vectors and production of transgenic tobacco plants. XXVI Reuniao anual da sociedade brasileira de bioquímica e biologia molecular, Caxambu, MG, Brasil, 03-06 May 1997, P 33.

Couñago R., Reis E.M. and Margis R., 1997. Purification of a functional grapevine fanleaf nepovirus proteinase expressed in *E. Coli*. XXVI Reuniao anual da sociedade brasileira de bioquímica e biologia molecular, Caxambu, MG, Brasil, 03-06 May 1997, P 45.

Margis R., Reis E.M. and Villeret V. Structural and phylogenetic relationships among plant and animal cystatins. Archives of Biochemistry and Biophysics. Submitted.

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**DEGRADATION OF PASTURE LANDS AND THEIR RECLAMATION USING
LEGUMES: A MAJOR ENVIRONMENTAL CHALLENGE FOR BRAZILIAN
AGRICULTURE**

Co-ordinator: University of London, Wye College, UK (G. Cadisch & K. E. Giller)

Objectives

The general goal is to provide a detailed assessment of the effect of pasture reclamation strategies both with and without legumes on the long-term productivity of pastures and to develop sensitive methods which can provide 'early warning' signals of degradation.

Activities

- * A network was initiated and successfully developed into an active collaboration between: i) the Centre for Research for the Cerrados (CPAC) and CIAT (Centre for Tropical Agriculture) in Ueberlândia, ii) the Centre for Bean and Rice Research (CPNAF) in Goiania and iii) CEPLAC in Bahia apart from the two original project partners at the Beef Cattle Research Centre in Campo Grande (CNPGC) and at the Centre for Agrobiology (CNPAB), Rio de Janeiro.
- * A set of experiments with comparable treatments were established at these stations studying: i) processes of pasture degradation and soil organic matter formation (^{13}C), ii) impact of legume residues (^{15}N labelled) and urine on nitrogen transformations, iii) effect of soil fauna on litter decomposition, iv) identification of limiting nutrients. AB-DLO provides assistance in the identification of 'early warning' signals (organic matter fractionation) while Wye College provides expertise in isotope based N and C cycling studies and model development.

Results so far

A minimum data set has been established to obtain 'early warning signals' for degradation of pastures. Separation of soil organic matter fractions based on their size and density has been shown to provide useful indicators of degradation. The results from the network further highlight the importance of maintenance fertilization in maintaining pasture productivity. Nitrogen is the most limiting nutrient followed closely by phosphorus deficiency. N deficiency is caused by a high N immobilization potential in degraded pastures as revealed by the ^{15}N gross-mineralization technique. Incorporation of legumes into these systems alleviated the N immobilization capacity. Legume persistence is a main limiting factor in the successful utilization of biological N_2 fixation in mixed pastures although some encouraging results with new accessions (*S. guianensis* cv. *Minerão*) or species (*Arachis pintoi*) were observed. An economic summary indicates that the use of a cash crop can compensate for some of the recuperation costs but does not fully account for all of them.

Follow-up

During the last phase of the project emphasis is given to completing sample analysis and data interpretation as well as integration of the data into computer simulation models.

Selected publications

Boddey, R. M., de Paula Resende, C., Pereira, J. M., Cantarutti, R., Alves, B. J. R., Ferreira, E., Richter, M., Cadisch, G., and Urquiaga, S., eds. (1995). Nitrogen cycle in pure grass and grass/legume pastures: Evaluation of pasture sustainability. *Nuclear Techniques in Soil-Plant Studies for Sustainable Agriculture and Environmental Preservation*, pp. 307-319. IAEA (International Atomic Energy Agency), Vienna.

Cadisch, G., and Giller, K. E. (1996). Estimating the contribution of legumes to soil organic matter build up in mixed communities of C₃/C₄ plants. *Soil Biology and Biochemistry* **28**, 823-825.

Cadisch, G., Imhof, H., Urquiaga, S., Boddey, R. M., and Giller, K. E. (1996). Carbon (¹³C) and nitrogen mineralization potential of particulate light organic matter after rainforest clearing. *Soil Biology and Biochemistry* **28**, 1555-1567.

Miranda, C.H.B.; Kichel, A.N. & Macedo, M.C.M. (1996). Recuperação de pastagens degradadas de *Brachiaria decumbens* com o cultivo simultâneo de milho. *Anais da XXXIII Reunião Anual da Sociedade Brasileira de Zootecnia (Annals of the XXXIII Annual meeting of the Brazilian Society of Animal Science)*. Fortaleza, 21 a 26 de Julho de 1996. Vol 2. p. 75-77.

Resende, C. d. P., Cantarutti, R. B., Pereira, J. M., Santana, J. B., Macedo, R. d. O., Tarré, R. M., Richter, M., Ferreira, E., Cadisch, G., Oliveira, O. C. d., Resende, A. S. d., Alves, B. J. R., Urquiaga, S., and Boddey, R. M. (1996). Avaliação do impacto da introdução de leguminosas forrageiras na sustentabilidade de pastagens de gramíneas através de estudos do ciclo de nitrogênio. Report No. 2, p. 1-11. EMBRAPA-CNPAB, Seropédica, Rio de Janeiro, Brazil.

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Contract number: CI1*CT940074

Period: January 1994 to December 1996

**COMPARISON OF MEXICAN AND EUROPEAN ISOLATES OF
*COLLETOTRICHUM LINDEMETHIANUM***

Co-ordinator: Université de Paris Sud XI, Orsay, France (Michel Dron)

Objectives

- ◆ Collect isolates of *C. lindemuthianum* from five Mexican states and three regions in France
- ◆ Determine the pathogenicity of these isolates on a set of differential bean genotypes developed at the International Centre for Tropical Agriculture
- ◆ Characterise the isolates, using molecular markers and electrophoretic karyotypes.
- ◆ Compare and analyse the data obtained by both the Mexican and French groups to determine relationships between pathogenicity, genetic characteristics, and the origin of the isolates.
- ◆ Include the data in a wider study involving Latin American and African isolates.

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DNA REPLICATION IN HIGHER PLANT CELLS : THE USE OF GEMINIVIRUSES AS A MODEL SYSTEM TO STUDY INTERACTIONS BETWEEN VIRAL AND CELLULAR PROTEINS

Co-ordinator: Universidad Autónoma de Madrid, Madrid, Spain (Crisanto Gutiérrez)

Objectives

The long-term goal of the research was to develop a useful model system to study DNA replication in higher plant cells. The model system is the replication of geminiviruses, causative agents of important crop reductions all over the world. Thus, in addition to benefits at the basic levels, the results could be of potential help for the rational design of strategies for disease control.

Activities

The research has focused on 2 major aspects :

- * Identify the viral and cellular proteins involved in geminiviral and cellular DNA replication. This included the molecular characterization of the pieces making-up the puzzle: the proteins involved in geminiviral and cellular DNA replication.
- * Study the coordination between viral DNA replication and cell cycle regulation. During this part of the project we have tried to put the different pieces together.

Results

- ⇒ In the course of this project, we have defined the structural and functional organization of the origin of geminivirus DNA replication as well as analyzed its possible connection to cell cycle regulators, and isolated a cDNA encoding a plant retinoblastoma protein, homologous to the human tumor suppressor protein.
- ⇒ We have also achieved a high-yield purification of several cellular factors involved in DNA metabolism. In the case of one DNA polymerase and PCNA, we have also cloned their cDNAs. A significant contribution is the availability of reagents, such proteins, antibodies and cDNA clones, as well as the contacts and exchange of personnel among the participating laboratories which have been achieved through the course of this project.

Publications

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Luque A.E., Benedetto J.P. and Castroviejo M. DNA Polymerase Cl : a homolog of rat DNA polymerase Q. *Plant Mol. Biol.* (submitted).

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A FUNCTIONAL APPROACH TO SALT TOLERANCE (FAST)

Co-ordinator: Universidad Politécnica de Valencia, Valencia, Spain, (Ramón Serrano)

Objectives

The final goal is to isolate plant genes that could improve the salt tolerance of sensitive crops by genetic engineering.

Activities

The project focuses on the utilization of the yeast *Saccharomyces cerevisiae* as model system to isolate plant halotolerance genes. One approach is to obtain plant homologues of previously identified yeast halotolerance genes (Valencia, Spain). Another approach is to isolate plant genes which confer halotolerance in yeast (Cuernavaca, Mexico). The selected genes would be expressed in transgenic tobacco plants to test for salt tolerance (Valencia, Spain).

Results so far

Three Arabidopsis homologues of the yeast halotolerance gene HAL2 and two homologues of yeast HAL3 have been isolated. A cDNA library from the halophytic plant *Mesembryanthemum crystallinum* has been constructed in the yeast expression plasmid pSAL, specifically constructed for this project.

Follow-up

Two plant cDNA libraries in yeast expression vectors, one from Arabidopsis and another from *Mesembryanthemum crystallinum* will be screened for salt tolerance in transformed yeast. Transgenic Arabidopsis plants with the plant homologues of yeast HAL2 and HAL3 genes will be constructed and their salt tolerance evaluated.

Selected publications

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DELIVERY OF TRANSGENES INTO TISSUES OF ADULT FISH

Co-ordinator: University of Southampton, England, U.K. (Norman Maclean)

Objectives

- ◆ Develop DNA vaccines for treatment of fish disease
- ◆ Improve the technique of administration to ensure an appropriate and persisting immune response.

Activities

- * The two centres at Southampton (England) and Valparaiso (Chile) have followed different lines, the former involving a bacterial disease-furunculosis, caused by *Aeromonas salmonicida*, and the latter a viral disease, IPN, involving the infectious pancreatic necrosis virus.
- * In Southampton we have followed two lines of approach. One has been to use the 'GCAT' gene (glycerophospholipid cholesterol acetyltransferase) from the bacterium. This is a soluble toxin, but a mutated and non-toxic form of the sequence was used. This was used in a form where it was spliced to a carp beta actin promoter, following up on our earlier experience that this promoter is well expressed as a transgene in fish. Secondly we have looked at reporter gene expression, using lacZ as a reporter, to test and improve on the method and site of administration. All of this work has been done with tilapia fish (*Oreochromis niloticus*).
- * In Valparaiso the work has concentrated on isolating a specific gene, VP2, from the virus, following growth of the virus in a chinook salmon cell line. The immunogenicity of different domains of the VP2 protein have been tested in the fish.

Results so far

- ⇒ We have concluded that the 'GCAT' gene from *A. Salmonicida* is probably not a good choice for our work since, although in its non-mutant form it is a potent toxin, it is a rather poor immunogen, and response to the toxin does not eliminate the pathogen (work from other laboratories). Meanwhile we have concentrated on the methodology of application to the fish. We have concluded that a circular rather than a linear construct gives better transgene persistence, and that the transgene product persists at least for months rather than weeks.
- ⇒ The work in Valparaiso has involved the immunization of rabbits with whole IPNV and recovery of polyclonal antibody. This is now being used to test the ability of different clones to express the VPZ fragments. The ability of each clone to induce an immune response in fish (following intra muscular injection in both tilapia (Southampton) and trout (Valparaiso)) is also now being tested. Also of note is the fact that in Valparaiso a PCR selected primer amplification system has been used to obtain good amounts of the

VP2 open reading frame clones. The immune response of the injected fish is currently being evaluated.

Follow-up

N.M. in Southampton is now attempting to set up a group of laboratories working on "molecular approaches to fish disease". Meanwhile the laboratory in Chile is also trying to apply the technology to fish rickettsial disease as well as IPNV.

Selected publications

Alam S., Popplewell A. and Maclean N., 1996. Germ line transmission and expression of a lacZ containing transgene in tilapia *Oreochromis niloticus*. *Transgenic Res.* **5**, 87-95.

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DISPOSITION KINETICS, TISSUE RESIDUES AND EMBRYOTOXIC EFFECTS OF ANTIPARASITIC DRUGS IN FOOD-PRODUCING ANIMALS

Co-ordinator: Universitat Aut3noma de Barcelona, Bellaterra(Barcelona), Spain
(Margarida Arboix)

Objectives

The general objective was to establish a correlation between the pharmacokinetic and metabolic behaviours of BZD compounds and the factors affecting them under field conditions, the pattern of tissue residues, and the potential embryotoxic/teratogenic effects of these anthelmintics.

Activities

- * The efforts of the Argentinean team were focused on the study of the biotransformation and disposition kinetics of benzimidazole anthelmintics in cattle: age influence, diet and nutritional status.
- * The pharmacokinetic profile and tissue residue levels determination of benzimidazole anthelmintics in cattle were carried out by the pharmacological team of Barcelona.
- * The characterization of the embryotoxic and teratogenic effects of benzimidazole anthelmintics was also done in Barcelona.
- * The Toulouse team was in charge of developmental bone abnormalities evaluation by means of morphometric techniques.

Results so far

- ⇒ The kinetic studies developed in cattle and sheep, intraruminally treated with ABZ, have shown differences in ABZ availability (absorption and metabolism) in the animals fed "ad libitum" and those submitted to fasting. The latter animals produced more ABZSO than the former. We also detected differences between the animals fed with concentrated stuff and those fed with grass. The first ones presented a higher availability and concentration of metabolites than the latter.
- ⇒ These studies were also carried out *in vitro*, thus observing that the metabolism was significantly reduced in the ruminal fluids from those animals fed with grass.
- ⇒ The plasmatic kinetics of ABZ and its metabolites were also determined in cattle, showing that ABZ quickly metabolizes and can only be found in plasma as sulphoxide and sulphone. Both present relatively short $t_{1/2}$. Tissue residues studies remarked that none of the metabolites were present in the marker tissues with levels higher than 100 ppm (MRL) after 14 days.
- ⇒ ABZ was shown to produce embryoletality and teratological effects in sheep and rat. We have found skeletal malformations, generally vertebral and costal defects, and developmental kidney anomalies. It is the first time that the teratogenic effect of BZD on blood vessels formation has been proved.
- ⇒ The morphometric technique allowed quantification of the effect of ABZ on the skeletal development of rat and chicken. It also was the first time that a significant delay of the skeletal development was demonstrated in chicken.

Follow-up

- During the last year, the Argentinean group carried out the study of the influence of animal age and development on BZD anthelmintics disposition kinetics and biotransformation.

- In Barcelona, the efforts were addressed to the evaluation of residues of ABZ, Febendazol and their metabolites in cattle. The characterization of malformations induced by ABZ has been mainly focused on the vascular system and has also been realized in Barcelona.
- The cause of the ossification delay was evaluated in Toulouse.
- Results orient the future investigation towards the study of the ABZ enantiomers as an alternative to the racemic currently used.

Selected publications

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Contract number: CII*CT940133

Period: April 1995 to April 1998

MODELLING THE TRANSMISSION DYNAMICS AND DETERMINING THE STABILITY OF *ECHINOCOCCUS GRANULOSUS* IN URUGUAY TO DETERMINE COST-EFFECTIVE METHODS FOR CONTROL

Co-ordinator: University of Cambridge, Cambridge, United Kingdom (S. Lloyd)

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Contract number: CII*CT920020

Period: September 1992 to December 1994

A COMPARATIVE STUDY OF THE EFFECT OF SULFHYDRIL INHIBITORS ON THE EXCITATION-CONTRACTION COUPLING OF SINGLE CARDIAC CELLS

Co-ordinator: Université François Rabelais, Tours, France (J.A. Argibay)

Selected publications

A. Lacampagne, J. Argibay. Alteration of L-type calcium currents by chemical modification of sulhydryl groups in guinea-pig isolated cardiocytes. *J. Physiol.* **467**: 357P.

A. Lacampagne, A. Duittoz, N. Peinau and J. Argibay. Modification of L-type calcium current by chemical interaction with sulhydryl groups in guinea-pig ventricular myocytes. Submitted to *Pflügers Archives*, *European Journal of Physiology*.

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Lacampagne A., Caputo C., Argibay J., 1995. The effect of ryanodine on cardiac calcium current and calcium channel gating current. *Biophysical Society Meeting*, San Francisco, USA.

Caputo C., Bolaños P. 1995. Effects of thapsigargin on isometric tension and fluo-3 calcium transients of amphibian skeletal muscle. *Biophysical Society Meeting*, San Francisco, USA

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BASIS FOR EVALUATING THE FERTILIZING ABILITY OF MAMMALIAN SPERMATOZOA

Coordinator : Consejo Superior de Investigaciones Científicas. Centro de Investigaciones Biológicas. Madrid. Spain (Pedro Esponda).

Objectives

The main objective of this project was to analyze different events of sperm physiology, that can be used as tools for evaluating normal and abnormal sperm fertilizing ability. The epididymal function, the influx of external conditions, the acrosome reaction, and the kinetics of sperm-zona pellucida interaction during fertilization were the main targets of study.

Activities

- * Evaluation of the function of human spermatozoa from fertile and infertile men, and the kinetics of the sperm-zona *pellucida* interaction was studied in the Chilean laboratories (Universidad de Chile and Universidad Católica de Chile).
- * Analyses of the influx of external factors, such as light and photoperiod, were developed together by the Spanish group and the University of Chile laboratory.
- * On the other hand, the cytochemical analysis of embryos (in order to locate nuclear antigens and enzymes) was carried out by the Spanish group and the group from Universidad Católica de Chile.
- * 11 publications and 16 congress presentations were made, using the results of this project.

Results so far

- ⇒ Human sperm fertilizing ability was examined in fertile and infertile men. Acrosome reaction analyses proved to be a reliable parameter of sperm fertilizing ability. This conclusion was reached when the kinetics of sperm-zona *pellucida* binding were observed in *in vitro* analyses. Also, the influence of cervical mucus and factors such as -GnRH seem to have a direct effect on the capacity of spermatozoa to bind the *zona pellucida*.
- ⇒ The *in vitro* tests seem to have direct applications for various clinical situations.
- ⇒ Besides, external factors such as photoperiod were investigated in relation to the secretions of epididymis and of male accessory sex glands. Results revealed that light and androgens control the epididymal activity (secretions), and hence the fertilizing ability of spermatozoa by different mechanisms.
- ⇒ The presence of antigens (fibrillarín) and enzymes (RNA polymerase I) were tested in gametes and early embryos and results showed that they appeared late after fertilization.

Follow-up

The Chilean groups are pursuing collaborative work particularly devoted to the analysis of human spermatozoa parameters. In addition, the Spanish laboratory and the group from University of Chile are working on the effects of agropesticides on mammalian sperm physiology.

Selected publications

Vigil P., Wöhler C., Bustos Obregón E., Comhaire F., Morales P. 1994. Assessment of sperm function in fertile and infertile men. *Andrologia* **26** : 55-60.

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DEVELOPMENT OF CELL POLARITY IN INTESTINAL EPITHELIA AND THE USE OF CELL LINES DERIVED FROM COLON CANCER : STUDIES ON WATER AND ELECTROLYTE PERMEABILITY

Co-ordinator: Commissariat à l'Energie Atomique, Direction des Sciences du Vivant, Gif-sur-Yvette, France (Jacques Bourguet)

Objectives

- ◆ Study the permeability properties of Caco-2 epithelial cells (derived from a human COLON cancer) and related cell lines.
- ◆ Compare the permeability properties of the mammalian intestine in vitro with to those described in intestinal cells cultured on permeable supports. To study the effects of enterotoxins.
- ◆ From cell to molecular biology: To determine the role of water channels in the studied epithelial barriers.
- ◆ Attempt the reconstitution of functional aquaporins in transfected cells cultured on permeable supports.

Activities

- ★ Development of a cell-molecular biology facility at Buenos Aires, including: Cell culture chamber (incubator, laminar flow); mRNA extraction and injection system; video-microscopy; espectro-fluorometric determinations; freeze-fracture operation; PCR training; net water flows measurements
- ★ Development of a new methodology to measure water movements in epithelial cells forming a monolayer on a permeable support.

Results and follow up

- ⇒ The publications listed below show the general scope of the results obtained so far.
- ⇒ This project ended in 1996; nevertheless, the co-operative programme between Saclay and Buenos Aires continues.

Selected publications

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- Parisi M., Escobar E., Huet C., Ripoche P., Louvard D., Bourguet J., 1993. Water handling in Caco-2 cells : effects of acidification of the medium. *European J. Physiology.* **423**: 1-6.
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Period: January 1993 to December 1995

***FASCICULIN AS A TOOL TO STUDY THE ROLE OF ACETYLCHOLINESTERASE
IN THE BRAIN IN BOTH CHOLINERGIC AND NON-CHOLINERGIC
TRANSMISSION***

Coordinator : University of Oxford, Oxford, United Kingdom (Susan Greenfield)

Selected publications

Dajas F., Silveira R., Costa G., Castello M.E., Jerusalinsky D., Medina J., Levesque D., Greenfield S. 1993. Differential cholinergic and non-cholinergic actions of acetylcholinesterase in the substantia nigra revealed by fasciculin-induced inhibition. *Brain Research*. **616**. 1-5.

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**STRUCTURAL AND FUNCTIONAL STUDIES OF THE ENZYMES FROM THE
NAG REGULON OF *ESCHERICHIA COLI***

Co-ordinator : Institut de Biologie physico-chimique, Paris, France (Jacqueline Plumbridge)

Objectives

The amino sugars are both essential components of the bacterial cell wall and outer membrane as well as sources of energy. Because they have these dual functions the flux of amino sugars between the biosynthetic and degradative pathways must be controlled. This is achieved by regulating both the synthesis and activity of the enzymes of the two pathways. Our goal is a detailed description of the catalytic properties of the enzymes involved in amino sugar degradation to better understand the regulatory mechanisms controlling the use of amino sugars in bacteria.

Activities

The project focuses on the molecular characterisation of the two enzymes of the amino sugar degradative pathway; glucosamine-6-phosphate deaminase (NagB) and N-acetylglucosamine-6-phosphate deacetylase (NagA). Physico-chemical approaches were first used to identify amino acid residues in the deaminase important for its activity and allosteric regulation. More recently the availability of the crystallographic structure has aided the identification of residues involved in both the catalytic and allosteric sites. Candidate aminoacids are subjected to mutational analysis and the mutated proteins analysed to assess their contribution to the enzymic activity. The Mexican laboratory specialises in the protein physical chemistry, enzyme purification and kinetic analysis while the Paris laboratory has undertaken the construction of mutants and their *in vivo* analysis.

Results

So far about 50 mutations have been constructed in the deaminase. The amino acid replacements involved changes to the catalytic site, the allosteric site and to the intersubunit contacts. The involvement of a series of amino acids in a catalytic loop has been confirmed although the precise mechanism has proven to be more complicated than initially predicted. Various mutations affect the allosteric activation and an interesting observation is that many result in a change from the normal K-type activation to an asymmetric V or mixed K-V type. Some residues important in coupling the binding of the allosteric effector to changes in the active site have been identified. Certain of these mutations have detectable effects on the growth of bacteria on amino sugars. The first purification and kinetic analysis of N-acetylglucosamine-6-phosphate deacetylase has also been achieved.

Follow-up

The crystallographic structure of deaminase, which was made in collaboration with laboratories in Brazil and Mexico, was a major advance in the study of the enzyme and will guide the future studies on this enzyme. Glucosamine-6-phosphate deaminase can now be considered the model system for a homopolymeric allosteric enzyme. Crystallographic studies are en route for the deacetylase. During the course of this project a clone of the human deaminase became available and enabled us to initiate a comparative study of this eukaryotic enzyme.

Selected publications

Altamirano M.M., Plumbridge J.A., Barba H. and Calcagno M.L., 1993. Glucosamine-6-phosphate deaminase from *Escherichia coli* has a trimer of dimers structure with three intersubunit disulphides. *Biochem. J.* **295**: 645-648.

Plumbridge J.A., Cochet O., Sousa J.-M., Altamirano M.M., Calcagno M.L. and Badet B., 1993. Coordinated regulation of amino sugar synthesising and degrading enzymes in *E. coli*. *J. Bacteriol.* **175**: 4951-4956.

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Souza J.-M., Plumbridge J.A. and Calcagno M.L., 1997. N-Acetylglucosamine-6-phosphate deacetylase from *Escherichia coli* : Purification and molecular and kinetic characterization. *Arch. Biochem. Biophys.* **340**: 338-346.

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Period: April 1993 to September 1996

**MOLECULAR STUDIES ON DESICCATION-INDUCED GENE PRODUCTS AND
THE REGULATION OF THEIR EXPRESSION IN THE DESICCATION
TOLERANT RESURRECTION PLANT *CRATEROSTIGMA PLANTAGINEUM***

Coordinator: Max-Planck-Institut, Köln, Germany (D. Bartels)

Objectives

The general and long-term objective is to develop plants that exhibit an improved tolerance to water deficit.

Activities

During this project, strategies have been developed in the collaborating laboratories to isolate and characterize genes encoding proteins regulating gene expression related to drought. To conduct these experiments, the desiccation-tolerant resurrection plant *C. plantagineum* was chosen as an experimental system for the following reasons:

The resurrection plant is being used as an experimental model system to dissect pathways leading to desiccation tolerance. This plant can recover from severe desiccation within 24 hours of contact with water. During drying or ABA treatment, novel gene products accumulate rapidly in leaves and other tissues. It is assumed that these gene products contribute to the protection of cellular structures during dehydration. Many of these transcripts encode proteins with significant homologies to LEA (=Late Embryogenesis Abundant) proteins, which are abundantly expressed in developing embryos of higher plants and which are developmentally regulated by ABA. It has been a major goal to isolate genes and determine parameters that regulate the expression of the response genes.

Results

- ⇒ In the course of the project, genes encoding putative transcription factors of the myb and leucine zipper family were cloned; a third class of genes may be involved in a signalling pathway. These genes have been selected because the encoded transcripts are responsive to ABA and/or drought. The first class of relevant genes which was isolated belongs to the *myb* gene family. Myb genes comprise a gene family with several members. Out of this family two transcripts were identified and characterized. One clone designated cpm10 encodes a transcript which is upregulated by ABA in undifferentiated callus tissue. The other clone cpm7 codes for an RNA responsive to dehydration in roots. These findings show that *myb* proteins are candidates for transcription factors acting tissue specifically and activating gene expression under stress.
- ⇒ In a search for transcription factors activating drought-stress-induced genes, DNA binding proteins were purified, which had previously been shown to be necessary in gene activation. In a biochemical approach, a protocol for the purification of the DNA-binding protein was developed, involving ion exchange chromatography and affinity chromatography.

⇒ In the third part, defined primers were used to isolate transcription factors known to be involved in ABA-mediated gene expression. As a result of this work, two highly interesting genes have been cloned and characterized. Both of these genes encode transcripts modulated by ABA or drought. One of the transcripts codes for a potential transcription factor of the homeodomain leucine zipper family, the other one encodes an enzyme (phospholipase D) potentially involved in the synthesis of second messengers.

Follow up

The investigation of the genes identified in this project is being pursued through support in an extended collaboration.

Selected publications

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Period: 1992 to 1994

STRUCTURE FUNCTION RELATIONSHIP IN LIGHT MODULATED ENZYMES OF HIGHER PLANT CHLOROPLASTS

Co-ordinator: CSIC, Granada, Spain (Julio López-Gorgé)

Objectives

Plants contain a wide variety of thioredoxins (small-molecular-weight proteins involved in redox regulation reactions) divided at least into three types, *m*, *f*, *h*, based on their subcellular localization, biochemical reactivity and amino acid sequence. In addition, some thioredoxins display a high specificity and tight interaction with their target enzyme (this is true for example of the FBPase/thioredoxin *f* couple). In addition, in plants, the three-dimensional structure of thioredoxins has not been solved so far and it was of high interest to obtain structural models that could help understand the peculiarities of these proteins in plants.

Activities

The project focused on the cloning of several types of thioredoxins in plants (chloroplastic and extra chloroplastic) as well as on some target enzymes (chloroplastic fructose-1,6-bisphosphatase, FBPase, was the prominent one).

Results

- ⇒ Protein sequences, cDNA and genomic clones have been isolated for thioredoxins of the *f*, *m*, and *h* types.
- ⇒ Expression systems that permit to obtain high-yields from recombinant proteins have been successfully set up.
- ⇒ Expression and mutagenesis of FBPase has been realized. It has been shown that the regulatory site of this enzyme is more complex than previously thought, involving three residues instead of the canonical two.
- ⇒ Attribution of the NMR resonances and secondary structures of thioredoxin *m* and *h* has been done.

Follow up

After the project was completed, several papers dealing either with structure or mutagenesis of components of the system were published by our group: Mittard et al., 1997, Eur J. Biochem, **243**:374-383 describe the 3D structure of *Chlamydomonas reinhardtii* thioredoxin *h* and the one by Jacquot et al., 1997, FEBS Letters **401**: 143-147 additional mutagenesis of the fructose bisphosphatase regulatory site. Experiments are now under way to uncover the expression pattern

of the components of the ferredoxin thioredoxin systems and to study the structural basis of the expression of the heat stability of the thioredoxin molecules.

Selected publications

- Stein M., Jacquot J.P., Jeannette E., Decottignies P., Hodges M., Lancelin J.M., Mittard V., Schmitter J.M., and Miginiac-Maslow M. 1995. *Chlamydomonas reinhardtii* thioredoxins : Structure of the genes coding for the chloroplastic *m* and cytosolic *h* isoforms - Expression in *Escherichia coli* of the recombinant proteins, purification and biochemical properties. *Plant Molecular Biology*. **28**: 487-503.
- Lancelin J.M., Stein M., Jacquot J.P. 1993. Secondary structure and protein folding of recombinant chloroplastic thioredoxin Ch2 from the green alga *Chlamydomonas reinhardtii* as determined by ¹H NMR. *J Biochem*. **114**: 421-431 .
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Contract number: CI1*CT920084

Period: March 1993 to March 1996

**MULTIDISCIPLINARY ANALYSIS OF PHENOTYPIC DIFFERENTIATION OF
NEURONAL CELL TYPES INDUCED BY CENTRAL GLIA**

**Co-ordinator: Consejo Superior de Investigaciones Científicas, Madrid, Spain
Washington Buño)**

Selected publications

- A. Araque, D; Cattaert and W. Buño. 1995. Cd²⁺-blockade of hyperpolarization-activated current in crayfish muscle. *J. Gen. Physiol.* **105**:725-744.
- A; Araque and W; Buño. 1995. Fast, persistent Ca²⁺-dependent K⁺ current controls graded electrical activity in crayfish muscle. 1995. *Eur. J. Physiol. (Pflugers Archives)*. **430**: 541-551.
- A; Araque, F.J. Urbano, C. Cerveñanski, L. Gandía and W. Buño. 1995. Selective block of Ca²⁺-dependent K⁺ current in crayfish neuromuscular system and chromaffin cells by sea anemone *Bunodosoma cangicum* venom. 1995. *J. Neurosci. Res.* **42**:539-546.
- M. Borde, J.R. Cazalets and W. Buño. 1995. Activity-dependent response depression in rat hippocampal CA1 pyramidal neurones *in vitro*. *J. Neurophysiol.* **74**:1714-1729.
- F. Sierra, D. Lorenzo, O. Macadar, and W. Buño. N-type Ca²⁺ channels mediate transmitter release at the electromotoneuron-electrocyte synapses of the weakly electric fish *Gymnotus carapo*. *Brain Res.* **683**: 215-220.

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**MOLECULAR BASIS OF EXTENSION RATE MODULATION BY
PHYTOCHROMES IN LIGHT-GROWN PLANTS**

Co-ordinator: University of Leicester, Leicester, United Kingdom (Harry Smith)

Objectives

Identify genes the expression of which was modified as a component process in the extension growth responses of plants exposed to light regimes differing in the relative amounts of red (R) and far-red (FR) light. The phytochrome family of photoreceptors mediates these growth responses, which are collectively known as the 'shade-avoidance syndrome'.

Activities

The project focussed on new approaches to identify genes the expression of which is regulated by light regimes that simulate the conditions found within dense plant canopies. Dr. Jorge Casal, at the University of Buenos Aires, undertook investigations at the protein/enzyme level, using the techniques of in vitro RNA transcription and 2-D protein electrophoresis. The Leicester group, under the direction of Professor Harry Smith, carried out investigations at the mRNA level. At Leicester, differential display was used in a search for mRNAs regulated by light regime, and a set of promoterless *Gus*-tagged mutants has been screened for defects in extension growth responses. Additionally, transcript levels of *Athb2*, a gene the expression of which is known to be light-regulated, were investigated under simulated shade-avoidance conditions.

Results

The principal conclusion from this collaboration was disappointing in that neither group was able to obtain evidence from marked modifications in gene expression as part of the shade-avoidance syndrome. Two-dimension protein electrophoresis of extracts of cucumber wild types and mutants lacking shade-avoidance reactions, showed small changes in polypeptide distribution, but the changes were not reproducible. Similarly, differential display of mRNA preparations of *Arabidopsis* seedlings exposed to simulated shade conditions yielded a number of positives, but most turned out to be false positives when checked by Northern analysis. Overall, it seems clear that gene-expression changes underlying the extension growth responses of plants to light signals in crowded communities probably involve only a small number of relatively low-abundance transcripts. The fact that *ATHB2* expression is markedly modified by such light signals suggests that other genes are similarly regulated. *ATHB2* encodes a HD-ZIP transcriptional regulator, which is expressed at very low levels. We concluded that a search for gene-expression changes mediated by shade-light signals will require more refined methodology than we had at our disposal at that time.

Follow up

The collaboration was useful, but difficult because of the huge distances involved. We agreed to follow up the work along separate lines. The Argentinean group concentrated on studies with novel photoreceptor mutants, whereas the Leicester group moved to use more refined differential display analyses and cDNA micro-array studies in an attempt to isolate genes the expression of which is regulated by light quality. This work was funded under the Biotechnology Programme of Framework IV.

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EVOLUTIONARY STUDY OF THE MOLECULAR BASIS OF NEURAL INDUCTION AND LIMB PATTERN FORMATION

Co-ordinator: European Molecular Biology Laboratory, Heidelberg, Germany (Rolf Zeller)

Objectives

The scientific objective of this collaboration was to study comparatively the distribution and conserved functions of different types of regulatory molecules during amphibian (*Xenopus laevis*) and amniote (chicken, mouse) neurogenesis and limb development.

Activities

Recent experimental evidence indicates that the main regulatory cascades controlling embryonic development are evolutionary-conserved. Therefore, a comparative study of specific members of the *Hoxb* gene family was initially performed using *X.laevis* and chicken embryos. For this purpose, not only reagents were exchanged, but the necessary methodology was established and improved ("whole-mount" detection of transcripts and proteins in embryos). An excellent exchange of technology and knowledge was achieved by several working visits of the Argentinean partner to EMBL and joint participation at international meetings. During the second part of this collaboration, the cell-cell signalling mechanisms regulating neural tube and limb development were studied by comparative analysis of specific signalling molecules (SHH, FGFs) using *X.laevis*, chicken and mouse embryos. As the mouse *formin* gene products have been implicated in positive regulation of SHH signalling, its previously unknown *X.laevis* homologue was also isolated. Continuing functional analysis of *formin* should provide insights into how morphogenetic signals such as SHH are regulated and function during embryonic pattern formation.

Results

Initially, the laboratory in Buenos Aires contained all necessary equipment to perform the proposed molecular embryology studies. Improvement of the whole-mount *in situ* hybridization technique (crucial to the proposed studies) resulted in the publication of the two most up-to-date molecular biology manuals used world-wide. Comparative analysis of *Hoxb7* proteins during *X.laevis* and chicken embryogenesis revealed their conserved distribution. Furthermore, experimental manipulation of *X.laevis* and chicken embryos established that endogenous retinoids regulate neural tube polarity and spatial distribution of homeobox genes such as *Hoxb7* during pattern formation. As these studies were in progress, others established that SHH encodes a morpho-regulatory signalling molecule that regulates aspects of both neural tube and limb bud patterning. Therefore, the *X.laevis* SHH homologue was isolated and its analysis provided evidence for a regulatory role during gastrulation and neurulation. Furthermore, molecular analysis of the mouse *limb deformity (ld)* mutation showed that the disrupted gene products (*formin*) positively regulate establishment of the SHH expression domain during limb pattern formation. In particular, the SHH/FGF-4 feedback loop and establishment of positional information, as marked by differential *HoxD* expression, are

disrupted in limb buds of *ld* mutant embryos. Taken together, these studies lend further support to the concept that the molecular mechanisms regulating gastrulation, neural tube development and limb bud patterning are rather similar. As *X.laevis* embryos are an excellent model to study gastrulation and neural induction, the *X.laevis formin* homologue has been isolated to study possible general roles of *formin* in regulation and transduction of the SHH signal. The morphoregulatory functions of this novel protein are now being studied using combined embryological and genetic approaches.

Follow-up

Continuing functional and genetic analysis of cell-cell signalling processes using both *X.laevis* and mouse embryos should further our understanding of how pattern formation is controlled in different types of vertebrate embryos.

Selected publications

- Carrasco, A.E. and López, S.L. 1994. The Puzzle of Hox Genes. *Int J. of Dev. Biol.* **38**:557-564.
- López S.L., Dono R., Zeller R. and Carrasco A.E. 1995. Differential Effects of Retinoic Acid and a Retinoid Antagonist on the Spatial Distribution of the Homeoprotein Hoxb-7 in Vertebrate Embryos. *Dev. Dyn.* **204**:457-471.
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- Uetz P., Fumagalli S., James D. and Zeller R. 1996. Molecular Interaction between Limb Deformity Proteins (Formins) and Src Family Kinases. *J. Biol. Chem.* **271**:33525-33530.
- Haramis, A.G. and Carrasco, A.E. 1996. Whole-Mount In Situ Hybridization and Detection of RNAs in Vertebrate Embryos and Isolated Organs. *Curr. Protocols in Mol. Biol.* **2**:14.9.1-14.9.18.

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Contract number: CII*CT930037

Period: January 1994 to June 1996

AXONAL mRNAs: IDENTIFICATION, ORIGIN AND ROLE

Co-ordinator: Universita di Napoli, Napoli, Italy (Antonio Giuditta)

Objectives

Proving the existence of a local system of protein synthesis in axons and nerve endings. .

Selected publications

Yong T. Chun, A.E. Gioio, M. Crispino, A. Giuditta, B.B. Kaplan. 1995. Characterization of squid enolase mRNA: sequence, analysis, tissue distribution, and axonal localization. *Neurochemical Research*, vol. 20, no. 8, 923-930

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FORM AND VARIABILITY IN CHILEAN NOTHOFAGUS SPP

Co-ordinator: Université de Montpellier II, Montpellier, France (Bernard Thiebaut)

Objectives

Nine species of southern beech (*Nothofagus* Blume) are found in Chile. These trees are of biological, economic and silvicultural importance. Our objective was to study their biological diversity. Our results should improve knowledge of the biology of these trees and help the forester develop of management plans for Chilean *Nothofagus* forests.

Activities

The different teams worked in different disciplines and collaborated on four principal subjects: (I) Tree morphology, (ii) Anatomy and phenology of vegetative and reproductive organs, (iii) Genetic variability and (iiii) Germination in *N. alpina*.

The following species were studied:

- 1 - *Nothofagus alessandrii* Espinosa, "ruil", deciduous
- 2 - *Nothofagus alpina* Oersted, "rauli", deciduous
- 3 - *Nothofagus antartica* Oersted, "niré", deciduous
- 4 - *Nothofagus betuloides* Oersted, "coigüe de Magallanes", evergreen
- 5 - *Nothofagus dombeyi* Blume, "coigüe", evergreen
- 6 - *Nothofagus glauca* Krasser, "hualo", deciduous
- 7 - *Nothofagus nitida* Krasser, "coigüe de Chiloé", evergreen
- 8 - *Nothofagus obliqua* Blume, "roble", deciduous
- 9 - *Nothofagus pumilio* Kasser, "lenga", deciduous

Results

4.1. Tree morphology:

According to Troll's architectural model, the 9 Chilean species develop in the same way as other beeches of the *Fagus* genus in the northern hemisphere and *Nothofagus* in the southern hemisphere. The diversity of canopies observed between species and, within species, between seasons and individuals represents plasticity within the same architectural model. Canopy development was examined in beech (*Fagus and Nothofagus*) and oak (*Quercus*). Oaks have a sympodial architecture characterized by a weak hierarchy between tree and branches. On the other hand, beeches follow a more strongly hierarchical monopodial organization

4.2. Anatomy and phenology of vegetative and reproductive organs in the Chilean beeches:

Leaf appearance is earlier and more rapid among the deciduous species. The secondary cambium is seasonally active in both cases. The wood anatomy of *N. alpina* and *N. dombeyi* confers mechanical properties useful for wood-working.

4.3. Genetic variability:

Ten enzyme systems were analyzed and 17 polymorphic genes identified in the Chilean beeches. The interspecific variability of the 9 Chilean *Nothofagus* was high. The within-population genetic diversity was also high in 2 Chilean species. With a few exceptions, the most frequent alleles were the same in all the populations studied, indicating the existence of significant gene flow between populations of the same species.

4.4. Germination in *N alpina*:

Field and laboratory studies were carried out in order to establish a methodology.

Follow-up

The methods established by this project are already being employed in new applications, namely, the study of seven Chinese beeches, modelling tree development and three-dimensional imaging. The genetic work carried out in Chile will be completed by studying the same genetic markers in a greater number of individuals and by searching for new genes.

Selected publications

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THE CONSTRUCTION OF A DETAILED REACTION MODEL OF THE Na,K-PUMP

Co-ordinator: University of Arhus, Arhus, Denmark (Jens G. Nørby)

Objectives

The goal of this project is to develop in detail an experimentally verified reaction model for Na,K-ATPase (the Na,K-pump).

Activities

Preparation of enzyme from pig kidneys and measurements of steady state ATPase reaction rates of the enzyme under a large variety of liganded conditions. The concentration of the liganded states like ADP-bound, substrate-site phosphorylated (EP), and enzyme with occluded (sequestered) Rb^+ , $E[Rb]$, was determined in parallel. Especially important has been the development of a special quenching-and-washing chamber (QWC) that has enabled for the first time the quantitative determination at room temperature of the crucial Rb^+ -occluded intermediate formed during the forward progression of the reaction.

Results so far

The binding of substrate and product (ATP, ADP) and the rate of hydrolysis of ATP are profoundly influenced by both ionic strength and specific anions. This characterised the electrostatics of the substrate binding site and serve as a control for experimental variations in ionic conditions. In the absence of K^+ , the enzyme is a Na-ATPase. The generally established reaction scheme was satisfactory to describe the mechanism for this enzymatic activity. The critical measurements in the presence of K^+ (Rb^+) of the ratio : reaction rate (v) divided by intermediate concentration (EP or $E[Rb]$) provided the kinetic constants v/EP and $v/E[Rb]$. These could not be explained by the existing reaction scheme for Na,K-ATPase. Furthermore there was a pronounced discrepancy between the kinetic constant ($= v/E[Rb]$) for deocclusion of Rb^+ and the same constant directly measured in the QWC. This indicates that other hydrolysis modes, than those predicted by the conventional scheme, take place.

Follow-up

The reaction modes described will be monitored by the intrinsic protein fluorescence changes concomitant with the biochemical events, using a newly installed Applied Photophysics stopped-flow fluorimeter. When intrinsic changes are too small, an extrinsic probe (e.g. eosin) will be used. Combined with studies of the "backwards" formation of the crucial $E[Rb]$ this will hopefully lead to elucidation of the discrepancies between the general model and our results, and thus to a more complete model for the mechanism of the Na,K-pump.

Selected publications

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ENVIRONMENTAL INFLUENCES ON THE ECOLOGY AND PHYSIOLOGY OF SUB-ANTARCTIC FISH

Co-ordinator: University of St Andrews, St Andrews, U.K. (Ian A Johnston)

Objectives

To investigate larval development, growth, reproduction and bioenergetics in Sub-Antarctic Notothenioids, with a view to providing a sound biological basis for the management and conservation of species which constitute an important artisanal fishery in Chile and Argentina.

Activities

The project focussed on three species : *Eleginops maclovinus*; *Patagonotothen tessellata*, *Champscephalus esox*.

C.esox is of particular evolutionary interest because it is the only haemoglobinless icefish found outside the Antarctic. Exchanges between the collaborating laboratories took place throughout the project and a high degree of interaction between partners was achieved; with an exchange of techniques and expertise between laboratories. During 1997, results from the project have been presented at meetings of the Society for Experimental Biology at Canterbury, UK and at an "International Seminar on Marine Biology Research in Antarctic and Sub-Antarctic waters" held in Punta Arenas, Chile. The publications of the project are now beginning to appear in international scientific literature.

Results to far

The main conclusion of the study was that the physiological and ecological characteristics of the sub-Antarctic Notothenioids closely resemble those of the Antarctic Notothenioids living at lower and more constant temperatures. Slow growth rates, moderate aerobic scopes for activity and moderate energy investment in reproduction are probably related to the relatively low temperatures and high seasonal variation of the coastal ecosystem. The development of embryonic and larval stages was shown to be extremely sensitive to small rises in average sea temperature. The results highlight the vulnerability of the endemic Notothenioid fauna to over-fishing, competition with non-native species (e.g. salmonids) and global climate change. The unique biological characteristics of the fauna of the sub-Antarctic must be considered in the development of any policy for the conservation and management of artisanal fisheries stocks in the Beagle Channel.

Selected publications

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Patagonotothen tessellata (sub-family Nototheniinae). *Comparative Biochemistry and Physiology*, **118**, N° 4, 1437-1445.

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**EFFECTS ON THE PHYSIOLOGY OF ORGANISMS OF CHANGES BROUGHT
ABOUT BY GENETIC ENGINEERING**

Co ordinator: University of Edinburgh, Edinburgh, U.K. (Henrik Kacser [‡])
Universidad de la República, Montevideo, Uruguay (Luis Acerenza)

Objectives

- ◆ The general and long term goal is to develop new theoretical tools for the analysis and design of *in vivo* metabolic changes to such a stage that the theoretical frameworks could be applied to practical problems of biotechnological interest such as the improvement on the amount and yield of molecules produced by micro-organisms.

Activities

- * We studied and classified the constraints that restrict the design of metabolic responses using the frameworks of Metabolic Control Analysis (MCA) and Metabolic Control Design (MCD). Some of their consequences were derived.
- * We extended the double modulation method for *in situ* determination of elasticities.
- * We combined MCD with optimization procedures to study the consequences of the constraints in the responses on the maximization of metabolic fluxes.
- * We applied MCD to design a monocyclic cascade with a high sensitivity of response.

Results

- ⇒ The constraints in metabolic responses were classified in structural and kinetic. The structural constraints depend on the stoichiometry of the network and the kinetic constraints on the types of rate laws of the component enzyme catalysed reactions. Combining some consequences of these constraints with experimental information available for the bacteria *E.coli* we have shown that in this and similar organisms only a small fraction of the responses are independent, the main contribution responsible for this restricted situation being the kinetic constraints.
- ⇒ In the framework of MCA, metabolic responses can be expressed as a function of coefficients, called elasticities, that describe the responses of isolated enzyme-catalysed reactions. The double modulation method is used for experimental *in situ* determination of elasticities. We have developed a matrix representation of this method so that it can be applied to systems of any structure and size.
- ⇒ The existence of constraints in the metabolic responses affects the outcome of optimization processes. For instance, we have shown that the structure of the metabolic network that results from an optimization process may depend on the strength of the constraints in the responses.
- ⇒ The application of MCD to a monocyclic cascade has allowed us to obtain, in a systematic way, all the parameter conditions in which the structure shows a highly sensitive response.

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Selected publications

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BIODIVERSITY WITH APPLICATIONS IN BIOTECHNOLOGY OF YEASTS IN TROPICAL ECOSYSTEMS

Co-ordinator: University of Perugia, Perugia, Italy (Alessandro Martini)

Objectives

- ◆ Isolate yeast cultures from different tropical ecosystems in Brazil,
- ◆ Describe new biotypes and species,
- ◆ Characterize the yeast communities associated with various substrates including fruits, soil and plant surfaces,
- ◆ Deposit the most representative isolates in culture collections
- ◆ Select yeasts with potential applications in biotechnology.

Activities

- * The project focused on the collection of samples from Brazilian tropical ecosystems as different as fecal pellets of small mammals, fresh water from bromeliad flowers and streams, flowers and fruits, insects, mushrooms, exudates from roots and trees, soil and others. Isolated cultures have been identified in strict collaboration in the laboratories of both partners, by means of conventional yeast taxonomy, while the classification of representative isolates was confirmed by comparative analyses of electrophoretic karyotypes as well as by nDNA-nDNA hybridization studies. Representative strains within each isolated species have been selected to conserve in the Industrial Yeast Collection DBVPG of the University of Perugia and the Coleção de Culturas do Instituto de Microbiologia da Universidade Federal do Rio de Janeiro.
- * An effective international cooperation between our European and Brazilian laboratories was established, with frequent visits, exchanges of graduate students and joint attendance of specialized meetings in Europe, Brazil, Canada and Australia.

Results so far:

- ⇒ In the course of the project, more than 1,400 yeast cultures have been isolated and identified. Yeast biodiversity in the tropical forest has been defined in relation to succession, distribution, dissemination within niches, community structures and food webs. Specifically distinct yeast communities have been associated with different microhabitats, and several new species identified and characterized by molecular taxonomy procedures. Particularly well identified was the structure of the yeast communities associated with substrates including fruits and vectors of fruit associated yeasts from diverse microhabitats of several Brazilian tropical ecosystems.
- ⇒ Altogether, an array of ca. 650 cultures from non-conventional tropical environments is presently available for screening surveys aiming at selecting strains endowed with commercially exploitable properties. About half of the available cultures belong to the *Basidiomycetes*, a group of yeasts so far very poorly studied for biotechnological applications.

Follow up

Screening surveys are currently under way in both laboratories in Brazil and Italy with the aim of eliciting yeast strains capable of producing extracellular compounds potentially utilizable by the pharmaceutical and biotechnological industries. Preliminary results indicate that the yeast cultures of basidiomycetous origin include good candidates for a series of biotechnological applications.

Selected Publications

Santos E. A., de Oliveira R. B, Mendonça-Hagler L. C, and Hagler A. N. 1996. Yeasts associated with cashew, caja, umbu, and mango fruits typical of the semiarid region of northeastern Brazil. *Rev. Microbiol.* **27**(1): 33-40.

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**STUDIES ON ADP-RIBOSYLATION IN PROTOZOA AND PLANTS, AND DNA
TOPOISOMERASE IN PROTOZOA**

**Co-ordinator: University of Sussex at Brighton, Brighton, United Kingdom,
(S. Shall)**

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MOLECULAR REGULATION OF VERTEBRATE GLUTAMATE RECEPTORS

Co-ordinator: University of Bristol, Bristol, United Kingdom (Jeremy Henley)

Objectives

The objectives were to study the molecular and cellular biology of AMPA-type glutamate receptors and elucidate some of the mechanisms regulating their neuronal distributions and functions.

Activities and results

Results include:

- ⇒ The characterization by the IIBCE group of the glutamate receptor-evoked acetylcholinesterase (AChE) release (GEAR) response, recorded both *in vivo* and *in vitro* at cellular and subcellular levels
- ⇒ The discovery of a modulation of AMPA receptors by an enzymatic action of AChE
- ⇒ The finding that AChE is initially protective, moderating motor neurone overactivation by degrading excess acetylcholine and promoting synaptic plasticity.
- ⇒ The finding that AChE is endocytosed by astrocytes and activates astroglial and microglial cells.
- ⇒ The finding that GEAR precedes GluR-mediated apoptosis and neurones.
- ⇒ The finding that the modulation of AMPA receptors by AChE *in vivo* may be an important mechanism in neuronal degeneration.
- ⇒ The use of the two-hybrid system to identify a novel interaction between the GluR2 AMPA receptor subunit and the fusogenic protein NSF.
- ⇒ Biochemical characterisation of the metabolic half-life and surface expression of AMPA receptors in cerebellar granule cells.
- ⇒ Determination of the subcellular distributions of AMPA and NMDA receptors and developmental changes in the proportion of receptors that are surface expressed.

Selected publications

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- Nishimune A., Isaac J.T.R., Molnar E., Noel J., Nash S.R., Tagaya M., Collingridge G.L., Nakanishi S., and Henley J.M. 1998. NSF binding to GluR2 regulates synaptic transmission. *Neuron*. **21**: 1-11.
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MEMBRANE SKELETAL MUSCLE RECEPTORS AND ANTAGONISTS OF THE HORMONE 1,25-DIHYDROXYVITAMIN D₃ (CALCITRIOL)

Co-ordinator: Universidad de Santiago de Compostela, Santiago, Spain (Antonio Mouriño)

Objectives

- ◆ The long term goal of the project was the understanding of the structure of VDR (receptor of the hormone 1,25-dihydroxyvitamin D₃) to design new vitamin D analogues of clinical interest.
- ◆ The short term goals of the project were : (i) Synthesis of new analogues of the hormone. (ii) Biological assays of new analogues to study their potential as tool to isolate and study the VDR via affinity columns and photoaffinity probes. (iii) Biological assays of new analogues to study their potential as drugs for the treatment of cancer, psoriasis and order disorders. (iv) Biological assays of new analogues to study their non-genomic properties. (v) Biological assays of new analogues to study their potential as antagonists of the hormone 1,25-dihydroxyvitamin D₃.

Activities

- ★ The synthesis of new analogues of the hormone 1,25-dihydroxyvitamin D₃ were carried out in Santiago de Compostela.
- ★ The biological assays of the new analogues were carried out in Bahia Blanca (Argentina).
- ★ Temporary additional studies on the expression and purification of recombinant non-fusion human 1,25-(OH)₂-D₃ receptor (VDR) were carried out in Salzburg and Vienna.

Results

- ⇒ Synthesis. In the course of this period 8 new vitamin D analogues **12** (P1) - **17** (P6), **18** (CLO 1) and **19** (CLO 2) were synthesized for biological testing. The vitamin D analogue 10 (YF95G72) was re-synthesized for biological testing. The preparation of **M** and **N** is in progress. The synthesis of these compounds was accomplished in high yield by the convergent Wittig-Horner approach using the A-phosphine oxide fragment synthesized by a new efficient route developed for these purposes in our laboratory.
- ⇒ Biological assays. The following biological assays have been carried out : (i) (Binding to the VDR). (ii) ICA (Intestinal calcium absorption). (iii) Rapid effects on enterocyte Ca (II) influx, (iv) Rapid effects on enterocyte [Ca(II)]_i levels. (v) Fast changes in enterocyte cAMP and IP3 levels induced by analogs. (vi) Rapid effects of analogues on myoblast [Ca(II)]_i levels. (vii) Fast changes in myoblast cAMP and IP3 levels. (viii) Effects on myoblastt DNA synthesis. (ix) Activity on osteoblast differentiation. (x) Binding to vitamin D binding protein (DBP).
- ⇒ Expression and purification of recombinant non-fusion human VDR
- ⇒ Conclusion : Groups of analogues could be identified which stimulate or inhibit intestinal calcium activity. The ICA assays show that **15** and **19** have potent antagonist activity. This is a noteworthy finding since these analogues are of potential interest for the treatment of hyperproliferative and hypercalcemic diseases. Relevant is the fact that compounds **10** and **19** bind well to the VDR. These compounds will be taken as models for the construction of other vitamin D analogues for the development of affinity columns and photolabelling probes to isolate the VDR and to study the structure of the VDR active site. Preliminary temporary studies in collaboration with Austrian research teams have led to the expression and isolation of highly pure

VDR. Based on the above results we conclude that C-22 might be a suitable position for the introduction of photolabile groups to study the active site of VDR or linkers for the construction of affinity columns for the purification of VDR. Analogues based on **19** are also promising.

Follow-up

We are now focusing our efforts on the synthesis of new vitamin D analogues of type A and B to explore their potential for the construction of affinity columns for the isolation of pure VDR and photoprobes to study the VDR active site. Analogues M and N are being prepared for biological testing.

Selected publications

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REGULATION OF THE CITRATE TRANSPORT SYSTEM FROM *LACTOCOCCUS LACTIS* BIOVAR *DIACETYLLACTIS*

Co-ordinator: Consejo Superior de Investigaciones Científicas, Madrid, Spain
(Paloma López)

Objectives

The use of citrate in milk by lactic acid bacteria leads to the production of aromatic and flavour compounds, which are responsible of the organoleptic properties of butter and butter cheese. The transport of citrate in *Lactococcus lactis* biovar *diacetylactis* (*L. diacetylactis*) is catalysed by the citrate permease P (CitP). This polypeptide is encoded by the *citP* gene (which is included in the *citQRP* operon) carried by plasmid pCIT264.

The main objective of the project was the elucidation of the regulatory mechanisms involved in the expression of the *citQRP* operon in *L. diacetylactis* as well as to determine the effector of this regulation. The accomplishment of this objective had contributed to the general knowledge on regulation of gene expression and stress response in lactic acid bacteria, and it had provided a better understanding of the control of citrate utilisation by the industrial micro-organism *L. diacetylactis*.

Activities

The project was focused on the molecular characterisation of the citrate transport operon from *L. diacetylactis*. The construction of plasmids and gene manipulation in lactococci was carried out in Tucuman (Argentina) with the help of the partners in Rosario (Argentina) and in Madrid (Spain). The transcriptional analysis and DNA sequence determinations were mainly performed in Madrid. Rosario did most of the biochemical analysis of the citrate transport system. All groups were involved in the physiological studies of citrate utilisation.

Results

- ⇒ In this project the fate and synthesis of *citQRP* transcript was analysed, an interplay of mRNA processing and of expression of *citQ* and *citR* genes identified, and the environmental conditions required for efficient expression and activity of CitP determined.
- ⇒ It was established that the *cit* transcript is processed at a complex secondary structure, which includes the overlapping region of *citQ* and *citR*. A RNase E-like endoribonuclease appears to be responsible for this processing. The *citQ* and *citR* gene products were identified. The first AUG of the *citQ* open reading frame seems to be the translational start codon for both genes. Provided that *citQ* and *citR* are in different frames, a frameshift even should take place to support synthesis of a CitQ-CitR polypeptide. The interruption of *citQ* upstream of *citR* resulted in an increase of expression of the distal gene *citP*. Therefore, translation of *citQ* versus mRNA processing of *cit* mRNA seems to regulate synthesis of CitP by controlling expression of CitR, which is a transcriptional repressor.

⇒ The citrate uptake is influenced by the external pH in lactococci. CitP has optimal activity within a narrow pH range of 4.5-5.5. Both acidification of the medium and shift to acidic pH result in transcriptional induction of *citQRP* as well as enhancement of the citrate transport activity. This stress response is inhibited by an excess of the CitR polypeptide, and seems to provide to *L. diacetylactis* a selective advantage based on co-metabolism of glucose and citrate at low pHs.

Selected publications

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Contract number: CI1*CT940017

Period: April 1995 to March 1998

**CELLULAR SORTING, COOPERATIVE MEMBRANE TOPOGENESIS AND
LIGAND SPECIFICITY OF PURINE AND PROLINE PERMEASES IN
*ASPERGILLUS NIDULANS***

Co-ordinator: Université Paris Sud, Orsay, France (Claudio Scazzocchio)

Objectives

Carry out a detailed study of the structure and function of 1) an amino acid permease 2) a group of purine permeases of overlapping specificity. This study took advantage of the specific characteristic of a model eukaryotic system, the fungus *Aspergillus nidulans*.

Activities

We have carried out studies by classical and directed mutagenesis in the specific proline permease (PrnB) and the major specific uric acid-xanthine permease (UapA). We have investigated the functionality of purine permeases by constructing chimeric proteins between UapA and UapC, a broad specificity purine permease. We have investigated in detail the regulation of the genes coding for the above permeases. We have attempted to obtain antibodies against synthetic peptides derived from the sequence of the three proteins, in order to determine which mutations affect topogenesis. We are attempting the cloning of the gene coding for the specific hypoxanthine-guanine-adenine permease AzgA.

Results so far

We have sequenced 13 classical mutations of the prnB gene and constructed 5 in vitro mutations in crucial regions. This is allowing us to determine which residues are essential for permease activity and substrate specificity. A surprising result is that one cold sensitive mutation affects mRNA stability rather than protein function or topogenesis. We have conducted a complete functional analysis of the UapA and UapC proteins, by classical mutations, in vitro mutagenesis and construction of chimeric proteins. This analysis have led to the identification of a hydrophilic loop responsible for substrate specificity. We have found this loops in ESTs of many organisms, from plants to humans, and this has allowed for the first time the identification of human purine permeases. Suppressor analysis of cold sensitive mutations in the uapA genes has revealed a gene coding for a protein possibly involved in membrane topogenesis of a number of permeases. We have analysed in detail the control of transcription of the uapA and uapC genes, and discovered a new form of control of transcription of the prnB gene. We have obtained antibodies against epitopes of UapA, UapC and PrnB.

Follow-up

Although the new programmes of the EU do not appear to support this kind of fundamental research, the three groups will continue to work in close contact, together with two other

groups in Greece, to dissect completely the function and topogenesis of permeases of this model organism and use the methodology we have developed to study cognate human permeases.

Selected publications

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EXOENZYMES OF *TETRAHYMENA*: PRODUCTION AND PRACTICAL USES

Co-ordinator: Westfälische-Wilhelms-Universität Münster, Münster, Germany (Arno Tiedtke)

Objectives

The general aim of this project was to develop cultivation and fermentation techniques for protozoa, to use these single-celled eukaryotes for the production of biological products. For the ciliated protozoan *Tetrahymena*, the conditions for producing extracellular enzymes, in particular phospholipase A1 (PLA1) - an enzyme not available on the commercial market - had to be created.

Activities

The project focused on the improvement of conditions for mass culture of cells and harvesting of spent culture medium containing the extracellular enzymes. Waste products of industrial nutrient production like turkey meat and feather waste or potato-fruit water from starch production were tested as supplements of growth media. In addition, screening protocols for the detection of mutagenized strains of *Tetrahymena* hypersecretory for PLA1 were developed in Münster. An easy and efficient purification protocol for PLA1 from spent media, using selective interaction chromatography and the physico-chemical characterization of purified PLA1, was developed in Buenos Aires.

Results

Waste products of meat production and potato-fruit water, for example, proved valuable in supplementing the basic culture medium, allowing a biological recycling of these waste products. The screening for mutant hypersecretory strains in PLA1 were successful: different strains releasing at least three times more PLA1 than the parent (wild type) strain were obtained. The PLA1 purified from the spent culture medium of the hypersecretory strains was purified 100 times, giving a yield of 14% pure enzyme. The purified enzyme shows no PLA2 activity and can be preserved as freeze-dried powder.

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STUDIES ON SUBSTRATE AFFINITY IN REACTIONS CATALYZED BY NATIVE AND RECOMBINANT ALPHA-AMYLASES

Co-ordinator : Université d'Aix-Marseille, Marseille, France (G. Marchis-Mouren)

Objectives

α -amylase is a key enzyme; it enables starch resources to be used for glucose production and for the production of energy. α -amylase can accommodate different types of substrate under different states. Our goal was to shed some light on the various processes implied here, and to gain insight into the function of this subsite enzyme, especially regarding its role in human health (diabetes, obesity) or in crop (red bean) storage.

Activities

- ★ The structure of porcine pancreatic α -amylase (PPA) and its complexes with several inhibitors (acarbose, a-AI...) was determined by X-ray crystallography.
- ★ Kinetic studies of the inhibition process have been carried out using different types of substrate. Acarbose is a pseudotetrasaccharidic inhibitor, a-AI is a protein inhibitor extracted from kidney bean. Up to now only the degradation of soluble substrates of various lengths (amylose chain, shorter dextrin and maltopentaose) has been studied in Marseilles. Starch degradation has been studied in Montevideo. Initial velocities of hydrolysis have been determined in Marseille and time course in Montevideo.

Results

- ⇒ Inhibition of amylose, maltodextrin and maltopentaose has been undertaken at fixed inhibitor concentration.
- ⇒ The effects of acarbose and the reaction products were analyzed using Cleland's rules. The inhibition kinetics were plotted according to Lineweaver-Burk. Straight lines were obtained from the double reciprocal plots. Both the slope s and vertical axis i increased with increasing acarbose concentration. Similar inhibition kinetics were obtained with the a-AI inhibitor. In both cases, the inhibition was concluded to be of the mixed non-competitive kind.
- ⇒ Secondary plots from amylose acarbose inhibited hydrolysis showed parabolic curves. Those from maltodextrin and maltopentaose showed linear curves. While secondary plots from substrate a-AI inhibitor hydrolysis showed parabolic curves.
- ⇒ One inhibitory model is proposed for each inhibitor, which take into account the kinetic data and the X-ray crystallography results : two additional carbohydrate binding sites are postulated for amylose acarbose inhibited hydrolysis and only one for maltodextrin hydrolysis. The model proposed for a-AI action shows two additionnal binding sites, whatever the substrate S used. The main difference is that the PPA- S complex does not accept any inhibitor molecule. Comparaison of the kinetic parameters shows that acarbose is a more efficient inhibitor than a-AI.

Follow up

- Efforts have been made to develop practical applications.
- Time course will be further carried out on gelatinized and insoluble substrates using PPA (Montevideo) new work using fish amylase is carried in Marseilles with a view to aquaculture applications.

Selected publications

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GENETICAL AND MOLECULAR STUDIES ON GENOMIC IMPRINTING

Co-ordinator: Consejo Superior de Investigaciones Científicas, Madrid, Spain
(Lucas Sánchez)

Objectives

- ◆ The long-term goal of this project was the elucidation of the mechanisms that bring about different chromosome organisations, by means of which gene expression is controlled.
- ◆ The short-term goal was the analysis of the processes of genomic imprinting, chromosome elimination and dosage compensation in sciarids from a molecular cytogenetic point of view

Activities

- * the fine cytological characterization of the chromosomes by modern banding techniques.
- * Cytological characterization of chromosome elimination in different *Sciara* cell types. Immunofluorescence of spindle/chromosomes interactions. This includes the analysis of the chromosome movements in the first and in the second spermatocyte divisions in *S. ocellaris*, the analysis of chromosome elimination from the soma of the embryo and the analysis of X chromosome elimination from embryonic germ cells.
- * Cytogenetic and molecular dissection of the imprinted chromosome regions.
- * Isolation and molecular characterization of the genes controlling dosage compensation.
- * Characterization of new mutations of *S. ocellaris*.

Results so far

- ⇒ During the course of this project the following major results have been obtained.
- ⇒ First, the heterochromatic region of the *S. ocellaris* chromosomes is poorly differentiable.
- ⇒ Second, during meiosis I and II a monopolar spindle is formed from a single polar complex (centrosome-like structure). g-Tubulin, centrin and actin are present in the bud as well as in the single polar complex of first meiotic spindle.
- ⇒ Third, it has been found that the X chromosome that is eliminated in the in germ cells of embryos is attached to the internal membrane of the nuclear envelope, which evaginates forming a long protuberance into the cytoplasm and later forms a cytoplasmic vesicle containing the X chromosome that is eliminated.
- ⇒ Fourth, it has been found that the X chromosome elimination in somatic cells during early embryogenesis is not due to the lack of kinetic activity of this chromosome, but that it depends on an alteration of telomeric separation during anaphase.
- ⇒ Fifth, the *mle* homologous gene of *S. ocellaris*, which encodes a very conserved protein at the helicases domains has been cloned and is being characterized.
- ⇒ Sixth, the *sepia* X-chromosome, which carries a maternal effect genetic alteration that affects the number of X chromosomes eliminated in somatic cells, has been characterized.

- ⇒ Seventh, three new mutations have been isolated: big eye-jerez (bej) and white (w) in the X chromosome; and short wing (sw) which is autosomal
- ⇒ Eight, UV irradiation of the egg cortex when the nuclei are in the yolk region produce gynanders (XX/X0 individuals in which the eliminated chromosome is of paternal origin), whereas if irradiation takes place at the syncytial blastoderm, when the nuclei reach the cortex, both gynanders and mosaic (XX/X0 individuals in which the eliminated X chromosome is the one inherited from the mother) are produced.

Selected publications

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Perondini, A. L. P. and Ribeiro, A. F. 1997. Chromosome elimination in germ cells of *Sciara* embryos: involvement of the nuclear envelope. *Invertebrate Reproduction and Development*. 32: 131-141.

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**PHOSPHATIDYLINOSITOL METABOLISM AND CONTROL OF PLASMA
MEMBRANE H⁺-ATPASE ACTIVITY IN YEAST**

Co-ordinator: Katholieke Universiteit Leuven, Leuven; Belgium (Johan Thevelein)

Objectives

The goal of this project was to elucidate the molecular mechanisms underlying glucose-induced stimulation of phosphatidylinositol turnover (PI) in yeast and its possible connection with glucose-activation of plasma membrane H⁺-ATPase activity

Activities

- ★ Measurement of glucose-induced PI turnover and activation of plasma membrane H⁺-ATPase have been performed in a variety of yeast mutants and using inhibitors of PI metabolism commonly employed in mammalian cells.
- ★ Multi-copy suppressors of growth arrest by such inhibitors have been isolated and characterised.
- ★ Measurements of GTP/GDP bound to Ras proteins have been performed under conditions leading to stimulation of cAMP synthesis, e.g. glucose addition and intracellular acidification. The results have led to the investigation of the possible involvement of the GPA2 gene product in glucose activation of the cAMP pathway.

Results so far

The major outcome of this project is the identification of PLC1 encoded phospholipase C as the enzyme responsible for glucose-induced PI turnover in yeast. It constitutes the first clear *in vivo* link between the function of this enzyme and PI metabolism. In addition *plc1* deletion mutants are also deficient in glucose-activation of plasma membrane H⁺-ATPase, supporting a causal link between the two phenomena. In the project reliable methods for the measurement of PI turnover have been developed. A new very potent inhibitor (3-nitrocoumarin) of phospholipase C has been synthesised and shown to be effective *in vivo* in yeast. Several multi-copy suppressors of growth inhibition by neomycin have been isolated and identified by sequencing. In related work the G-protein, encoded by the GPA2 gene has been shown to be required for glucose activation of the cAMP pathway, whereas the GTP/GDP ratio on the Ras proteins was not enhanced upon glucose addition.

Follow-up

Research on the molecular mechanisms underlying glucose-activation of signal transduction pathways in yeast, in particular phosphatidylinositol metabolism and the Ras-cAMP pathway, is continued in the three laboratories in several national and international projects. The laboratory in Milan concentrates on phosphatidylinositol turnover, the laboratory in Ouro Preto on glucose activation of plasmamembrane H⁺-ATPase. The laboratory in Leuven continues the studies on glucose activation of the Ras-cAMP pathway and the mechanism by which Gpa2 plays a role in glucose sensing.

Selected publications

Brandão R.L., N.M. de Magalhães-Rocha, R. Alijo, J. Ramos and J.M. Thevelein, 1994. Possible involvement of a phosphatidylinositol-type signaling pathway in glucose-induced activation of plasma membrane H⁺-ATPase and cellular proton extrusion in the yeast *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta* 1223, 117-124.

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Colombo S., P. Ma, L. Cauwenberg, J. Winderickx, A. Teunissen, D. Nauwelaers, J.H. de Winde, M.-F. Gorwa, D. Colavizza and J.M. Thevelein, 1998. Involvement of distinct G-proteins, Gpa2 and Ras, in glucose- and intracellular acidification-induced cAMP signalling in the yeast *Saccharomyces cerevisiae*. Manuscript in preparation.

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Ca²⁺ CELLULAR DYNAMICS, PROTEIN PHOSPHORYLATION (WITH SPECIAL REFERENCE TO THE GLIAL FIBRILLARY ACIDIC PROTEIN 6FAP) G-PROTEIN ACTIVATION AND GLUTAMATERIC NEUROTRANSMISSION IN THE VERTEBRATE BRAIN

Co-ordinator : Consejo Superior de Investigaciones Científicas, Madrid, (Galo Ramírez)

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**MOLECULAR, CELL BIOLOGY AND MODELLING STUDIES OF THE
NICOTINIC ACETYLCHOLINE RECEPTOR AND ITS MEMBRANE
ENVIRONMENT**

Co-ordinator: University of Bath, Bath, United Kingdom. (G. G. Lunt)

Objectives

The general objective was to transfer the techniques of molecular modelling from the University of Bath to the Institute for Biochemical research in Bahia Blanca (INIBIBB), Argentina and to develop further models of the nicotinic acetylcholine receptor protein with particular reference to its membrane-environment.

Activities

The nicotinic acetylcholine receptor is the best studied member of an important group of cell-signalling molecules - the fast acting Ligand Gated Ion Channels (LGICs). Direct experimental data on the receptor protein are very few and the Bath group has developed hypothetical structural models which are in accord with many published experimental data from sources such as electrophysiology, pharmacology, protein chemistry and biochemical binding studies. The group in INIBIBB, headed by Prof F. J. Barrantes has a long history of work on the relationship between the receptor and the membrane lipid environment. And has developed hypothetical models of protein - lipid interactions. The project sought to bring together these activities and to establish some core competencies in molecular modelling studies in Bahia Blanca.

Results

⇒ The bulk of the work during the life of the project has been carried out in Bahia Blanca. A new model of the receptor was developed which represents the receptor protein in the channel-open state and this has been compared with a previous closed-channel model developed in Bath. Attempts have been made in Bath to express chimeric proteins constructed from elements of the receptor and bacterial pore-forming toxins. Modelling studies in both Bath and Bahia Blanca identified certain bacterial pore-forming toxins as possible structural templates for receptor channel modelling. The expression studies progressed well in that large quantities of authentic chimeric protein were expressed but the protein does not appear to have any of the functionality associated with either the receptor or the toxin. In Bahia Blanca a molecular modelling centre has been established and several young Argentine scientists have commenced training in this area. An international workshop in this area was held in Bahia Blanca which has helped to consolidate INIBIBB as a national centre for modelling studies. One of the key workers on the contract, Dr Marcelo Ortells has now moved from Bahia Blanca to Buenos Aires and has established a second modelling centre supported by grants from the charitable foundation, ANTORCHAS.

⇒ Work in Bath has resulted in the award of a major grant from the Wellcome Trust on the design and synthesis of novel channel-forming peptides. Work on the expression of chimeric proteins involved collaboration with the Centre for Applied Microbiology Research (CAMAR) at Porton Down, Wilts. UK and aspects of the work continues within the CAMAR organisation.

Follow-up

The contract has now come to an end. Prof Barrantes has developed a collaborative programme with colleagues in Brazil as a result of the contract; Dr Ortells has established a second modelling centre in Buenos Aires and has obtained grant support; the University of Bath has secured further funding from the Wellcome Trust. The publications originating from the contract have aroused much attention in the field and the work has been very well received at several International Conferences. The collaboration between Bath and Bahia Blanca continues with a PhD student from Bahia Blanca about to start to write up his thesis in Bath. The contract has been an excellent example of technology transfer and has initiated a major new research area in Argentina.

Selected publications

Ortells M. O. & Lunt G. G., 1996. A mixed helix-sheet model for the transmembrane region of the nicotinic acetylcholine receptor. *Prot. Eng.* **9** 51 - 59

Ortells M. O. , Barrantes G. E. , Wood C. , Lunt G. G. & Barrantes F. J., 1997. Molecular modelling of the nicotinic acetylcholine receptor transmembrane region in the open state. *Prot. Eng.* **10** 511 - 517

Barrantes G. E. , Ortells M. O. & Barrantes F. J., 1997. Screening structural-functional relationships of neuropharmacologically active compounds at the nicotinic acetylcholine receptor: a modelling study. *Neuropharmacology* **36** 269 - 279

Bouzat C. B. & Barrantes F. J., 1997. Assigning functions to residues in the acetylcholine receptor channel region. *Membr. Molec. Biol.* **14** 167 - 177

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Period: February 1995 to February 1998

REGULATION OF INTRACELLULAR CALCIUM IN MUSCLE

Co-ordinator: Unité mixte CNRS URA-614/INSERM U 153, Paris, France
(François Rieger)

Objectives

To investigate the mechanisms which control cytoplasmic calcium concentration at rest and during activity and the mechanisms involved in calcium release in skeletal and heart muscle and in neuronal cells.

Activities

The project focused on understanding intracellular calcium regulation in muscle and neuronal cells using biochemical, biophysical, pharmacological and ultrastructural approaches. Various biological models were used for this purpose including rat embryo skeletal muscle cells in primary culture, prefusional L6 cells, myoblast from the mutant *muscular dysgenesis (mdg)*, dystrophic DMD cell lines in culture (human and mice *mdx*), frog muscle fibres, rabbit ventricular muscle, isolated T-tubule vesicles, isolated sarcolemmal vesicles from cardiac muscle, cerebellar Purkinje cells and isolated cholinergic synaptosomes. During the project, all the main goals proposed were accomplished and important progress towards new aims have been made. Different aspects of the research were facilitated by this grant (including equipment and supplies for the Chilean and EU groups). Furthermore, it allowed important interactions between the different laboratories. The visits of several scientists allowed concrete developments in particular areas, for example J. Molgo (CNRS, Gif sur Yvette) made possible the use of some marine toxins affecting intracellular calcium levels in skeletal muscle cells and began with E. Jaimovich and J. Hidalgo (University of Chile) to study nucleoplasmic calcium signals in adult muscle. T. Shimahara and R. Bournaud (CNRS, Gif sur Yvette) together with J. Hidalgo started exploring in nerve cells, the same type of calcium signals and calcium regulation systems that are present in muscle cells. M.A. Carrasco (University of Chile) allowed to determine IP₃ mass levels in pure cholinergic synaptosomes at CNRS in Gif sur Yvette and M. Pincon-Raymond (Paris) facilitated the study of the role of dihydropyridine receptors in slow calcium signals using dysgenic muscle cell lines. B. Drouet (Paris) introduced at the University of Chile molecular biology techniques to study the nature of IP₃ receptors in muscle cells. Furthermore, S. O'Neill (Liverpool) worked on the calibration of the intracellular calcium measurement system of the Chilean groups. Finally, the project partly financed an international Symposium held during the Annual Meeting of the Chilean Biological Society in 1997 attended by more than 100 Chilean scientists in which all the work presented there was related to results supported by this EU project.

Follow up

The new and basic information for both calcium regulation in skeletal muscle and neuronal cells will allow new developments in particular the characterization of slow calcium waves associated to cell nuclei in skeletal muscle cells and the distinct population of IP₃ receptors to such nuclei. The finding of a possible role of IP₃ receptors in regulating nucleoplasmic calcium in skeletal muscle cells will prompt us to study the nature and location of these receptors.

Selected publications

- Carrasco M.A., Morot Gaudry-Talarmain Y. & Molgo J., 1996. Ca^{2+} -dependent changes of acetylcholine release and IP3 mass in *Torpedo* cholinergic synaptosomes. *Neurochemistry International* **29**, 637-643.
- Kamp F., Donoso P. & Hidalgo C., 1998. Changes in luminal pH caused by calcium release in sarcoplasmic reticulum vesicles. *Biophysical Journal* **74** : 290-296.
- Melliti K., Bournaud R., Bastide B., Hidalgo J. & Shimahara T., 1996. Effect of SR33805 on barium current and asymmetric intramembrane charge movement in freshly dissociated mouse cerebellar Purkinje neurons. *Neuroscience Letters* **216**, 1-4.
- Overend C.L., Eisner D.A. & O'Neill S.C., 1997. The effect of tetracaine on spontaneous Ca^{2+} release and sarcoplasmic reticulum content in rat ventricular myocytes. *Journal of Physiology (London)* **502** : 471-479.
- Reyes R. & Jaimovich E., 1996. Functional muscarinic receptors in cultured skeletal muscle. *Archives of Biochemistry & Biophysics* **331** : 41-47.
- Seigneurin-Venin S., Parrish E., Marty I., Rieger F., Romey G., Villaz M. & Garcia L., 1996. Involvement of the dihydropyridine receptor and internal Ca^{2+} stores in myoblast fusion. *Experimental Cell Research* **223** : 301-307.

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ISC

Chemical Sciences

SYNTHESIS OF CHIRAL BIOLOGICALLY ACTIVE COMPOUNDS AND/OR KEY-INTERMEDIATES FOR THEIR PRODUCTION USING TRANSITION METAL ASYMMETRIC CATALYSIS

Co-ordinator : Consorzio Pisa Ricerche, Pisa, Italy (Carlo Bertucci)

Objectives

The main objectives of this project consisted in the development of efficient methodologies for the production of therapeutically active compounds and for the characterisation of their stereochemistry.

Activities

The target drugs, i.e. 2-arylpropanoic acids (non-steroidal anti-inflammatory agents) and pheniramines (antihistaminic agents), and key intermediates for their preparation, were synthesized employing enantioselective processes catalysed by transition metal complexes with selected chiral ligands. Enantioselective HPLC, NMR and circular dichroism methods were developed to determine the enantiomeric composition and the absolute configuration of the prevailing enantiomer of the obtained compounds. The Venice (Italy) and Ribeirão Preto (Brazil) partners were involved in the synthesis of the chiral ligands and of the chiral drugs, employing catalytic processes (mainly enantioselective hydrogenation, hydroformylation and cross-coupling reaction). The group in Pisa was mainly involved in the stereochemical characterisation of the obtained compounds.

Results

In the course of the project, selected catalytic processes starting from easily available material proved efficient for the preparation of the following pharmacologically active compounds : pheniramine, chlorpheniramine, brompheniramine, ibuprofen, ketoprofen and flurbiprofen. In some cases very encouraging enantiomeric excesses were reached. Finally, efficient enantioselective HPLC methods were developed, using UV and circular dichroism as detection systems.

Follow-up

This project allowed the development of new topics for the Brazilian research group in the field of synthetic organic methodologies for the production of fine chemicals. In particular, topics connected with catalysis and stereochemical characterisation, which had previously been almost completely neglected, were introduced in the education programme of the Faculty and became the subject of several master's theses. It is also worth mentioning that the expertise achieved in this area can be addressed to the preparation not only of more complex and valuable pharmaceuticals, but also of other biologically active compounds such as herbicides, insecticides, and food additives. Even if the project expired in 1995, the

collaboration in the same area is continuing among the groups in Italy and the University of Ribeirão Preto.

Selected publications

Chelucci G., Cabras M.A., Botteghi C., Marchetti M., 1994. (-)-(4S,5R)-4-(2-Pyridyl)-5-(diphenylphosphino)methyl-2,2-dimethyl-1,3-dioxolane a new chiral ligand for enantioselective catalysis. *Tetrahedron : Asymmetry* **5** : 299.

Botteghi C., Chelucci G., Del Ponte G., Marchetti M., Paganelli S., 1994. New Synthetic Route to Pheniramines via Hydroformylation of Functionalized Olefins. *J. Org. Chem.* **59** : 7125.

Botteghi C., Cazzolato L., Marchetti M., Paganelli S., 1995. New Synthetic Processes to Pharmacologically Active 1-(N,N-dialkylamino)-3,3-diarylpropanes via Rhodium-catalyzed Hydroformylation of 1,1-Diarylethenes. *J. Org. Chem.* **60** : 6612.

Botteghi C., Bertucci C., Del Ponte G., Alberico E., Marchetti M., 1997. Optically active Pheniramine by enantioselective hydrogenation of unsaturated amines, esters and acids using Rh(I)-complexes with (-)-BINAP as catalytic precursors. *J. Mol. Catal.* **125**, 109.

Botteghi C., Del Ponte G., Marchetti M., 1997. Contribuções recentes da reação de hidroformilação na síntese de produtos farmacêuticos. Parte II. *Química Nova* **20** : 30.

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FLAVOUR CHEMISTRY OF SOME COLOMBIAN FRUITS HAVING ECONOMIC VALUE

Co-ordinator : Universität Würzburg, Würzburg, Germany (Peter Schreier)

Objectives

The general target of the project was the study of the aroma chemistry of selected tropical fruits, comprising (i) free volatiles, (ii) 'bound' aroma constituents (aglycons), and (iii) glycosides as flavour precursors.

Activities

Supported by an extensive exchange programme between the Colombian partner and Würzburg, a high number of fruits and other plant-derived raw materials have been investigated, i.e. badea, blackberry, curuba, fig, granadilla, lulo, mango, mangosteen, melon, melocotón, mamey, maracuja, papayela, pineapple, guava, pinuela, rose apple, and tamarillo. The analytical techniques mostly comprised the simultaneous distillation-extraction (SDE) at pH 7 (free volatiles) with subsequent SDE at pH 2.5 (aglycons), as well as the separation of sugar conjugates by XAD adsorption and methanol elution followed by (a) both enzymatic (Rohapect) and acid hydrolysis (each yielding information on aglycons) and (b) multi-step analysis of complete glycosidic structures using various chromatographic (countercurrent chromatography, HPLC) and spectroscopic techniques (MS, NMR). Configurational studies were carried out particularly by enantioselective multidimensional gas chromatography (enantio-MDGC).

Results

- ⇒ Among the above-mentioned plant material mangosteen, melon, melocoton, papayela, pinuela and tamarilla has been studied most extensively leading to a high number of newly described aglycons and glycosidically bound flavour precursors. The results are well documented in eight common papers (from 16 in total). The results are helpful to reinforce the Colombian agricultural industry to develop, in cooperation with the industry from abroad, (A) 'traditional' and (B) 'alternative' plant products. (A) means the evaluation of half-technologies of fruit homogenates, concentrates etc.; (B) comprises new flavour types, e.g. from free volatiles or after suitable enzymatic hydrolysis. In such a way, the high-value market of 'natural' flavours will be opened. Concluding, we can recommend Colombia to significantly increase the production of fruits such as lulo, papayuela and blackberry. These fruits, according to the results obtained in the project, possess excellent, attractive and exotic flavour properties, which make them to high-quality raw materials and offer possibilities in developing new agricultural products.
- ⇒ As a consequence of the project, the existing infrastructure of the Colombian partner has been improved substantially, leading, e.g., to the introduction of a PhD programme

Selected publications

- Suarez M., Duque C., Bicchi C., Wintoch H., Full G., Schreier P., 1993. Volatile constituents from the peelings of lulo (*Solanum vestissimum* D.) fruit. *Flav. Fragr. J.* **8**: 215-220.
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- Parada F., Krajewski D., Herderich M., Duque C., Schreier P., 1995. 3,4-Dimethoxyphenyl β -D-glucopyranoside from pinuela (*Bromelia plumieri* Karstens) fruit. *Nat. Prod. Lett.* **7**: 69-72.
- Parada F., Krajewski D., Duque C., Jäger E., Herderich M., Schreier P., 1996. 1-O- β -D-glucopyranosyl anthranilate from pinuela (*Bromelia plumieri* Karstens) fruit. *Phytochemistry* **42**: 871-873.
- Krajewski D., Duque C., Schreier P., 1997. Aliphatic β -D-glucosides from fruits of *Carica pubescens*. *Phytochemistry* **45**: 1627-1631.

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PRODUCTION AND ADVANCED STRUCTURAL CHARACTERIZATION OF COAL TAR PITCHES

Co-ordinator: Consejo Superior de Investigaciones Científicas, Oviedo, Spain
(Sabino R. Moinelo)

Objectives

- ◆ Investigate the relationships between the chemical composition of coal tar pitches and their carbonisation behaviour, particularly their ability to form mesophase. Aspects of chemical composition that were investigated include the nature of aromatic and heteroaromatic groups.
- ◆ Investigate the effect of thermal processing parameters (temperature, heating rate, residence time, etc.) on the yield and composition of the quinoline insolubles and β -resins using a pilot plant facility. The information will be used to specify, produce and test a number of well-characterised pitch binders

Activities

- * The research programme effectively combines the capacity to produce coal tar pitch under controlled conditions at USIMINAS with the strengths of INCAR and Strathclyde in coal conversion chemistry and structural elucidation and the sophisticated equipment available at UNICAMP for high temperature treatment and testing of binder pitch and essay of electrodes.
- * The project focuses mainly on the production of binder pitches for the manufacture of carbon electrodes. Several sets of pitches were obtained under different conditions by INCAR and UNICAMP (lab scale) and USIMINAS (pilot plant). Pitches were characterised at INCAR (chromatographic methods, Ir-spectroscopy and TGA) and Strathclyde (NMR spectroscopy). INCAR developed new methodologies for pitch characterization which allow to analyse them in terms of classes of PACs (three kinds of cata-PACs and one of peri-PACs) and thermal reactivity. The University of Strathclyde developed NMR spectroscopic methods (^1H y ^{13}C in solution and solid state and with high temperature probe) which allow to quantify the amount of different carbons (bridgehead, quaternary, etc.) and also to monitor and quantify the mesophase formation. USIMINAS and UNICAMP evaluated the essay electrodes obtained with the different pitches produced at USIMINAS.

Results

- ⇒ The new methodologies developed for pitch characterization (HPLC and NMR) allow to study the mechanisms and kinetics of polymerization and condensation of pitches and to predict their thermal behaviour.
- ⇒ The peri-condensed PACs are much more reactive under air stream than the cata-condensed ones.

- ⇒ Binder pitches of better quality for carbon electrodes manufacture and in higher yields were produced under air stream at 350°C rather than under nitrogen, vacuum or atmospheric distillation.
- ⇒ Correlations were obtained between the chemical composition (in terms of classes of PACs) of pitches and the quality parameters of the electrodes.

Selected publications

J. M. Andresen, P. R. Dennison, M. M. Maroto-Valer, C. E. Snape, R. Garcia and S. R. Moinelo, 1994. Characterisation of Pitch Fractions by Quantative Solid State ¹³C NMR. Prep. Am. Chem. Soc., Div. Fuel Chem., **39(3): 777**.

Y. Martin, R. Garcia, R. Solé and S. R. Moinelo, 1996. Structural Characterization of coal Tar Pitches Obtained by Heat Treatment under Different Conditions. Energy & Fuels **10: 436**.

Y. Martin, R. Garcia A. Solé and S. R. Moinelo. Characterisation of Pitch by HPLC. Chromatographia (in press).

J. M. Andresen, Y. Martin, S. R. Moinelo, M. M. Maroto-Valer and C. E. Snape. Solid State ¹³C NMR and High Temperature ¹H NMR Determination of Bulk Structural Properties for Mesophase Containing Semi-cokes Prepared from Coal Tar Pitch. Carbon (in press).

J. M. Andresen, C. A. Luengo, R. Garcia, S. R. Moinelo and C. E. Snape. Structural Uniformity of Toluene-insolubles from Heat-treated Coal Tar Pitch as Determined by Solid State ¹³C NMR Spectroscopy. Energy & Fuels (in press).

Furthermore, this research gave rise to three PhD theses (J.M. Andresen, Y. Martin and R. A. Sole) and two patents (Brazil).

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Contract number: CI1*CT920030

Period: April 1993 to December 1997

**SYNTHETIC, STRUCTURAL, KINETIC, AND CATALYTIC STUDIES ON
TRANSITION METAL CLUSTERS CONTAINING NOVEL UNSATURATED
PHOSPHORUS LIGANDS AND THEIR COMPARATIVE BEHAVIOUR WITH
ORGANIC ANALOGUES**

Co-ordinator: University of Sussex, Brighton, UK (John F. Nixon)

Objectives

To develop a new field of chemistry involving activation of novel unsaturated phosphorus compounds, typified by phospho-alkenes and phospho-alkynes on poly-nuclear transition metal carbonyl complexes.

Activities

- ★ The project focussed on the interaction of tetrairidium carbonyl complexes and triruthenium and osmium carbonyl complexes with phospho-alkynes and phospho-ferrocene molecules. Several novel reactions have been studied, both spectroscopically and structurally (X-ray crystallography).
- ★ New modes of ligation of the triphosphaferrocene complex have been observed and an unusual activation of dihydrogen at the trimetallic centres and alkyne-phospho-alkyne coupling reactions are among the highlights obtained so far.

Results

- ⇒ Papers have appeared on the novel alkyne and phospho-alkyne coupling on an Ir₄ cluster, as well as two papers concerned with structural and synthetic aspects of several triphospha-ferrocene complexes of tetranuclear iridium and an interesting C-H activation.
- ⇒ The activation of dihydrogen and alkynes on triangular metal clusters has also been achieved and is being written up.
- ⇒ Studies have been augmented recently with para hydrogen to afford unusual information about H scrambling reactions in clusters.

Follow up

The project has just been concluded and the final report is being prepared. Several publications have already appeared, as well as presentations at international meetings, both in the UK and abroad.

Selected publications

Benvenuti M.H.A., Hitchcock P.B., Nixon J.F., and Vargas M.D. 1994. Novel alkyne and phospho-alkyne coupling on an Ir₄ cluster : synthesis and molecular structure of [Ir₄(m-CO)(CO)₇{m⁴-h³-Ph₂PC(H)C(Ph)PCBut} (m-PPh₂)]. *J. Chem. Soc., Chem. Commun.* 1869.

Benvenuti M.H.A., Hitchcock P.B., Nixon J.F., and Vargas M.D. 1996. Novel C-H activation in a bis-tetra-iridium carbonyl complex of the sandwich compound [Fe(h⁵-(C₅H₅)(h⁵-P₃C₂But₂)]. *Crystal and molecular*

structures of $[\text{Ir}_4(\text{CO})_{11}\text{Fe}(\text{h}5\text{-C}5\text{H}5)(\text{h}5\text{-P}3\text{C}2\text{But}2)]$ and $[\text{Ir}_4(\text{CO})_{10}\text{-m}\{\text{Fe}(\text{h}5\text{-C}5\text{H}5)(\text{h}5\text{-P}3\text{CH}2(\text{CMe}2)\text{Cbut})\}\text{Ir}_4(\text{CO})_{11}]$ *J. Chem. Soc., Chem. Commun.* 441.

Benvenuti M.H.A., Hitchcock P.B., Nixon J.F., and Vargas M.D. 1996. Syntheses and structural characterisations of novel complexes of the type $[\text{Ir}_4(\text{CO})_{11}\text{L}]$, $[\text{Ir}_4(\text{CO})_{10}\text{L}_2]$; and $[\text{Ir}_4(\text{CO})_{10}(\text{m-h}2\text{-L})]$, (L = $[\text{Fe}(\text{h}5\text{-C}5\text{H}5)(\text{h}5\text{-P}3\text{C}2\text{But}2)]$ and L = $[\text{Fe}(\text{h}5\text{-P}3\text{C}2\text{But}2)(\text{h}5\text{-P}2\text{C}3\text{But}3)]$ and L = $[\text{Ir}_4(\text{CO})_{11}\{\text{m-Fe}(\text{h}5\text{-C}5\text{H}5)(\text{h}5\text{-P}3\text{C}2\text{But}2)\}\text{Ir}_4(\text{CO})_{11}]$ and its facile conversion $[\text{Hir}_4(\text{CO})_{10}\{\text{Fe}(\text{h}5\text{-C}5\text{H}5) - (\text{h}5\text{-P}3\text{CH}2(\text{CMe}2)\text{Cbut})\}\text{Ir}_4(\text{CO})_{11}]$ via an unusual C-H activation. *J. Chem. Soc. Dalton Trans.* 739.

De Araujo M.H., Avent A.G., Hitchcock P.B., Nixon J.F., and Vargas M.D. Organometallics. Submitted for publication. Hydrometallation of *PcBut* and activation of the P-C bond by a tetra-iridium carbonyl cluster. Crystal and molecular structure of the phosphinidene complex $[\text{Hir}_4\text{Pt}(\text{dppe})(\text{m-CO})\text{CO}]\text{7}(\text{m-PCH}2\text{BUT})\text{m-PPh}_2]$ and of the partially encapsulated phosphide complex $[\text{Hir}_4\text{Pt}(\text{dppe})(\text{m-CO})(\text{CO})\text{7}(\text{m-PCH}2\text{But})\text{m-PPh}_2]$ and of the partially encapsulated phosphide complex $[\text{Ir}_4\text{Pt}(\text{dppe})(\text{m-CO})(\text{CO})\text{7}(\text{m-P})(\text{m-PPh}_2)]$.

De Araujo M.H., Hitchcock P.B., Nixon J.F., Al-Resayes S.A., Freire R.S., Martins A.A., Vargas M.D., and Ziglio C.M. Submitted for publication. Reactions of iridium carbonyl clusters with the phosphirene ring system *PPhC}2\text{Ph}2*. Crystal structure of $[\text{Ir}_4(\text{CO})_{10}\text{PPhC}2\text{Ph}2]$.

Braga D., Grepioni F., Nixon J.F., Vargas M.D., and Zigli C.M. Submitted for publication. Oxidative addition reactions of derivatives of $[\text{Hir}_4(\text{CO})_{10}(\text{m-PPh}_2)]$. Cyclometallation of $[\text{Hir}_4(\text{m-CO})(\text{CO})_8(\text{PPh}_3)(\text{m-PPh}_2)]$ and H_2 addition to the clusters $[\text{Hir}_4(\text{m-CO})(\text{CO})_9\text{-n}(\text{Ln})(\text{m-PPh}_2)]$ (L = PMe_3 , PPh_3 , and dppm). Molecular structures of $[\text{H}_3\text{Ir}_4(\text{CO})_9\text{-n}(\text{PMe}_3)(\text{m-PPh}_2)]$ (n = 1,2).

Braga D., Grepioni F., Nixon J.F., Vargas M.D., and Zigli C.M. Submitted for publication. Synthesis and characterisation of derivatives of $[\text{Hir}_4(\text{CO})_{10}(\text{m-PPh}_2)]$ with PPh_3 , PMe_3 and diphosphines. X-ray molecular structures of $[\text{Hir}_4((\text{CO})_8(\text{PPh}_3)_2(\text{m-PPh}_2))]$ and $[\text{Hir}_4((\text{CO})_8(\text{Ph}_2\text{CH}_2\text{PPh}_2)(\text{m-PPh}_2))]$ and studies of the interconversions of the isomers of these compounds and of the PMe_3 derivatives in solution.

Braga D., Grepioni F., Nixon J.F., Vargas M.D., and Zigli C.M. Submitted for publication. Oxidative addition reactions of HCCPh with $[\text{Hir}_4((\text{CO})_{10}\text{-n}(\text{PMe}_3)_2(\text{m-PPh}_2))]$ (n=1,2). Crystal and molecular structure of the novel trinuclear iridium cluster $[\text{Ir}_3(\text{CO})_6(\text{PMe}_3)(\text{m}^3\text{-h}2\text{-HCCPh})(\text{m-PPh}_2)]$.

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NEW CATALYSTS FOR IMPROVING THE REDUCTIONS OF SULFUR AND NITROGEN COMPOUNDS IN ENVIRONMENT

Coordinator : Institut de Recherches sur la Catalyse, Villeurbanne, France (Michel Vrinat)

Objectives

- ◆ In many countries, environmentally-driven regulations require significant improvement in the quality of mid-distillate fuels, in particular a decrease in sulfur- and nitrogen-containing molecules. However, even if technology could play a major role in meeting the low-sulfur diesel fuel specifications; there is still a challenge to discover more active and more selective catalysts able to achieve deep desulfurization and hydrogenation in the presence of nitrogen compounds.
- ◆ Taking into account the results available in literature, new catalysts could be developed using additives, new active phases, or new supports. The latter approach was chosen in this project as a continuation of previous collaboration and partners' expertise.

Activities

- ★ The project focuses on the preparation of molybdenum, cobalt-molybdenum and nickel-molybdenum hydrotreating catalysts over $\text{TiO}_2\text{-Al}_2\text{O}_3$ supports of various compositions, their characterization by various physico-chemical techniques and their catalytic evaluation in different reactions.
- ★ Supports (so-gel method) and some catalysts were prepared in Mexico, concentrating on the characterization of catalysts by temperature programmed reductions (TPR), thermal desorption of ammonia (TPD), acidity, UV visible diffuse reflectance spectroscopy and FTIR analysis of adsorbed NO.
- ★ Catalytic activities in the hydrodesulfurization of thiophene at atmospheric pressure and dibenzothiophene under high pressure were carried out at Villeurbanne, where the characterization of the most interesting samples (in the sulfided state) was made by XPS and electron microscopy (HREM).
- ★ Madrid was mainly concerned with catalytic activity measurements in a trickle bed reactor which allow the use of a real feed and simultaneous reaction of hydrodesulfurization and hydrodenitrogenation.

Results

⇒ The coprecipitation method used here allowed the synthesis of supports with large surface area even in the TiO_2 rich samples. For CoMo and NiMo catalysts prepared over the support of intermediate composition the lower activity (as compared to catalysts prepared over Al_2O_3) was correlated to a loss of cobalt (or Ni) in the amorphous Al_2O_3 . However, for the catalysts with a high TiO_2 content in the support, promising intrinsic activities were obtained. The interest of such supports ($\text{TiO}_2/(\text{TiO}_2 + \text{Al}_2\text{O}_3) > 0.9$) was confirmed by the experiments with the real feed-stock. However, catalyst ranking is strongly dependent on

the support composition and also on the reaction conditions such as nature of the model molecule or H₂ and H₂S partial pressures.

⇒ This work produced insight into the understanding of the so-called “support effect” and also demonstrated that the choice of a catalytic test for the development of new catalysts appears of vital importance, particularly for systems prepared over new supports.

Selected publications

Olguin E., Vrinat M., Cedeno L., Ramirez J., Borque M. and López-Agudo A. 1997. The use of TiO₂-Al₂O₃ binary oxides as support for Mo based catalysts in hydrodesulfuration of thiophene and dibenzothiophene. Appl. Catal. A : general . **165**: 1-13.

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Period: November 1993 to May 1996

TRANSITION METAL CARBENE COMPLEX MEDIATED SYNTHESIS OF ORGANIC COMPOUNDS OF PHARMACOLOGICAL INTEREST

Co-ordinator: Université Pierre et Marie Curie Paris VI, Paris, France (Henri Rudler)

Objectives

The general goal of the project was the synthesis of elaborate and pharmalogically valuable, nitrogen, oxygen and sulfur-containing heterocyclic compounds from carbene complexes of chromium and tungsten pentacarbonyl and from iron tetracarbonyl.

Activities

- ★ The project focused on the synthesis, structure and reactivity of carbene complexes of transition metals. These complexes were obtained both in Paris and in Mexico, and their structures determined at the crystallographic facilities of both Universities.
- ★ The Mexico team focused investigations on the synthesis of aminocarbene complexes of chromium, on ketene complexes of iron, and on reactivity towards various substrates; the Paris group studied the chemistry of aminocarbene complexes of chromium.

Results

- ⇒ During the course of the project an impressive number of new and original heterocyclic compounds were obtained, including lactams, pyrrolinones, aminolactones, pyrroles, exotic aminoacids, furans and more complex alkaloid-related structures.
- ⇒ The screening of these new compounds for possible biological activities has been undertaken and is still under way.
- ⇒ From a more fundamental point of view, new reaction intermediates of these complexes were isolated and thoroughly characterized at Paris University. Moreover, these new intermediates could in turn be used as starting material for the synthesis of valuable organic compounds with original structures. The fundamental role of the metal in these reactions could also be established.

Follow-up

Most of the goals of this project have been achieved or were in close sight by its end. However, efforts to develop practical applications of these new compounds are still in progress. Both groups remain active in this field of organometallic chemistry applied to organic synthesis. Thanks to this contract, new horizons for the application of carbene complexes were opened up.

Selected publications

Alvarez-Toledano C., Cano A.C., Toscano R.A., Parlier A., Rudler H., 1993. Reactivity of a ketene complex of iron towards saturated and unsaturated amines. *Bull. Soc. Chim. Fr.*, **130**, 601.

Bouancheau C., Parlier A., Rudler M., Rudler H., Vaissermann J., Daran J.C., 1994. Reaction of aminocarbene complexes of chromium with alkynes. *Organometallics*, **13**, 4708.

Bernes S., Toscano R.A., Toledano-Alvarez C., Rudler H., Daran J.C., 1995. Formation and X-ray structure of the alkylidene complex $\text{Fe}_2(\text{CO})_6\text{C}(\text{CH}=\text{CHPh})_2$ obtained upon deoxygenation of $\text{Fe}_2(\text{CO})_8\text{C}(\text{O})(\text{CH}=\text{CHPh})$ with methyllithium. *J. Organomet. Chem.*, **498**, 15.

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DETERMINATION OF FULL STRUCTURE AND PARTIAL SYNTHESIS OF NEW POLYETHER TOXINS OF MARINE ORIGIN

Coordinator: Universidad de La Laguna, Tenerife, Spain (Julio Delgado Martin)

Objectives

The transport of ions and molecules *in vivo* is a precisely regulated biological process that is accomplished almost exclusively with the aid of proteins believed to have multiple transmembrane spans. Our own specific interest in transport processes relates here to the isolation and synthesis of organic compounds that emulate the biofunctional properties of selected marine polyethers and their ability to selectively alter the permeability of membranes.

Activities

The development of synthetic flux-promoting compounds with regulated membrane permeability is a complex problem. The models must be designed in consideration not only of structure but also of function. Once the design is decided, the molecules must be synthesised. After preparation, permeability induced by the model must be assessed in phospholipid bilayer if the effort is to be biologically relevant. Altogether, this requires a variety of techniques and resources. This project represents a multidisciplinary approach covering the following aspects:

- * chemical synthetic studies using the methodology we have developed, and applying new organic reactions to facilitate access to these sophisticated structures;
- * study of the pharmacological activity and relationship between the chemical structure and bioactivity, in order to identify the active centre and importance of the different functional groups within the activity observed;
- * study of the molecules and receptor substrate in diverse animal tissues, to determine which characteristics favour, to a greater or lesser extent, toxin affinity with the receptor,
- * chemical analysis and bioactivity of new components isolated from marine sources.

Results so far

In the course of this project, the bioactivity of extracts and individual components of twenty-two species of Uruguayan seaweeds were tested with the *Artemisia salina* shrimp assay. These results, including the cytotoxic and antihelminthic activity of all the compounds isolated, have been published.

In relation to the synthetic work carried out by the Uruguayan group, a successful synthesis of a trans-fused oxatricyclic system has been achieved. The molecule synthesised possesses useful appendages to be used in the construction of more complex polyethers. New convergent strategies for the synthesis of trans-fused polyethers based on hydroxy-ketone cyclization of C-linked oxacycles and O-linked oxacycles have been developed by the Spanish Group.

Pharmacological studies using the natural polyether okadaic acid have shown that at low, non-contractile, concentrations mimicks the effects derived from protein kinase C activation in rat uterus. This important discovery suggests that type-1 and/or -2A protein phosphatases might be enzymes involved in the regulation of protein kinase C-mediated mechanical responses in oestrogen-primed rat uterus.

Follow-up

At this moment we have available a significant number of important molecules obtained both from marine sources and synthetically, that deserve attention from the pharmacological standpoint.

Selected publications

Alvarez E., Díaz M.T., Pérez R., Ravelo J.L., Regueiro A., Vera J.A., Zurita D., Martín J.D., 1995. Simple design for the construction of complex *trans*-fused polyether toxin frameworks. A linear strategy based on entropically favored oxirane ring enlargement in epoxycycloalkenes followed by carbon-carbon or carbon-oxygen bond-forming reactions. *J.Org.Chem.*, **59**, 2848-2876.

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Arteche E., Strippoli G., Loirand G., Pacaud P., Candenas L., Moltó L., Fernández J., Norte M., Martín J.D., 1997. An analysis of the mechanisms involved in the okadaic acid-induced contraction of the oestrogen-primed rat uterus. *J. Pharmacol. Exp. Ther.*, **282**, 201-227.

Manta E., Scarone L., Hernández G., Mariezcurrena R., Suescun L., Brito I., Brouard I., González M.C., Pérez R., Martín J.D., 1997. A facile synthesis of an oxatricyclic *trans, syn, trans*-substituted oxepanyl framework. *Tetrahedron Letters*, **38**, 5853-5856.

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THE EXTRACTION OF GOLD USING A NATURAL RESOURCE FROM PERU

Co-ordinator : The University of Surrey, Guildford, United Kingdom
(A.F. Danil de Namor)

Objectives

- ◆ Extraction of gold using peat.
- ◆ A study of the binding properties of gold (I) and gold (III) with amino-acid-type ligands as models for the metal binding sites of humic and fulvic acids which are major constituents of peat (Dublin).

Activities

A researcher from Peru initially carried out research at the Royal College of Surgeons in Ireland and then moved to the laboratory of the co-ordinator at the University of Surrey. The work in Dublin involved synthesis and characterization of gold complexes with amino-acid-type ligands, while the work in Surrey involved solution (calorimetry, pH-metry, conductimetry, nuclear magnetic resonance spectroscopy) and solid-state studies of these and many other systems. A large number of extraction studies have also been carried out at the University of Surrey.

Results

The reactions of ethylenediaminetetraacetic acid, propylenediaminetetraacetic acid and cyclohexanediaminetetraacetic acid derivatives with gold(III) have been investigated. Several diacid-diamide and tetraamide derivatives (L) were prepared and characterized, and these were reacted with Na[AuCl₄] and Na[AuBr₄]. Several complexes of the type [AuLCl₂]Cl or [AuLCl₂] [AuCl₄] were obtained. In the case of the tetra (N-methylamide) of ethylenediaminetetraacetic acid, a novel complex of formula [Au(LH₁)Cl][AuCl₄] was obtained and its crystal structure determined. This contains gold(III) co-ordinated to the two amine nitrogens and a deprotonated amide nitrogen of L. Since it contains a deprotonated amide group it is a model for gold(III)-peptide interactions. Only two crystal structures of gold(III)-peptide complexes had previously been reported. The results of this work have furthered our knowledge of gold(III) chemistry considerably, regarding methods of synthesis, preference for donor atoms, stability, etc.

Follow-up

During the last stages of the project efforts will be made to develop practical applications of the work carried out in Dublin and in Surrey. From our combined efforts an improved understanding has been obtained of the coordinating properties of gold(III) in both the solid state and in solution and on the factors favouring its extraction from aqueous solution.

Selected publications

Adolfo F. Aguilar Cornejo, Alfonso Castineiras, Kevin B. Nolan. Synthesis and structure of a gold (III) complex containing a deprotonated amide ligand. A model for gold (III)-peptide/protein interactions. Submitted for publication *J. Chem. Soc., Dalton Trans.*

A.F. Danil de Namor, Felix J. Sueros Velarde, and Mercedes C. Cabaleiro. Reaction of tetrakis[(3-pyridylmethyl)oxy]P-Tert-butylcalix(4)arene with $KAuCl_4$ and K_2PtCl_6 . new pyridinocalix(4)arene adducts of gold (III) and platinum (IV). *Polyhedron*

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OXIDE SURFACE SCIENCE RELATED TO LATERITE CATALYSTS

Co-ordinator: Manchester University, Manchester, United Kingdom
(Geoff Thornton)

Objectives

To study fundamental chemical reactions at oxide surfaces which are related to catalytic processes in the Venezuelan oil industry, such as hydrodesulfurisation and hydrocracking. The catalyst involved was the complex mineral laterite, which is found naturally in abundance in Venezuela. The role of thermal activation, point defects, steps and the "perfect" surface in the reactivity was of particular interest.

Activities

We focussed on the surfaces of two constituents of laterites, namely TiO_2 and NiO . H_2S , SO_2 and thiophene were used as probe molecules to mimic the hydrodesulfurisation, and benzene for the hydrocracking process over a catalyst. Several surface analytical techniques such as UPS, XPS, STM, AES, NEXAFS, LEED and TDS were employed to characterise the surface structure as well as the adsorption and desorption of the probe molecules.

Results

The STM study investigated several phases of the (100) and (110) surfaces of $\text{TiO}_2(110)$, which have a varying degree of O-deficiency. Studies of the structure of the (110)1x1 surface also employed surface x-ray diffraction. The low temperature adsorption of benzene on $\text{TiO}_2(100)$ results in molecular adsorption on both the 1x3 and 1x1 surfaces. It was found that point defects on the (100)1x3 surface give rise to dissociation of benzene, although they do not affect thiophene adsorption. The study of SO_2 reaction with TiO_2 surfaces evidenced a local structure domination of adsorbate bonding to oxide surfaces in contrast to the bonding to metals. As for work on $\text{NiO}(100)$, STM identified O vacancy sites on the *in situ* cleaved surface. This may play a role in the reaction with H_2S , which results in the formation of an ultrathin layer of $\text{Ni}(100)c(2x2)S$. These results throw significant light on some important features of laterite catalysis.

Selected publications

- Raza H., Harte S. P., Muryn C. A., Wincott P. L., Thornton G., Casanova R., Rodríguez A. 1996. NEXAFS Studies of the reaction of SO_2 with $\text{TiO}_2(100)$ 1x1 and 1x3. *Surface Sci.* **366**: 519-530.
- Charlton G., Howes P. B., Nicklin C. L., Steadman P., Taylor J. S. G., Muryn C. A., Harte S. P., Mercer J., McGrath R., Norman D., Turner T. S., Thornton G. 1997. Relaxation of $\text{TiO}_2(110)1x1$ Using Surface X-Ray Diffraction, *Phys. Rev. Lett.* **78**: 495-498.

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Raza H., Wincott P. L., Thornton G., Casanova R., Rodríguez A. Photoemission studies of the reaction of benzene with TiO₂(100)1x1 and 1x3. *Surf. Sci.*, in press.

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**CRYSTAL CHEMISTRY AND CHEMICAL BONDING OF NON
STOICHIOMETRIC 2201 BISMUTH CUPRATES AND RELATED PHASES IN
CONNECTION WITH THEIR SUPERCONDUCTING PROPERTIES**

Co-ordinator : CNRS, Caen, France (B. Raveau)

Objectives

- ◆ Try to understand the structural mechanisms that govern non stoichiometry and chemical bonding in the 2201 bismuth cuprates and related tubular phases in connection with their physical properties.
- ◆ Contribute to the training of young researchers from Bolivia.

Activities

The project focussed on the study (synthesis, structural and physical properties) of new cuprates involving bismuth.

- ★ Synthesis and preliminary characterization of new materials were performed independently in the two European laboratories (Barcelona and Caen) with the collaboration of Bolivian researchers.
- ★ Complementary study techniques were used at Barcelona (thermogravimetric analysis, X-ray thermodiffraction experiments, etc.) and Caen (electron diffraction and high-resolution electron microscopy, EDS analysis, X-ray absorption spectroscopy, etc.).

Results

- ⇒ Two large families of cuprates exhibiting double [BiO] layers have been isolated. The first one concerns 2201-related compounds with a single copper layer. The structure of these compounds derives from the 2201's either by shearing mechanism along the c axis of the structure (collapsed phases), shearing mechanism associated with a translation (double collapsed phases) or by periodical introduction of perovskite-type "walls" perpendicularly to the layers of the 2201 structure (tubular phase). The study has been extended to 2212-related oxides which form the second family. These materials are not superconductors but they are important because they can play the role of pinning centers for vortex if they are introduced as defect in superconducting bismuth materials.
- ⇒ The influence of bismuth substitution for Hg and Tl on the structural and physical properties of 1201 cuprates has also been studied. In the case of Bi for Hg substitution, single crystals can be obtained, that allow good structural determination and magnetic study. A great difference is observed between the superconducting Bi-doped 1201 and Bi-2201 materials; it concerns the bismuth formal charge which is larger than +4 and lower than +3 in 1201, and 2201 respectively.
- ⇒ New perovskites related to the superconducting $Ba_{1-x}K_xBiO_3$ have also been isolated.

Selected publications

- Raveau B., Hervieu M., Caldes M.T., Fuertes A. and Michel C. 1995. Generation of Tubular and Shear-Like Structures from Layered Bismuth Cuprates and Oxycarbonates. ECERS 10.
- Luhrs C.C., Caldes M.T., Gómez-Romero P., Fuertes A., Bassas J., Martínez S. 1995. Study of the Formation of Sr_2CuO_2 from Sr_2CuO_3 by X-ray Thermo-diffraction. European Journal of Solid State Inorganic Chemistry. **32**: 1039.
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CHARACTERIZATION AND MODE OF ACTION OF DRACULIN, AN ANTI-COAGULANT PROTEIN FROM VAMPIRE BAT SALIVA AND ISOLATION OF OTHER ANTICOAGULANT AND ANTIPLATELET FACTORS FROM THE SAME SOURCE

Co-ordinator : University of Maastricht, Maastricht, The Netherlands (H.C. Hemker)

Objectives

The main objective is to isolate the factor(s) responsible for the anticoagulant effect of vampire bat saliva. Once isolated, to study its biochemical properties, structural as well as kinetics and from the results delineate the possible mechanism of action. In addition, since there exist some scarce reports on an antiplatelet action of vampire bat saliva, use the same material to study this potential activity.

Activities

The capture and maintenance of vampire bats (*Desmodus rotundus*) is carried out in Venezuela, as well as the isolation and biochemical characterization of the protein (Lab. Thrombosis Experimental, IVIC). Protein sequencing was done in USA, by the Protein Chemistry Group from Rhône-Poulenc Rorer. Biological activity on human blood plasma and whole blood is assayed in Maastricht (Biochemistry Dept.). Experiments leading to the expression of the anticoagulant factor through the use of molecular biology approaches are conducted in Venezuela (Lab. Thrombosis Experimental, IVIC).

Results

We have isolated and purified the anticoagulant factor from vampire bat saliva. It was given the name "Draculin"; is a 78 kDa glycoprotein that it inhibits activated factor IX (FIXa) and activated factor X (FXa), in purified systems as well as in citrated human plasma. Its primary sequence has been determined and the corresponding cDNA has been obtained (unpublished). Efforts to demonstrate the presence of an antiplatelet factor have been not completely successful due to high variability in results.

Follow-up

During the last year of the project efforts have been directed to clarify relevant aspects of structure/function relationship and the kinetic characterization of Draculin. We have shown that native Draculin is secreted as a "microheterogeneous" glycoprotein, that is, it is composed of a unique polipeptide backbone with variable degree of glycosilation. The biological activity (measured as FXa inhibition) is strictly dependent on the appropriate glycosilation of the protein. Lost of biological activity correlates with degree of glycosilation, and "active" and "inactive" Draculin are significantly different in the degree and in the quality of glycosylation. Kinetically, Draculin behaves as a "noncompetitive" inhibitor of FXa. In this

regard, this is a unique behaviour among the known natural inhibitors of FXa. This behaviour may explain the ability of Draculin to inhibit, independently, both coagulation factors FIXa and FXa. Kinetics constants have been obtained and will be useful for future studies directed to evaluate the possibility of obtaining active glycopeptides derived from the native molecule. We initiated this year the study directed toward obtaining biologically active recombinant Draculin. One important difficulty is that being a heavily glycosylated molecule, its expression needs of certain vectors and cells with a competent glycosylation system. In this regard we choose the yeast *pichia pastori* as the most effective system for expression of glycoproteins. Most of the work done so far in this aspect has been devoted to the building of the appropriate insert.

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Period: April 1993 to April 1997

**PREPARATION STUDY OF THE PROPERTIES AND ANALYSIS OF
BIODEGRADABLE SURFACTANTS FROM VEGETABLE ORIGIN**

Coordinator: Université Paul Sabatier, Toulouse, France

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MODIFIED NOBLE METAL CATALYSTS FOR THE SYNTHESIS OF HIGHER ALCOHOLS

Co-ordinator : Université des Sciences et Technologies de Lille, Villeneuve d'Ascq, France
(Ginette Leclercq)

Objectives

- ◆ Develop a ruthenium-containing catalyst with properties modified by additives or support interaction in order to switch selectivity from an alkane-producing metal to an oxygenate-producing catalyst in CO+H₂ reactions.
- ◆ Obtain a better appreciation of the principles underlying the modification of noble metals by promoters, which is still not very well understood in catalysis.

Activities

- * The project concerned, first, the synthesis of dispersed ruthenium on various oxide and mixed oxide supports (shared out among the three groups). The catalysts were characterized by several physico-chemical methods :
spectroscopic techniques: FTIR study of CO adsorption (Caracas), XPS (Lille, Brunel), Raman Spectroscopy (Brunel);
 - X-Ray Diffraction (three universities), Transmission Electron Microscopy (Caracas);
 - Thermo Gravimetric Analysis (Lille) and Temperature Programmed Desorption (Brunel);
 - CO and H₂ adsorption (Lille and Brunel).
- * A probe reaction (the hydrogenolysis of lower alkanes) was used at Brunel University to characterize metal support interactions.
- * Finally the activities and selectivities of these catalysts for the CO+H₂ reactions have been studied in flow fixed bed reactors at medium pressures (about 50 bars) at Lille and Caracas.

Results

- ⇒ The chemical probe (hydrogenolysis of light alkanes) has proved to be a good and sensitive indication of the composition and of the properties of the catalyst surface. Its rates, selectivities, energies of activation are highly structure-sensitive. A performing model of the reaction rate has been developed and is able to calculate separately all the kinetic parameters in order to determine the sensitivity of these parameters to support and modifier effects.
- ⇒ Selectivity for the formation of oxygenates on Ru supported on single oxides seems to be linked to the reducibility of the support, since with non-easily-reducible supports (SiO₂, Al₂O₃, ZrO₂), the alcohol production was very low. On the other hand, with WO₃ and especially with MoO₃ as supports, the alcohol production is better. The best selectivity was obtained with Ru/MoO₃ with up to 80% of alcohols in the products at low temperature and low conversion (in the alcoholic fraction there was about 1/3 of higher alcohols : C₂+). K addition did not seem to improve greatly the alcohol production, but

higher alcohols were more favored since 45 % of the alcohols were C₂+ . Since it is commonly admitted that a mixture of oxygenates containing at least 50 wt% of higher alcohols could be directly used in fuels, such results seem promising.

⇒ When Ru is deposited on mixed oxides associating an oxide (ZrO₂ or WO₃) supported on Al₂O₃, the selectivity in oxygenates is improved in comparison with that obtained for Ru deposited on the single oxides. Such a phenomenon is believed to result from a better reducibility of oxides when they are deposited on high surface area supports.

In conclusion, the results obtained are very positive and encouraging since they have shown that support interaction or modifier effect can transform Ru from an alkane-producing metal when deposited on a non reduced support to an oxygenate-producing catalyst when supported on a partly reduced support. We are trying to continue working together in order to improve further the selectivity in oxygenates of Ru in the CO+H₂ reaction

Selected publications

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DEVELOPMENT OF NADH ELECTROCHEMICAL BIOSENSORS FOR ANALYTICAL PURPOSES

Co-ordinator: Università degli Studi di Firenze, Firenze, Italy (Marco Mascini)

Objectives

The objectives were the practical application of NADH biosensors for solving specific analytical problems, such as L- and D-lactate determination in milk samples, the salicylate determination in blood of rheumatoid arthritis patients and malic acid in fruit juice.

Activities

The project aimed to extend a highly sensitive NADH sensor in the submicromolecular range and to investigate the suitability for metabolite determination in real samples. It was necessary to develop an appropriate enzyme immobilization procedure, to eliminate matrix interference by general cleanup procedures of the sample and to perfect a sampling technique using a microdialysis procedure.

Results

In the course of this project, L- and D-lactate determination in milk samples as a rapid detector of microbial contamination of sterilized milk has been achieved. The malate biosensor for malic acid determination orange juice was also developed. We have studied the optimum experimental conditions to measure malate by using NADH oxidation and immobilized malate dehydrogenase. The results obtained with our system present good agreement in comparison to the results obtained with spectrophotometric commercial kits. An amperometric biosensor for salicylate was developed by immobilizing the enzyme salicylate hydroxylase via glutaraldehyde onto a polypyrrol film doped with hexacyanoferrate, supported on a glassy carbon electrode surface. The procedure was quite innovative and new results were then obtained.

Follow-up

In the last period we devoted our efforts to developing new strategies for printing electrodes with a screen printing technology based on carbon and mixing carbon with noble metals. This will improve the procedures of measurements by this new printed electrodes which are very cheap and very sensitive to NADH oxidation.

Selected publications

G. Marrazza, A. Cagnini and M. Mascini, 1994. NADH Electrochemical Sensors for the Enzymatic Determination of L- and D-lactate and 3-hydroxybutyrate using Flow Injection Analysis. *Electroanalysis* **6**: 221-226.

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**CHEMOMETRICS AND QUALIMETRICS IN PHARMACY AND FINE
CHEMISTRY**

Co-ordinator: Vrije Universiteit Brussel, Brussels, Belgium (Desire Luc Massart)

Objectives

The work performed during the period of the project aimed the development of chemometrics and qualimetrics techniques for analytical methods, and the transfer of these techniques to the Brazilian partners.

Activities

The project involved basically the development of techniques concerning expert systems, method development and validation and experimental designs. Concerning validation and computer programs, a knowledge-based system for validation was developed. The aim of such system is to assist the analyst to demonstrate the method's suitability to produce useful data. The system contains the knowledge necessary for the planning of the experiments and the evaluation of the experimental results. It was conceived in such a way that it guides the analyst in a logical order to achieve the conclusions of the method studied and should be used to contribute to the global quality assurance of the analytical data. Still in validation ruggedness testing of analytical methods were studied. From this a strategy for the selection of an experimental design to treat the results obtained when performing ruggedness testing was developed. In HPLC the necessary knowledge to build a knowledge-based system for selectivity optimization of the separation by varying the pH and the mobile phase composition (OPTIPHORM) was acquired. The strategy proposes linear and non linear models and includes multicriteria decision making techniques (MCDM) specially developed for the selection of rugged optima in HPLC. In mixture analysis the attention was focused on the detection and quantification of chromatographic peaks in HPLC. As long as all compounds are well separated, no special difficulties arise in quantifying them. However, when peaks overlap, chemometrical tools may be applied to resolve the overlapped peaks into individual concentration profiles. For this purpose several chemometrical techniques were developed to be applied to liquid chromatography coupled with diode array detection (HPLC-DAD).

Results

⇒ Evaluating the work planned to be performed during the 3 years of the project, we think it was successfully completed. The strategy for validation of trace metal determination in biological matrices by atomic absorption (AAS), the recommendations for method validation, the strategy for the selection of experimental design and the one for multivariate data analysis were developed and tested. They have been used as basis for new research and developments made in the laboratory. These strategies have been applied on the work developed by both Brazilian partners. In order to train the Brazilian partners on the application of some of these techniques, 2 courses; one about method validation and the other about neural networks, were given by the Belgium partners in Brazil.

⇒ The immediate consequence of the interaction Belgium/France/Brazil is that a first book in Portuguese about experimental designs was written, chemometric classes are officially implemented in the pos-graduation courses at the University of Campinas (UNICAMP) and in the undergraduated courses at the University of Rio de Janeiro (UFRJ). This achieves the ultimate aim of the project that is the transferring the knowledge gathered in this laboratory to Brazil.

Selected publications

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DESIGN, SYNTHESIS AND CHARACTERIZATION OF HOMOGENEOUS AND HETEROGENEIZED CATALYTIC PRECURSORS, CONTAINING SULFUR LIGANDS

Co-ordinator : Consejo Superior de Investigaciones Cientificas, Madrid, Spain
(Pilar Terreros)

Objectives

The aim of this project was to design a group of active catalysts with sulfur-containing ligands such as thiolates and thioethers. The use of specifically modified sulfur ligands (fluorinated thiols and chiral dithiols) instead of the classical phosphine systems constituted the major novelty. Sulfur ligands are cheaper and easier to modify chemically than phosphines and, due to their co-ordination properties, could confer to the complexes novel properties as catalysts in hydrogenation, hydroformylation and hydrodesulfuration reactions in homogeneous conditions, and heterogeneized in various supports.

Activities

The project focused on the design and preparation of new ligands containing sulfur. These could be mono-, bi-, poly-dentate either with S-donor groups or assisted by other basic functions such as phosphine, amine, etc. in order to modulate their electronic and steric properties. Complexes of different platinum-group metals, namely Pd, Pt, Rh and Ir, were synthesised and their performance in catalytic reactions were explored, especially with regard to the activity and chemo-, regio-, and enantioselectivity. The preparation of new chiral dithiolate ligands was carried out mainly in Barcelona and Tarragona. These ligands were often derived from cheap, optically-pure molecules from the chiral pool. The preparation of the corresponding dimeric complexes containing these ligands was performed. The groups in Mexico and Madrid devoted their attention mainly to the synthesis and characterisation of metal complexes containing different fluorinated thiolates. The synthesis of metal complexes containing both thiolate and tripodal phosphines ligands was performed in Florence. The anchorage of these complexes in inorganic oxide supports and functionalised resins was done in Barcelona and Tarragona. The Barcelona, Tarragona, Florence and Madrid groups did most of the catalytic studies of these complexes in either homogeneous or heterogeneous catalytic tests (hydrogenation, hydroformylation, asymmetric hydroformylation, HDS).

Results

During the three annual meetings held in Florence, Mexico and Barcelona, the discussions and exchange of results and ideas were very useful, as well as information about new techniques and facilities in the catalysis field. Human mobility has been very active, including a number of stays of pre- and post-doctoral students from the participating groups and, in some cases, from other laboratories. A great number of new functionalized chiral thiolate ligands and new optically-pure polydentate ligands with P,N-donor atom sets were prepared. A wide family of Pt, Pd, Rh, Ir complexes containing these ligands and perfluorinated thiolates was studied and fully characterised, and the reactivity in reactions related to the catalytic processes was analysed. The catalytic performance (activity, chemo-, regio- and enantio-selectivity) of these

complexes focused on two reactions; namely hydrogenation and hydroformylation of alkenes. Some thiolate complexes showed to be active as HDS catalysts in homogeneous and biphasic conditions. Some compounds were immobilised, either in functionalised polymeric matrixes or in inorganic supports (SiO₂, TiO₂), and studied as catalysts in hydroformylation and Fischer-Tropsch reactions. There was an exchange of products between groups to complement the different parts of the work.

Follow-up

The groups are trying to improve the catalytic activity and selectivity of the prepared compounds, searching for practical applications in the pharmaceutical and petrochemical industries.

Selected publications

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**ISOLATION, PRODUCTION, CHARACTERIZATION OF
GLYCOSYLTRANSFERASE ENZYMES FOR NEW CARBOHYDRATES**

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BIOCORROSION, BIOFOULING OF STAINLESS STEELS IN INDUSTRIAL ENVIRONMENTS. PREVENTION AND PROTECTION

Co-ordinator : University of Portsmouth, Portsmouth, United Kingdom (Iwona B. Beech)

Objectives

The research aimed to gain a better understanding of deterioration phenomena of carbon and stainless steels in aqueous marine environments, due to the formation of microbial biofilms and to consider appropriate prevention measures.

Activities

Field studies carried out at Portsmouth (UK) focused on characterisation of biofilms formed on 304 and 316 stainless steel and on the analysis of steel surfaces prior to and following their exposure to evaluate the susceptibility of both alloys to biocorrosion. A comparison of physical and chemical surface features of both types of steel was performed by partners in Sheffield (UK) and in La Plata (Argentina). Laboratory investigations undertaken by Argentinean and both UK teams were conducted to determine the efficacy of biocide glutaraldehyde in controlling biofilm development on surfaces of 304 stainless steel. At Portsmouth work was carried out to develop a new medium facilitating the isolation and laboratory growth of different isolates of sulphate-reducing bacteria (SRB). Partners at Portsmouth and La Plata undertook the assessment of the role of exopolymers produced by SRB in the corrosion of carbon steel.

Results

It has been demonstrated that susceptibility of stainless steel to biocorrosion differed between steel alloys depending on the environment and type of microbial consortium. Contrary to other reports polished surfaces were more prone to fouling compared to rough surfaces, regardless of the type of steel. It was found that glutaraldehyde formed a conditioning film on surfaces of stainless steel coupons. The layer had a biostatic effect on microbial population and was effective in controlling the build-up of a biofilm, indicating that pre-treatment of surfaces could be beneficial as a biocorrosion prevention measure. A new medium has been developed for the isolation and growth of SRB. Compared to other SRB-isolation broths the new medium proved to be superior regarding SRB recovery and biomass production. It was revealed that exopolymers (EPS) produced by different isolates of SRB varied in their chemical composition and ability to chelate metal ions. EPS proved to influence the corrosion behaviour of carbon steel. A high molecular weight, iron-binding protein-carbohydrate complex was isolated and showed to be capable of accelerating the corrosion rate of carbon steel in aerated solutions. A new mechanism of SRB-influenced corrosion, which involves EPS, has therefore been proposed.

Selected publications

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BIFUNCTIONAL ZEOLITE CATALYSTS FOR THE SYNTHESIS OF SPECIALTY OR FINE CHEMICALS. SYNTHESIS OF CARBONYLATED COMPOUNDS

Co-ordinator: CNRS/ UMR 6503, Poitiers, France (Michel Guisnet)

Objectives

The objective of this project was to develop bifunctional metal acid zeolite catalysts for the selective synthesis in one step of functional compounds (ketones) the synthesis of which normally requires several successive steps.

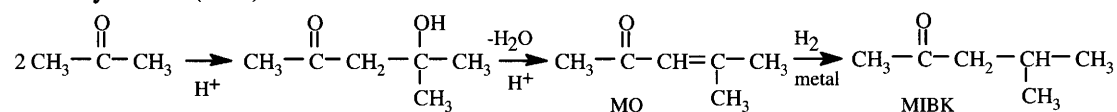
Activities

The project focused on the transformation of carbonylated compounds such as acetone, cyclohexanone, acetophenone into methylisobutylketone, 2-cyclohexylcyclohexanone, 1,3-diphenylbutan-1-one, which present an industrial interest. These syntheses require three successive steps: aldolisation, dehydration then hydrogenation, that can be catalyzed in one step on bifunctional catalysts Pt or Pd zeolites with different pore structures (FAU, BEA, MFI,...). To guide the choice of the catalysts, relationships are established between the activity, stability and selectivity of bifunctional zeolite catalysts for the synthesis of carbonylated compounds and :

- i) the properties of the acid and metal sites and more particularly the balance between these sites;
- ii) the pore structure of the zeolite : diffusion path of the molecules, space available near the acid sites.

Results so far

The transformation of acetone into methylisobutylketone (MIBK) was investigated in Caracas over series of Pt and MFI zeolite catalysts with metal contents between 0.03 and 1.0 wt % under the following conditions: flow reactor, temperature: 70-190°C, $p_{H_2} = 0.1-0.9$ bar, acetone = 0.1-0.75 bar. Whatever the operating conditions, acetone is directly transformed into MIBK; only traces of mesityloxide (MO) are observed :



Propane and 2-methylpentane, that are also formed through bifunctional catalysis, are the main side-products. The best results, especially the highest selectivity to MIBK (> 90%) are found with PdHMF1 catalysts at 160°C and low hydrogen pressure. A correlation can be established between the catalytic properties and nM/nA, the ratio between the number of accessible metal sites and the number of acidic sites.

The same type of experiments was carried out at Lisboa and Poitiers with bulkier reactants: cyclohexanone, acetophenone and 2,5-hexanedione, and correlations between the catalytic properties and nM/nA were established. Contrary to what was found with acetone transformation, large pore-size zeolites are required to obtain high activities and selectivities. Other types of side reactions are observed in particular acid cracking of the intermediate product

(equivalent to MO) formed from acetophenone and cyclization of 2,5-hexanedione into 2,5-dimethylfuran.

Follow-up

During the last six months of the project, the emphasis was placed on the understanding of the relatively rapid catalyst deactivation caused by blockage inside the pores of heavy secondary products (coke). Coke composition was determined and optimal conditions for coke removal through oxidative treatment was established. Furthermore, bimetallic catalysts (PdCu etc.) were prepared, with a view to improving the selectivity to C=C hydrogenation necessary to the formation of the desired product compared to C=O hydrogenation which leads to the main side-products.

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MECHANISTIC STUDIES OF HYDRODESULFURIZATION AND HYDRO-DENITRIFICATION REACTIONS OF HETEROCYCLES IN MEXICAN CRUDE OIL

Co-ordinator: The University of Sheffield, Sheffield, United Kingdom (P. M. Maitlis)

Objectives

Mexico is one of the world's leading suppliers of oil (21% of which is exported to the EU) and has some of the largest reserves; however, much of this oil is of the heavy ("Maya") type, with high levels of sulfur and other impurities. When burnt, sulfur-containing compounds give sulfur dioxide, an extremely toxic and polluting gas, thus it is vital for the combusted fuel to be as sulfur-free as possible. The removal of sulfur (hydrodesulfurization, HDS) is accomplished industrially with a sulfided cobalt molybdenum catalyst. This is very effective in removing sulfur down to the 1000 ppm level, but it is not able to remove sulfur impurities down to the < 10 ppm level which is *now being demanded* by new EU legislation. The problem compounds are sulfur-containing heterocycles: thiophenes, benzo- and dibenzothiophenes, and their alkyl-derivatives. A new process for HDS using platinum/palladium on an acidic alumina for a "second stage" hydrotreating has very recently been developed. The joint EC-Mexican research project was designed to probe the chemistry underlying this new process.

Activities

- * Platinum(0) phosphine complexes react with thiophenes to give the corresponding six-membered thiaplatinacycle rings in which platinum has oxidatively inserted into one C-S bond of the heterocycle. These thiaplatinacycles have been isolated and fully characterised, both spectroscopically and by many X-ray structure determinations. The formation reactions are reversible, and some equilibrium and competition data have been determined.
- * The reactions of the thiaplatinacycles have been investigated, in particular those which lead to cleavage of C-S bonds and the formation of hydrocarbons derived from the heterocycles by extrusion of sulfur (HDS). Related reactions with palladium and nickel complexes are also being studied.

Results

- ⇒ Formation and characterisation of thiaplatinacycles from oxidative insertion of Pt(0) into the C-S bonds of thiophenes. Thiophenes bearing functionalities in either the 2- or the 3-position react much more easily with Pt(0) than the parent thiophenes or their alkylated derivatives. The formation of similar thia-palladia- and thianickela-cycles from oxidative insertion of Ni(0) and Pd(0) into thiophenes.
- ⇒ The fundamental chemistry of thiaplatinacycles : the equilibria between the thiophenes and the thiaplatinacycles and also between thiaplatinacycles formed from different thiophenes; the cleavage of Pt-C bonds in thiaplatinacycles by acids.

- ⇒ The demonstration of HDS by hydrogen alone in the thiaplating cycles resulting in the formation of the parent hydrocarbons. It has therefore been possible to model a substantial part of the platinum-catalyzed HDS reaction in terms of real chemical reactions. These HDS reactions are further promoted by a) palladium salts, and b) by alumina, results that illustrate the relation between these model processes and the second stage industrial HDS over Pt-Pd on acid supports.
- ⇒ The training of Mexican scientists (one PhD, five MSc, plus student projects) in advanced organometallic and catalytic chemistry. Substantial funding has also been obtained from the Mexican CONACYT which has warmly supported the project and has provided equipment and consumables of an approximately equal value to that brought in by the EU-ISC grant.

Follow up

EU funding for the project has now unfortunately finished. The immediate aims of the project have been realised but the longer-term objectives of improving the new Pd/Pt catalysed HDS processes are not likely to be achieved unless extra research funding can be provided: the source of that is not immediately obvious, since the Mexican oil economy is now in financial crisis and there seems no mechanism by which the EU can provide a further grant.

Selected publications

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MODELS FOR ADSORPTION ON METAL OXIDES

Co-ordinator: University of Sevilla , Sevilla, Spain (Javier Fernández-Sanz)

Objectives

The main goal of this project was to develop theoretical models for the adsorption of simple probe molecules and atoms on the surfaces of metal oxides usually employed as catalysts and supports in heterogeneous catalysis.

Activities

Research involved both theoretical and experimental techniques specially suited for the study and characterization of surfaces. The computational strategy was based on complementary studies performed using quantum chemistry methods and classical molecular dynamics (MD) simulations. *Ab initio* calculations were used to analyze the nature of the surfaces and adsorbate-substrate interactions, as well as to determine some pair potentials, which were later used in the MD simulations. Whenever possible, complementary XPS and DRIFT spectra were recorded.

Results

Three solids were studied: γ - Al_2O_3 , MgO and TiO_2 . For γ - Al_2O_3 , bulk, surface and hydration process models were obtained. The MD simulation of a microcrystal shows that the surface features a noticeable reconstruction and the presence of four and five-fold coordinated Al atoms, which are likely to be responsible for the experimentally observed Lewis acidity. For MgO, adsorption of acetone and surface enolization were analyzed. The theoretical calculations allow for the interpretation of the DRIFT spectra revealing the presence of an enolate surface species. The MD simulations of the surface hydroxylation show that the protons penetrate into the bulk in agreement with ERDA and DRIFT data. For TiO_2 , the effects of Na and K deposition were analyzed. The *ab initio* calculations indicate an efficient surface reduction and the MD simulations permit us to discern and confirm previous interpretation of experimental data.

Selected publications

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**ENSURING INTERNATIONAL COMPARABILITY OF CHEMICAL
MEASUREMENTS OF CHILEAN LABORATORIES THROUGH THE
INTERNATIONAL MEASUREMENTS EVALUATION PROGRAMME (IMEP) OF
THE EUROPEAN COMMUNITIES**

**Co-ordinator : Institute for Reference Materials and Measurements, Geel, Belgium
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ISC

Earth Sciences

**THE INFLUENCE OF SHALLOW STRATIGRAPHY ON LONG DURATION
GROUND MOTIONS IN MEXICO CITY BASIN: EXPERIMENTS AND
MODELLING**

Co-ordinator: Université de Liège, Liège, Belgium (Denis Jongmans)

Objectives

The main objective of the project was to understand the processes responsible for the space variability and the long duration of the observed ground motion in Mexico City which caused huge damage during the 1985 Michoacan earthquake. To this end, *in situ* seismic experiments were conducted in Mexico City during the spring of 1994 and modelling techniques were developed in order to provide tools for the data interpretation.

Activities

Three types of experiments were conducted by the three partners in Mexico City in the spring of 1994 :

- * seismic tests in Texcoco Lake and on basalt outcrops in order to determine the dynamic characteristics of these two formations.
- * recording earthquakes at a small aperture array in the botanical garden in order to identify the incoming waves contributing to the records.
- * deployment of broad-band stations along a North-South line crossing Mexico City to study a possible reported regional amplification.

On the other hand, developments of indirect boundary element method techniques were performed in close collaboration between the Instituto de Ingenieria (UNAM - Mexico) and the LGIT (Université Joseph Fourier - Grenoble).

Results

The seismic experiments conducted in the lake bed zone of Mexico City show very low shear wave velocity values ranging from 30 m/s at the surface to 115 m/s at 40 m depth while Q_s values increase from 8 to 60. These relatively low attenuation values support the idea that surface waves generated at the edges of the Mexico basin can not propagate very far within the lake bed zone and that another process is responsible for the long duration of the observed ground motion. The origins of this long duration were investigated by the two seismological experiments. The deployment of a small aperture array in the hill zone inside the valley of Mexico allowed us to identify the direction of arrival of the different waves that participate in the ground motions. The array analysis clearly indicated that very little of the energy from wave resonance within the lake zone is transmitted to the hill zone. On the other hand, long-duration ground motions were observed at the array, with evidence of multipathing probably resulting from single forward scattering in the upper crust. This interpretation is supported by the second experiment which measured the surface wave propagation across the Mexican Volcanic Belt (MVB). The study allowed us to distinguish in the MVB a low-velocity zone

responsible for the regional amplification and long duration observed in the vicinity of Mexico City. Numerical simulations confirmed that such a low-velocity layer induces a regional amplification of an order of magnitude close to the observations.

Selected publications

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METAMORPHISM IN EXTENSIONAL CONTINENTAL SETTINGS : A CASE STUDY FROM THE BASIN AND RANGE PROVINCE

Coordinator: Université Aix-Marseille III, Marseille, France (Jean-Jacques Cochemé)

Objectives

- ◆ NW Mexico, a part of the Basin and Range Province, appeared to have good potential as a site to explore the inter-relationships between extension and metamorphism at different levels in the crust and to test the conceptual models proposed earlier by partners. The discovery of zeolites in sedimentary rocks of the Baucarit Formation was taken as indicative of the development of a low-grade metamorphism synchronous with the Cenozoic extensional process.
- ◆ The project was established to investigate these areas of research with the following aims:
 1. Determine the regional extent of the very low grade metamorphism (zeolite facies);
 2. Determine if there is a continuum into higher grades with increasing depth;
 3. Develop P-T-t paths of the metamorphic evolution and relate these to the geodynamic models of the tectonic setting of this area during the Miocene period.

Activities

- ★ Characterization of zeolites and extent of the heulandite-cemented sediments over Sonora and adjacent areas was completed during the first and second years (field work in Sonora with participants of the UNISON, laboratory work in Bristol and Marseille). We presented a model of high heat flow during the deposition of the lower member of the sedimentary pile, but we concluded that the probability of finding a transition into higher grade metamorphic facies within the extensional basins is low because of the restricted vertical interval of the Baucarit Formation. Accordingly, subsequent parts of the work were reassessed during the second year of the project.
- ★ We then focused our work on :
 - (I) zeolitized tuffs in the upper sediments and their potential economic interest (Lab. work on zeolites in Marseille);
 - (II) higher-grade metamorphic rocks: we refound (field work by the UNISON team, datings at UNAM) in the basement rocks of the metamorphic core complex zone for any possible evidence of Tertiary metamorphism associated with the extensional processes.

Results

⇒ Infilling sediments (the Baucarit Formation) in the basins are composed of two units; a lower member with heulandite as the main authigenic mineral and an upper member with different zeolites species comparable to those found in saline alkaline lakes of SW USA. Zeolites occur in sediments as a pore-filling cement and also in the intercalated volcanics, both in basaltic flows and rhyolitic tuffs.

- ⇒ Based on the reported stability fields from the literature and the presence of unreacted glass, zeolites and associated minerals from the upper member are of low temperature. The widespread distribution of heulandite in sandstones of the lower member of the Baucarit Formation of eastern and central Sonora suggests:
- (I) a uniform process of alteration.
 - (II) a constant temperature of alteration over a wide area.
- As estimated temperatures for heulandite are between 85 and 125°C and as there is only a low burial, it is proposed that hot fluids are responsible for the alteration of the volcanic glass.
- A decrease with time in the initial permeability of the tuffs is consistent with the observed evolution of the changing Al-smectite toward a more magnesian composition. A comparison of chemical compositions of the zeolites found in the basalts with published data on zeolites from different settings (Shoppard et al., 1970; Sameshima, 1978; Alberti and Brigatti, 1985; Nativel et al., 1994), suggest a hydrothermal origin for chabazite and erionite.
- ⇒ Deeper levels in the crust are exposed in the metamorphic core complex zone. We found evidences of tertiary HT-LP metamorphism in response to extension by dating phyllosilicates. The highest grade of metamorphism is the amphibolite facies.
- ⇒ This high-temperature - low pressure regional metamorphism is related to the underplating of basaltic magmas in the no-slab window area after the collision of the East Pacific Rise with the North America Plate.

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**PALAEOBIOGEOGRAPHY AND TECTONIC EVOLUTION OF THE UPPER
SILURIAN-DEVONIAN SEQUENCES IN ARGENTINA. GEOLOGICAL
CORRELATIONS WITH NORTHERN AND SOUTHERN PALAEOCONTINENTS.
RELATIONS WITH THE GENESIS OF MINERAL RESOURCES.**

Coordinator : University of Wales, Cardiff, United Kingdom (Dianne Edwards)

Objectives

- ◆ Collection, description and identification of plants from Argentina and Bolivia, from rocks deposited during the early evolution of higher plants.
- ◆ Assessment of their affinities, evolution and geographical distribution.
- ◆ Assembly of geological data relevant to sedimentology, biostratigraphy, tectonics, structural geology and mineral resources.

Activities

- ★ Extensive collecting in the Argentine Precordillera and southern Bolivia has yielded major new collections of plants, some associated invertebrates, and trace fossils. The former have been examined in La Plata and Cardiff, using a wide range of techniques. Particularly important has been the detailed analysis of the fossils in relationship to entombing sediments.
- ★ Fieldwork and literature searches have allowed reconstructions of palaeogeography and tectonics, with impact on biogeography and climate.
- ★ All such data have been incorporated into a detailed illustrated final report that is nearing completion.

Results

- ⇒ The record of the earliest higher-land plant, *Cooksonia*, in the Silurian of Bolivia was the first for the southern hemisphere, and for the palaeocontinent Gondwana. *Cooksonia*, plus abundant associated fragmentary plants, some fertile, indicates that plants colonised high latitudes very early in terrestrialisation, with little indication of phytoprovincialism. Abundant vegetation also characterised high Gondwana in the succeeding Devonian. Here, taxa cannot be placed in northern hemisphere, taxa necessitating the erection of a number of new taxa and suggesting the development of a cold high-latitude phytoprovince. Prior to this study the Argentine rocks had been interpreted as turbidites (storm deposits) associated with a deep submarine fan, but have now been identified as shallow marine deposits formed on the inner shelf.
- ⇒ Silurian-Devonian rocks (particularly Villavicencio Format) are currently being assessed as petroleum reservoirs. In the same area, barite is currently mined. Ore deposits produced by epithermal mineralisation associated with megafaults and Cenozoic magmatism in San Juan and La Rioja include pyrite, calcopyrite and arsenopyrite, but exploitation of Cu, Hb, Sb, Mo, Pb-Ag-Zencontaining ores is not currently viable. Iron ores characterise the Silurian-Devonian of N.W. Argentina and S. Bolivia, and are still

mined in the Lipeon (= Kirusillas) formation, but not in the Sierra Grande formation of Rio Negro Province, Argentina.

Follow-up

Development of a biostratigraphic framework (and hence age control) will continue in both countries (grant application to CONICET and RS): geological evolution of ore deposits.

Selected publications

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THE PALAEOZOIC EVOLUTION OF THE ANDEAN LITHOSPHERE (30-32°S)

Coordinator: British Antarctic Survey, Natural Environment Research Council,
Keyworth, United Kingdom (R.J. Pankhurst)

Objectives

A multi-disciplinary investigation of the Palaeozoic geology through the eastern Andean region of western Argentina, to yield:

- ◆ correlation between plutonic, volcanic and sedimentary events,
- ◆ the chemical and isotopic signature of the continental crust and underlying lithospheric mantle,
- ◆ a geotectonic model of terrane accretion, crust-mantle interactions and continental growth, and
- ◆ consequences for the metallogenic potential of the region.

Activities

- ★ Fieldwork and mapping was organized by the Argentine team (La Plata, Córdoba); collecting for laboratory work was mostly carried out by the full team over three Spring seasons.
- ★ Geochemical analyses were obtained commercially and geochronology was carried out at the Keyworth laboratory and in Canberra, Australia.
- ★ Two research students were occupied on the project (Salamanca, La Plata).

Results

The new database has allowed the development of tectonic models incorporating the idea, made just as the project was starting, that the Argentine Precordillera and western Sierras Pampeanas are an allochthonous terrain derived from Laurentia (the present North American continent). The Pampean orogeny, during which the eastern zone of the Sierras de Córdoba was accreted to cratonic Gondwana, was essentially an Early Cambrian event (c. 530-520 Ma) rather than Precambrian, as had been previously supposed. In latest Precambrian times (c. 540 Ma), passive margin sedimentation took place in a marine basin, formed during rifting away of a local part of the Mesoproterozoic supercontinent (Rodinia). This was followed by subduction-related calc-alkaline magmatism (c. 530 Ma) as the early ocean gradually closed. Terrane collision was the main cause of crustal thickening and high-grade metamorphism associated with Pampean orogeny, culminating by about 523 Ma with extensive crustal anatexis and the production of peraluminous cordierite-rich granites. The Famatinian cycle of granitoid magmatism in the central zone of the transect also started earlier than previously considered, in earliest Ordovician time, c. 490 Ma. This implies that a new and very intense subduction-related magmatic arc, continuing until about 455 Ma, on the continental slope of newly-enlarged Gondwana; underlying Mesoproterozoic crust, indistinguishable from that of the eastern Sierras Pampeanas, was involved. There is no direct evidence from either the style or geochemistry of the granitoids that the Famatinian orogeny was related to the collision of the Precordillera (or any other) allochthonous terrane, which we now believe to have been a later event. In the western zone, the Sierra de Pie de Palo has a composite geological structure: medium- and high-grade gneisses and granitoids in the west are of late Mesoproterozoic age (c. 1000 Ma), similar ages to the Laurentian unexposed basement of the Precordillera. Knowledge of this clear zonation is

important with respect to the genesis of mineralized rocks, and the fluorite deposits of Córdoba were formed in Cretaceous rather than the Palaeozoic times. Further work is now being supported by CONICET (Argentina) and an application has been made for continued Spanish funding.

Selected Publications.

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**STUDY OF VOLCANIC COLLAPSE CALDERAS DEVELOPED IN
TRANSCURRENT FAULT ZONES (THE CALAMA-OLACAPATO-TORO
VOLCANO-TECTONIC LINEATION, CENTRAL ANDES, ARGENTINA), AND
THEIR ASSOCIATED MINERAL DEPOSITS (AU, AG, SB, PB, ZN, CU, AND
BORATES)**

Coordinator : Consejo Superior de Investigaciones Científicas, Barcelona, Spain
(Joan Martí)

Objectives

The main objective of this research project was the development of a valid methodology for the analysis and identification of tectonised volcanic calderas from an area of the Central Andes (Northern Argentinean Puna), that have associated ore deposits of high strategic and economic interest (Ag, Au, Sb, Pb, Zn, Cu and borates). This has included the reconstruction of the original caldera structures of the studied area, the identification of the changes caused by further tectonic and volcanic events, their volcanological characterisation and their relationship with the origin and distribution of ore deposits.

Activities

The research developed in the project was undertaken by a multidisciplinary research group including scientists from Argentina, Spain and Italy. This group carried out a complete geological and volcanological study of several volcanic calderas related to transcurrent faults and associated with ore and evaporitic deposits. Argentinean and Spanish researchers carried out a detailed geological and volcanological study of the selected caldera structures, that also comprised a comprehensive petrological, geochemical and mineralogical study of the volcanic rocks and associated ore deposits. The Italian researchers were in charge of the isotopic and geochronological study that allowed to determine a precise stratigraphy of each caldera and the timing of the emplacement of the ore deposits.

Results

The use of a methodology specifically developed for this research project has permitted the geological reconstruction of the studied area, even where it had been affected by significant tectonic and volcanic processes. In detail, this study included the reconstruction of the original geological structure of the studied area, the identification of the changes caused by further tectonic and volcanic events, their volcanological characterisation and their relationship with the origin and distribution of ore deposits. The relationships between caldera-forming processes and the formation and distribution of ore deposits have been established. The results obtained will be used as guideline for prospecting other volcanic areas of the Central Andes that show similar characteristics.

Follow-up

Interpretation of results has continued after the completion of the project. Collaboration between the research groups involved in project continues and during the last year has concentrated on the location of similar areas in the same region which could be of interest for prospecting in the near future.

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**EVOLUTION OF MESO-CENOZOIC MAGMATISM AND METAMORPHISM IN
THE NORTHERN PATAGONIAN ANDES (44°-47° S LAT.)**

Coordinator : Université Aix-Marseille III, Aix en Provence, France (Alain Demant)

Objectives

- ◆ Establish the petrological and geochemical characteristics of the Meso-Cenozoic magmatic episodes in the North Patagonian Andes and determine their chronology;
- ◆ Understand the peculiarities in the geodynamic development of the region, to compare the NPA with others known sections of the Andes and to learn about the causes of its alongstrike segmentation.

Activities

- * Many field campaigns were undertaken by participants. The Pisa group focused its activity mostly on the recent arc lavas to compare the characteristics of the stratovolcanoes (Kay, Maca and Hudson) with the small monogenetic cones located along the Liquiñe-Ofqui fault zone. Study of the channel area of the Tertiary to Quaternary fore-arc sequences was made in October 1994 and 1995 (Universidad de Chile, British Antarctic Survey and Aix-Marseille III University). The detailed stratigraphy of the Coyhaique region was established thanks to participation in the regional mapping programme of Sernageomin.
- * Geochemical analyses to characterize the volcanic sequences were made at the same time in Santiago and Marseille, with the aim of developing a good analytical laboratory at Universidad de Chile. Isotopic determinations were done at the British Antarctic Survey. Low grade metamorphism which affected the Mesozoic and the Tertiary sequences was studied in collaboration with Universidad de Chile and Universidad de Cadix.

Results

- ⇒ The multidisciplinary approach used in the course of this research programme contributed to a better understanding of the magmatic, tectonic and metamorphic evolution of the northern Patagonian Andes.
- ⇒ The Northern Patagonian batholith is a complex structure formed by successive plutonic intrusions. The main magmatic episode is Lower Cretaceous in age (125-90 Ma) but more recent dispersed plutons were emplaced at shallower depth along the Liquiñe-Ofqui strike-slip system until the Upper Miocene (20 to 10 Ma). Such an occurrence of recent plutons at the surface underline a rapid uplift of the Cordillera during the plio-quaternary. Most of the Patagonian plutons are typical subduction related calc-alkaline magmas but some of the recent Miocene rocks are more primitive ($^{87}\text{Sr}/^{86}\text{Sr}\sim 0.7034-0.7041$) calc-alkaline I-types tonalites to granites. The granitoids seems to have been generated by re-melting of basaltic material underplated at the base of the crust.
- ⇒ The Jurassic Ibañez Formation and Cretaceous Divisadero Formation are calc-alkaline volcanic sequences made of andesitic to dacitic lavas and ignimbrites. These sequences are not always easily distinguishable in the field. A study of the secondary mineral associations evidenced differences in the physical conditions of low-grade metamorphism and constitute an interesting tool to discriminate the Jurassic and Cretaceous Formations. The frequent

occurrence of sills, dykes and hypabyssal intrusions in the Divisadero Formation indicates that these outcrops correspond to the roots of a volcanic succession heavily eroded during cordilleran uplift.

- ⇒ A major extensional event begin at the end of the Upper Cretaceous and during the Paleocene, in the back-arc region. Plateau basalts were emplaced and continental molasses filled afterward these bassins. The progressive change in the geochemistry of the basalts seems to be due to a shift from a lithospheric to an asthenospheric source region. This back-arc extension correlates with the collision of the Chilean ridge with the South American Plate.
- ⇒ During the Quaternary, volcanic activity was mainly located westward in the arc region. Stratovolcanoes are made of calc-alkaline andesites to rhyolites suites but dispersed basaltic volcanic centres are present in the Puyuhuapi, Isla Colorada and Murta regions. The contrasted geochemical signatures of these basalts underline the complexity of the geodynamic context and the diversity of the sources.

Selected publications

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**MODELLING WAVES OF THE MEXICAN SEAS, USING ERS-1 WIND AND WAVE
DATA TO RUN A THIRD-GENERATION WAVE MODEL**

**Co-ordinator: University of Southampton, Southampton, United Kingdom,
(I.S. Robinson)**

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ANDEAN TECTONICS OF ARGENTINA

Co-ordinator: Université de Rennes I, Géosciences, France (Peter Cobbold)

Objectives

The general objectives are to study the distribution of Cenozoic stresses and deformation at the scale of continental South America, by collecting new data in Argentina, analyzing them and comparing them with experimental and numerical models.

Activities

New data have been collected in the Andes of Argentina. Mapping of tectonic structures and sedimentary basins has been of prime importance. Laboratory studies have included analysis of satellite imagery, fission track dating, paleomagnetic measurement of block rotations, interpretation of seismic reflection profiles, interpretation of gravity data and small-scale physical modeling.

Results

For the Central Andes (northwestern Argentina to southern Peru), the paleomagnetic database now demonstrates that its arcuate shape is due to oroclinal bending, in operation since the late Cretaceous (100 million years). Fission track ages reflect a history of continuous uplift and exhumation over the same period. Across the high plateau of northwestern Argentina, deep seismic reflection profiles show alternating Paleozoic ranges and Cenozoic sedimentary basins, separated by thrust faults. Differential rotations of these crustal blocks have been taken up by scissor-like motions on the thrust faults. In the Southern Andes, the Patagonian orocline formed in response to strike-slip motions at the plate boundary between South America and Scotia. We have identified a system of Neogene rift valleys, trending NE-SW along the Magellan Strait. Structures in the northern Andes of Colombia and Venezuela reflect strikeslip motions along the Caribbean boundary of South America. The overall pattern of Cenozoic deformation, including the oroclines of the Central Andes and Patagonia, has been reproduced in small-scaled physical models. The models show that reactivation of pre-existing crustal weaknesses, such as the Paleozoic foreland basin of the Central Andes, leads to an irregular plate margin, so accounting for the sinuous shapes of the subduction zone and of the Andes themselves

Follow-up

Ongoing cooperation with the petroleum industry in Argentina is leading to new insights into the development of sedimentary basins and hydrocarbon traps.

Selected publications

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GEOLOGICAL STUDY OF ANDEAN SALINE BASINS: CHILE (17-27°S Lat)

Co-ordinator: The University of Birmingham, Birmingham, UK (Peter Turner)

Objectives

To establish detailed geological and geochemical knowledge of saline basins in the Andes of northern Chile. Three basins, namely the Salar Grande and Salar de Llamara (Coastal Cordillera) and Salar de Surire (High Andes) were chosen for study since these represent end members of all the evaporitic basins in the Chilean Andes and provide insights into the evolution of Andean brines and salars through geological time. The ultimate objective is to determine the relative influence on tectonics, volcanism and microclimatic variation on the evolution of these saline basins.

Activities

The project has proceeded by collaboration of a team comprising partners in Antofagasta, Chile (Chong), Barcelona (Pueyo) and Birmingham (Turner). A key aspect of the work has been fieldwork in remote, hyperarid parts of the Atacama Desert, some at high altitude (Surire is at 4,200m alt.). Chong has coordinated this activity and the sampling of evaporitic material and brines. The geochemical work has been done mainly at Barcelona and Birmingham have coordinated petrological and geological activity.

Results so far

Work on the Salar Grande-Salar de Llamara system is virtually complete. The results indicate that the Salar grande is composed entirely of very pure halite (>95%) with minor sulphates (glauberite, thenardite and polyhalite) and terrigenous components. Vertical variations in trace element composition reflects how brine content decrease upwards and terrigenous input increases reflecting increased aridity through time. The original source of the brines were the High Andes to the east. These groundwaters were enriched by water-volcanic rock interaction and drained into the Central Depression. Subsequent supply to the Salar Grande was through structural pathways including E-W neotectonic faults. Work on Surire shows the importance of borates, boric acid, native sulphur and bisulphates in the brines of the High Andean salars. The results of mineralogical and chemical analysis indicates the importance of volcanism as the source of these brines.

Follow-up

During the final work of the project attention will be paid to completing the analytical work and discussing the significance of the results. In particular we aim to develop a general model for Andean brine evolution which will address the original objective of determining the relative importance of volcanicity, microclimate and tectonic activity in the evolution of the Chilean Salars

Selected publications

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Contract number: CII*CT940075

Period: January 1995 to December 1997

GEOCHEMISTRY AND ALTERATION PATTERNS OF THE OUTFLOW AND PERIPHERAL ZONES OF A FOSSIL HYDROTHERMAL SYSTEM

Co-ordinator: Universitat de Barcelona, Barcelona, Spain (Àngels Canals)

Objectives

- ◆ The general goal is to investigate the origin and evolution of an Ag-bearing epithermal mineralization (La Guitarra deposit, Temascaltepec (Mexico)) in order to evaluate its economic possibilities and to find prospection tools useful to investigate other geologically similar areas.
- ◆ A detailed study of the geothermal system of La Primavera (Guadalajara, Jalisco) is also carried out to compare the geochemical characteristics of the fluids and of the alteration zones in both systems.

Activities

- ★ The Spanish team (UB and UAB partners) has focused its work on the La Guitarra (Temascaltepec, Mexico) Ag-Au deposit, while the Mexican team has undertaken its main work in the La Primavera geothermal field.
- ★ During the first year of the project the work was focussed on the geologic field work and sampling in both areas. The second year was devoted to laboratory work: fluid inclusion and stable isotope analysis (UB and UAB), and lithochemical analysis (UNAM).

Results so far

- ⇒ La Guitarra is a polymetallic and multi-stage, low-sulfidation epithermal vein system. The Ag-Au ore is mainly displayed in silica bands. Stage-I fluids show salinities from 5 to 12 wt. % NaCl eq.; T from 162 to 212• C; $\delta^{18}\text{O}_w$ from +1.2 to +5.2‰; X_{FeS} in sphalerite from 0 to 25%. Stage-II (Ag-bearing stage): salinity from 4 to 6 wt. % NaCl eq.; T from 161 to 210• C; $\delta^{18}\text{O}_w$ from -2.0 to +6.0‰; X_{FeS} in sphalerite from 0 to 16%. Time-space distribution of the above variables convey to magmatic and meteoric fluid recognition. Coupled with mineralogical evidences of boiling, those characteristics point to a mixing-boiling model for ore deposition.
- ⇒ In La Primavera, geothermal field major elements concentrations show that thermal fluid leaches a significant percentage of the main cations, and the enrichment shown by altered surface samples in iron, magnesium and titanium may be a result of this leaching. Deep samples show an enrichment in barium, cobalt and strontium with respect to shallow samples, and in all trace elements except rubidium with respect to surface samples. Only one surface sample presents enrichment in Cu, Cr, Ni and Co with respect to deep samples, the same

sample has the largest enrichment with respect to unaltered samples in Ti, Fe, and Mg. This sample comes from a fumarola vent where sulphur deposition takes place.

Selected publications

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- Camprubí A., Canals À., Cardellach E. & Prol-Ledesma R.M. 1997b. Características del sistema epitermal Ag-Au La Guitarra (Temascaltepec, estado de México). Mem. XXII Conv. Nal. AIMMG. 15 p. (In Spanish)
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COTCOR - COMPARATIVE GEOPHYSICAL TRANSECT ACROSS COSTA RICA

Co-ordinator: GEOMAR, Research Center for Marine Geosciences, Kiel, Germany
(Ernst R Flueh)

Objectives

The goal of this project was to determine the deep crustal structure of the Middle American isthmus in Costa Rica. Special emphasis was put on the influence of morphologically different segments of the oceanic Cocos Ridge subducting underneath Costa Rica. These are reflected in the seismicity and volcanic activity. Other aims were to investigate the lateral extension of the ophiolites within the forearc that can be found onshore.

Activities

- ★ In March 1996, two wide angle transects were acquired in North and South Costa Rica, completing the transects begun by the onshore-offshore TICOSECT project in 1995. The first profile starts at the Middle America Trench (MAT), the Pacific coast close to Marbella, crosses the Nicoya Peninsula and extends to the Nicaraguan border, close to Los Chiles. It was occupied by 120 PDAS landstations, which recorded 8 explosive shots fired at five different locations in drillholes with a maximum depth of 46 m.
- ★ The southern transect begins at the Pacific coast (Dominical) near the Osa Peninsula, crosses the Terraba Basin, the Talamanca Cordillera and extends to the Limon Basin at the Atlantic (Caribbean) coast. With 60 landstations recording 5 shots in 10 drillholes at depths down to 35m, the first complete wide angle transect across the Middle American isthmus was completed.

Results so far

The southern profile shows an anomalously thick oceanic crust (max. 12 km, velocities from 6 to 7.2 km/s) on the northern flank of the Cocos Ridge, subducting under Southern Costa Rica with an angle of app. 12 °. The landbridge itself shows no strong lateral velocity variations, but the average velocity is above 6.3 km/s, indicating an oceanic origin. On the Pacific coast, a high velocity wedge reaches into the shelf, indicating that rocks building up the landbridge extend close to the trench. In front of the wedge, a small prism with velocities around 3 and 4 km/s is interpreted as accreted material, mainly from the shelf, and the image of the high velocity wedge shows structures that may indicate tectonic erosion. A thick, low velocity zone (max. 8 km) occurs at the boundary between the subducting slab and the high velocity backstop. It may be composed of subducted sediment and wedge material eroded from the frontal part of the wedge. The Terraba basin could be modeled with velocities of 4.2 km/s to a depth of app. 4 km. The basin of Limon shows a sediment cover with velocities of 2.2 km/s to a depth of 2 km, followed by a layer of up to 4.5 km/s velocity, reaching 6 km depth.

Follow-up

The geologic interpretation of the calculated velocity models is still under way, since the comparison with the TICOSECT datasets is not yet finished. Follow up projects could extend the southern profile on- and offshore from the Limon area on to the Caribbean crust to image the eastward part of the Middle American isthmus, which is not yet covered by seismic wide angle data. They will also contribute to an improved definition of centers of geologic activity.

Selected publications

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**GENESIS OF EMERALD DEPOSITS IN THE EASTERN CORDILLERA OF
COLOMBIA: STRUCTURAL GEOLOGY, GEOCHEMISTRY AND
ENVIRONMENTAL IMPACT OF MINING**

Co-ordinator: CNRS - Centre de Recherches Pétrographiques et Géochimiques, Vandoeuvre-
lès Nancy, France (Alain Cheilletz)

Objectives

Unlike the other emerald deposits in the world, the Colombian deposits show no connection with magmatic phenomena. This observation has led to much confusion about the genetic model of these unique mineralizations. Moreover, the intense exploitation of these deposits since pre-Colombian times raises the problem of the renewal of these reserves, the discovery of new deposits, and of mining effects on the environment.

Activities

The project was based on geological and geochemical studies of the Colombian deposits in order to identify the principal concentration factors for beryllium and for the emerald deposits. Geological mapping of the principal emerald mines of the eastern cordillera (Coscuéz, Muzo and Chivor districts) was carried out with MINERALCO S.A. and the logistic support of the principal local mining firms. The integration of results on the mines at the level of the eastern Cordillera was carried out using cuts and regional geological maps supported by micropaleontological and radiometric (K-Ar et $40\text{Ar}/39\text{Ar}$) dating. The geochemical study of fluids (P/T/X parameters by fluid inclusion, Cl-Br-I geochemistry, O-S-C isotopic geochemistry, rare gases and fluids in emerald channels) and of black shales hosting the mineralizations (major and trace elements) were carried out with ORSTOM.

Results

An original model of the formation of the Colombian deposits has been developed, definitively discarding the hypothesis of a magmatic origin of emerald. Precipitation of emeralds and accompanying minerals (essentially pyrites and carbonates) is controlled by a thermochemical reduction of sulphate (TRS) following the interaction of high-temperature ($\sim 300^\circ\text{C}$) alkaline brines with the organic matter contained in the black shales. This reaction reduces the sulphates of evaporitic origin and oxidises the organic matter. It is generated following tectonic movements at a continental level during two episodes characterized by shearing of the sedimentary series at the level of the evaporites in turn at the Cretaceous-Tertiary boundary (65 Ma) and the Eocene-Oligocene (38-33 Ma.) boundary. In the deposits, the principal mineralization site has been identified: it is hydrothermal tectonic breccia limiting the tectonic layers formed during the deformation of the sedimentary series. This important discovery will represent a sure guide to miners for the discovery of new mineralizations at the local and regional scales.

Follow-up

The scientific results of the project will be presented in the First World Congress on Emeralds to be held in Bogotá in early 1998. Apart from the numerous scientific publications and doctoral theses available or in preparation, the results are already being put to practical use at the Chivor deposit.

Selected publications

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THREE-DIMENSIONAL MODELING OF THE NORTHERN GULF OF CALIFORNIA

Co-ordinator : Istituto per lo Studio della Dinamica delle Grandi Masse, Venice, Italy
(Andrea Bergamasco)

Objectives

The general objective is to get further understanding of the mechanism that controls the seasonal circulation of the Northern Gulf of California (NGC), using three-dimensional modeling to complement a national observational, *in situ* programme.

Activities

The project focuses on increasing the modeling effort on termohaline and wind-driven circulation, in particular during the winter season when interesting water-mass formation events take place. A numerical model was set up and, using a data set of *in situ* observations acquired in the national programme, many simulations were performed.

Results

Numerical simulations were performed with realistic bathymetry, initial hydrographic conditions and realistic wind, heat flux and evaporative forcing.

- ⇒ Water-mass formation experiments: The results showed that water from extensive shallow regions of NGC increases in density as a result of cooling and evaporation. Most of the sinking occurs during the relaxation period, after the strong cooling and evaporation events have ceased. This water sinks to an intermediate depth of about 75 m, then moves towards northwest where it encounters larger slopes and curvature of the isobaths. In the southern part it sinks further depth of about 120 m and moves towards southeast.
- ⇒ Winter circulation experiments: The seasonal winter circulation was studied by three numerical experiments using realistic bathymetry and hydrography but under very simple forcing conditions.
 - In the first experiment, horizontally uniform temperature and salinity fields were used, and the basin was forced with a constant along-gulf wind from N-W. Results show a large anticyclonic circulation that flows to the southeast and then returns towards northwest.
 - The second experiment consisted of a simple geostrophic adjustment with no forcing, starting from rest and using winter hydrographic initial conditions. Again, the circulation shows a broad anticyclonic gyre covering the northern part, but there is also a smaller gyre embedded within the larger scale cyclonic circulation.
 - In the last experiment we combined the initial conditions of the previous one with a sinusoidal wind of annual frequency, Results are similar to those from the second experiment. The fission of the core of the anticyclonic circulation forming two eddies

is evident and could be related to a baroclinic instability. The eddy appears to have an important barotropic structure and becomes more localized and robust with depth.

Follow up

Comparison with the general circulation of a European semi-enclosed sea (the Adriatic sea, for example) could be carried out, in particular, focusing on process study as deep water or dense water formation and spreading.

Selected publications

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**PILOT PROJECT FOR REGIONAL EARTHQUAKE MONITORING AND
SEISMIC HAZARD ASSESSMENT IN THE ANDEAN REGION**

Co-ordinator: Swiss Federal Institute of Technology, Zurich, Switzerland
(Domenico Giardini)

Objectives

- ◆ Improvement of monitoring capabilities and seismic hazard assessment as building blocks for the implementation of efficient risk-mitigation strategies in areas prone to significant earthquake disasters
- ◆ Use of modern technologies for earthquake surveillance.
- ◆ Development of efficient procedures for location and quantification of regional earthquakes, using analog and digital data for the implementation of rapid warning systems, in Europe and in the Andean region.
- ◆ Implementation of procedures for rapid data exchange and for building up a regionally distributed databank for parametric and digital seismic data.
- ◆ Compilation of a uniform, regional catalogue of earthquakes, a unified model of seismic source zones and a regional map of seismic hazard.

Activities

- * Determination of the location and magnitude of earthquakes by exchanging parametric data from seismic stations in bordering countries, as well as by using the waveform data from broad-band stations.
- * Seismic hazard assessment.

Results

- ⇒ Development of protocols and software for rapid retrieval and exchange of data from digital seismographic stations and national communication nodes
- ⇒ Drawing up of procedures for the quasi real-time, automatic determination, from regional telemetered waveforms, of source parameters for significant earthquakes (moment tensor, seismic moment, source extension and directivity)
- ⇒ Drawing up of procedures for the integration of parametric data collected by standard analog, short-period arrays, and by modern digital networks for implementation in national and regional activities of earthquake monitoring and in integrated national databanks.
- ⇒ Compilation of geophysical and geological databases required by probabilistic seismic hazard assessment

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Contract number: CII*CT940104

Period: April 1995 to March 1998

ASSESSMENT OF SEISMIC HAZARD IN EL SALVADOR

Co-ordinator: Universidad Complutense de Madrid. Spain (A. Udías)

Objectives

The objective of this project was to carry out an assessment of seismic hazard in El Salvador. This included a compilation of seismic data from the past, information of damage from recent and historical earthquakes, strong motion records, list of earthquakes etc.; installation of a network of digital strong motion instruments near San Salvador, preparation of seismicity and seismic risk maps, training of local personnel in seismic data process and preparation of graduate courses in Engineering Seismology.

Activities and Results

- ⇒ All earthquakes in the region Central America after 1894, many of them not known from other sources, have been identified and their locations have been determined. An earthquake catalogue for El Salvador and surrounding areas has been compiled at based on the regional catalogues of ISC, NGDC, NEIC and a number of special studies, including those that have arisen from this project. Historical seismicity starting from the time of the Spanish Colonial administration to 1894 is being studied. A search from contemporary documents at the Archivo de Indias, Sevilla has been studied including documents for the earthquakes of 1576, 1594, 1651, 1659, 1671, 1719 and 1798.
- ⇒ A special study has been made of the earthquakes series of 6 May 1951 that caused very heavy damage and more than 400 casualties in the region of Jucuapa. An important part of the studies of earthquakes of a region is that of their source mechanism. Source mechanism was obtained from seismograms for the shocks of May 5, 1965, October 10, 1986, June 19, 1982 and July 22, 1996. Results are consistent with tectonics of the region.
- ⇒ A new digital accelerograph network of 10 stations was established in El Salvador during the first year of the project. Until March 1997, a total of 9 earthquakes have been recorded producing total of 27 accelerograms. Work on the compilation and cataloguing of a data base of several hundred strong motion record from Central America has been done. A list of more than 1000 reports of strong motion records in Central America have been obtained from a number of sources.
- ⇒ An important component of the project was the regional seminar in San Salvador from 22 to 29 September 1997, under the title "Assessment and Mitigation of Earthquake Risk in the Central America Area". Papers were presented about current research in seismic risk in all countries. Proceedings will be published. Progress has been made in the establishment of a group of well trained personnel in seismology and earthquake engineering in order to give a continuity to the program after the support of this project is finished and in the preparation of graduate courses in Earthquake Engineering, at the Universidad Centro Americana, San Salvador.

Selected publications

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THE SEISMIC CYCLE IN SOUTHERN CHILE : EVOLUTION AND MONITORING

Co-ordinator: Institut de Physique du Globe de Paris, Paris, France (Raul Madariaga)

Objectives

To study the seismicity of the south-central Chile area between Constitución and Concepción, which is a region that has a very high seismic potential, and to determine whether it is currently an active seismic zone.

Activities

The main was a field investigation of the seismicity of the Constitución Concepción area that lasted from March to May 1996. During this period, 25 seismic stations of European origin (mostly French) were deployed and maintained in the field by a multinational team of more than Chilean, French and Italian researchers who continuously maintained and read the digital data produced. An area of 200x100 km stretching from the Maule River in the north, down to the latitude of Concepción was covered. In a second part of the project, a network of GPS sites (satellite geodesy) was set up in the same area and extended down to the BioBio river south of Concepcion. This network was resurveyed in 1997 and will be covered again in the near future. Although this experiment was of short duration and the team would have liked to do another field experiment they think that they identified the main features of the seismicity of the area.

Results

Local earthquakes were found to occur inside the downgoing slab of the Nazca plate all the way from the Chile trench (150 km offshore) to about 150 km under the Chile Argentina border. 90% of all recorded events took place inside the slab or on its upper boundary at the contact with the South American plate. There is practically no crustal activity, except for a small nest of earthquakes clearly associated with 2 major volcanoes in the area, and a few, very strange local events that took place on the border of the Chile central valley. As far as is known, this is one the rare places in Chile where seismic activity occurs on the western end of the central valley and is a subject that the team would like to pursue in the future. More than 400 events were carefully located; for about 50 of them, the team determined fault plane solutions of excellent quality. The region under Chillan at the southern end of the study zone was the site of a very large and destructive earthquake that claimed more than 15,000 lives in 1939. The team is convinced that this event took place inside the slab at a depth of about 90 km under the central valley, and not offshore at shallow depths as most large Chilean earthquakes do. If this is the case, then this region is a seismic gap, a serious candidate for a future large earthquake in south central Chile. In any case this region should be carefully surveyed.

Follow-up

The most important follow-up will be the permanent deployment of a set of broad-band seismic stations in the area. The Geology Department of the Universidad de Concepción as well as the new office of the Chilean mine and geological survey (SERNAGEOMIN) in Temuco, will collaborate in future work in the area. These activities are essential in order to survey the seismicity of the area, and search for any possible precursors, or increase in seismic activity that could be a prelude to a larger earthquake like that of 1835.

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GEOPHYSICAL STUDY OF THE SOUTHERNMOST CHILEAN MARGIN

Co-ordinator: CSIC, Barcelona, Spain (M. Torné)

Objectives

The general and long-term goal of the project was the study of the present-day lithospheric structure and style of subduction of the southernmost Chilean Margin between 52°S and 57°S, approximately between the Pacific end of the Strait of Magellan and Cape Horn. Within this project, this was achieved through the processing and interpretation of marine geophysical data (multichannel seismics and gravity), and the integration of these results with the current geological knowledge of the area.

Activities

The area of study lies in a region of active subduction where an approximately 15 million year-old piece of the Antarctic plate is being subducted beneath the Scotia plate at a rate of about 13 mm/yr, and where the convergence direction with respect to the continental margin varies from normal (obliquity = 0) at 52°S, to highly oblique (obliquity > 60) at 57°S. Thus the Chilean convergent margin south of the strait of Magellan represents an excellent opportunity to study an end-member style of subduction where obliquity plays an important role. To understand this margin we have carried out: a) Processing and interpretation of marine multichannel seismic lines, including waveform modelling of the Bottom Simulating Reflector (BSR) and pre-stack depth migration of the most representative seismic profiles. b) Compilation and modelling (2D and 3D) of marine and satellite gravity data, and c) Tectonic interpretation considering the geology of the area and the constraints provided by geophysics.

Results so far

- ⇒ The analysis of seismic data has allowed us to identify unambiguously the major structures of the margin: Sediment filled trench, accretionary prism, forearc basin and continental slope and shelf.
- ⇒ Gravity modelling shows that there are differences in sediment densities across the margin. There is a relatively dense accretionary prism (2.17 gr/cc), flanked by lighter sediments (1.75 gr/cc) in the trench and forearc basin. The shape of the subducting slab has been also constrained by this modelling, particularly in the continental platform area where the seismic data is not conclusive. The slab geometry shows two distinct segments: A shallow dipping (~ 3°) western flank until the back stop front of the accretionary complex, followed by a much steeper (~ 23°) eastern slab.
- ⇒ The analysis of the gas hydrate Bottom Simulating Reflector (BSR) seen in the accretionary prism section of the multichannel profiles, has allowed us to estimate a heat flow of approximately 60 mW/m², which is about half of the flow supplied by the underlying subducting oceanic crust. This discrepancy is likely to be related to the blanketing of the accreted sediments and to the fact that part of the heat is advected by fluids within the prism. Waveform modelling has yielded a detailed velocity-depth model in the vicinity of the BSR which can be used to estimate the concentration of gas (methane) within the sediments.
- ⇒ We propose a preliminary tectonic evolutionary model to explain the present morphology along the southernmost Chilean Margin. In this model, deformation within the Scotia plate can be understood as a complex shear zone controlled by tectonic forces driving the Antarctic and South American plates. The model incorporates not only the Magellan fault along the Northwest Strait

of Magellan but also secondary faults that can be traced along some fjords in Tierra del Fuego. This left lateral strike slip fault system conforms a “horse tail” with a master fault along the Strait of Magellan, and a series of counterclockwise rotating blocks.

Follow-up

During the last stage of the project we will concentrate on the synthesis and publication of the results. We want to analyse the applicability of current mechanical models of accretionary wedges with special emphasis to include oblique convergence as an independent parameter. We also want to explore the possibility of continuing cooperation between Chile and Spain to study the southernmost Chilean margin. We think that a multibeam bathymetric study would be the most effective way of achieving this goal in terms of time and cost. High resolution bathymetry in conjunction with the available geophysical and geological information, could provide a far more complete picture of the geotectonic configuration of the subduction complex.

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PALAEOMAGNETIC CONSTRAINTS AND STRUCTURAL BLOCK ROTATIONS IN OROGENIC ZONES IN MEXICO AND SPAIN

Co-ordinator: Universidad Complutense de Madrid (Maria Luisa Osete)

Objectives

- ◆ The main goal of this project was to quantify structural block rotations in different geodynamic situations in Mexico and Spain : 1) Continental-oceanic convergence (Mexico), 2) intraplate deformation (Mexico-central Spain) and 3) Continental-convergence.
- ◆ The final objective was to determine which are the parameters that control the rotational deformation of the upper continental crust.

Activities

To contrast and quantify block rotations five different areas have been selected :

1. The Trans-Mexican Volcanic Belt (TMVB) (Mexico).
 2. The Sierra Madre Oriental (Mexico).
 3. The Iberian Ranges (Spain).
 4. The Central System-Plasencia Dyke (Spain).
 5. The Betic Cordillera.
- * Palaeomagnetic surveys have been carried out in these regions in collaboration between the palaeomagnetic groups of Madrid, Mexico, Barcelona and Plymouth. Several (15) field work campaigns were carried out in these regions for sampling rocks suitable for palaeomagnetic studies. Sampled rocks were distributed among the palaeomagnetic groups for laboratory experiments. Magnetic results obtained from different laboratories were compared and discussed. As a result of this comparison magnetic measurements from the the Mexico laboratory have been correctly calibrated.
 - * In addition, several stays of doctoral and post-doctoral students between different laboratories have been partially financed by this project. These stays have contributed to the training of young scientists.

Results

- ⇒ Palaeomagnetic (231 samples analyzed) and radiometric results from the western end of the Mexico basin (Sierra de la Cruces) have revealed southern (SSE) migration of the volcanic activity. The rate of the migration seems to be according to the Cocos plate movement.
- ⇒ A total of 282 cores from 26 sites were sampled in the eastern part of the TMVB. Results indicate a small divergence between palaeomagnetic declination and the expected direction. Block rotations if they exist are of about 10° (or smaller) from the Pliocene to the present.
- ⇒ Palaeomagnetic analyses of 20 sites (214 samples) of Jurassic and Cretaceous age from the southern part of the Sierra Madre Oriental indicate counterclockwise rotations of about

20-30° with respect to stable North America. In addition a widespread remagnetization has been evidenced which could be located at the end of the Laramide Orogeny. The observed rotations took place after the remagnetization event.

- ⇒ The palaeomagnetic investigations carried out on the Peridotites about (120 samples) from the Internal part of the Betics indicate the existence of systematic and large clockwise rotations (of about 60°). Similar to the rotations observed in the Jurassic limestones from the External subbetics. Therefore strong rotational deformation has affected this region since the Neogene to the present.
- ⇒ A palaeomagnetic profile has been performed in the Central part of the Subbetics. Results indicate that the northernmost area has not been rotated (north to Carcabuey) while sites located to the south exhibit about 30-40° of dextral rotation.
- ⇒ Tertiary sediments from the Ebro basin have not been affected by rotations. In addition results from Triassic redbeds from the western part of the Iberian Ranges do not reflect significant rotations with respect to stable Iberia.
- ⇒ A directional discrepancy has been observed between the southern and northern sector of the Plasencia-Dike. The anomaly reflects differential tilts which could be related to the deformation of the Central System. A north-south compression without significant shear component seems to have affected this region since the Jurassic with respect to stable Iberia.

Follow-up

- Field work and laboratory analyses have ended. Now we are interpreting the Jurassic palaeomagnetic results from the Iberian Ranges (about 500 samples) where block rotations seem that have not played a significant role in the geological history of this Cordillera. Only locally directional anomalies have been found. Palaeomagnetic data obtained from the northern part of the Sierra Madre Oriental are under interpretation.
- A final meeting for discussion of latest palaeomagnetic results will be celebrated in Spain next 18th May.

Selected publications

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Period: May 1995 to April 1998

ICE AND MAGMA INTERACTION PROCESSES

Co-ordinator: CEMAGREF-Grenoble, St. Martin d'Hères, France (François Valla)

Objectives

- ◆ Improvement of the knowledge of ice-magma interactions.
- ◆ Collection of glaciological data on the Nevado.
- ◆ Specific equipment supply for Colombian teams.
- ◆ Technical and scientific training for Colombian scientists.

Activities

- * Annual field campaign of radar ice thickness measurement.
- * Morphological measurements and studies on the eruption deposits.
- * Making of technical equipment (snow sampler, ice radar and vapor drilling system).
- * Aerial survey for ice surface evolution mapping.

Results

- ⇒ Determination of the glacier bed-rock hiding the summit of Nevado del Ruiz.
- ⇒ Publication of the ice thickness map.
- ⇒ Colombian PhD in mathematical modelling of ice-heat interaction.
- ⇒ Summer school for young Latin-American scientists in glaciology (Manizales, 1999).

Follow-up

- Association of Russian specialists in the volcano-glacier field.
- Cycle of lectures in mathematical modelling for Colombian students.
- Conclusive symposium with partner, open to the South American scientific community (Bolivia, Ecuador, Argentina, Chile, Peru).
- Colombian-French glacier field campaign.
- Exchange between the most technical specialists (France, Colombia, Switzerland and Russia). Attempt to find new support for this useful and asked collaboration.

Selected publications

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Period: February 1995 to June 1997

MODELLING OF WATER AND SOLUTE TRANSPORT (RECHARGE AND POLLUTION) IN VOLCANIC AND SEDIMENTARY DEPOSITS UNDERLYING GUATEMALA CITY

Co-ordinator: Agence Nationale pour la Gestion des Déchets Radioactifs, Orléans, France
(Scott Altmann)

Objectives

The Guatemala City urban and industrial development is located above the aquifer that supplies its drinking water. Approximately 50 to 200 metres of unsaturated volcanic and sedimentary geologic formations separate the ground surface from the aquifer layer. The overall objective of the project was to demonstrate, through technology transfer, how a comprehensive research program involving field studies, laboratory measurements and numerical modelling could be used to evaluate the risk of aquifer pollution by trace metals transported by infiltrating ground water.

Activities

The main activities carried out jointly by the Guatemala (INSIVUMEH, EMPAGUA) and French (BRGM, Paris University XI) teams were: geological mapping and characterisation of the lithological sequences at the study site; detailed identification of the minerals present in the different strata; grain size distribution of the sediments; determination of surface and ground water geochemical and stable isotope (^{18}O , ^2H , ^{13}C) composition to determine mixing and losses; determination of the characteristic laws describing the unsaturated flow behaviour (suction vs. humidity, permeability vs. humidity) of one of the principal sedimentary layers (Diamicton); laboratory determination of the capacity of various horizon sediments (Diamicton R, in particular) to adsorb a model trace metal ion (Cd^{2+}) under differing chemical conditions (pH, total calcium concentration); development of a numerical model for estimating the rate of water and solute (Cd^{2+} in particular) transport from the ground surface toward the aquifer (including hydrodynamic and adsorption data for the other principal formations obtained in a previous study (Muñoz, 1995)).

Results

While other sedimentary layers were also characterised, this study focused principally on the diamicton R containing layer selected for installation of the unsaturated zone hydraulic parametre measurement station "Ojo de Agua". This formation is the most important in terms of overall thickness (many tens of metres) in the unsaturated zone underlying Guatemala City. The rock contains mostly plagioclase and amphiboles with trace amounts of illite and smectite, the latter mineral probably being responsible, along with trace iron oxides, for the significant capacity of this material to adsorb trace amounts of Cd at near neutral pH values ($K_d < 150$ ml/g). The K_d value decreases rapidly with decreasing pH, as well as with increasing total dissolved calcium concentration (for the same pH). A 2-dimensional, multi-

layer numerical ground and soil water model was used to represent the hydrodynamic behaviour of the various formations making up the unsaturated zone. Model parameters for the diamicton layer were determined by fitting the experimental data obtained at the test station specifically constructed during this project. The model was then used to calculate estimated transport through the unsaturated zone of solutes released at the ground surface with average annual climatic conditions over long periods of time (up to 10,000 years). Concentration profiles of a conservative tracer (i.e. $K_d = 0$), with a constant imposed concentration at the ground surface of 10 mg/l, showed a 1 mg/l break-through at the aquifer level (160 metres below ground surface) after only 50 years. With the same simulation conditions, but using the K_d values measured for cadmium for each of the different geological formations, the cadmium 1 mg/l break-through requires more than 10,000 years. This “de-contaminating” effect is due to the adsorption of the toxic metal on the minerals making up the different horizons. This very beneficial process can help protect the aquifer from pollution input of cationic metal ions as long as the sorption capacity of the rock is not exceeded and pH values remain near neutral. The observed pH dependency of K_d for these ions shows however that a subsequent decrease in pH would cause release of sorbed metals and movement through the formation.

Follow up

Potential follow-up actions that could be undertaken include carrying out tracer tests under laboratory and controlled field conditions to test model predictions of transport of different pollutants. The “Ojo de Agua” test station could be adapted to this purpose.

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Environmental Sciences

EFFECTS OF WASTEWATER REUSE ON URBAN GROUNDWATER RESOURCES

Co-ordinator: British Geological Survey, Wallingford, United Kingdom (John Chilton)

Objectives

The objective was to determine the effects of wastewater reuse for irrigation on groundwater resources under conditions typical of semiarid regions, and where the underlying groundwater was being used for municipal supply. This overall objective included the development of methods and strengthen the capabilities of partners for investigating these impacts. The city of León in Mexico was chosen for the study, which was co-funded by the UK ODA.

Activities

After collection and review of existing data, regular measurements of groundwater levels and sampling of both deep and shallow groundwater was established. The electromagnetic (EM) geophysical method was used to estimate the depth and lateral penetration of poor quality water. Soil profiling and the drilling of deep cored boreholes completed as multiple-level piezometers were used to study subsurface hydrochemical processes and to examine the fate and behaviour of the contaminants from the wastewater and to examine the vertical distribution of water quality. A simple mixing cell model was developed to evaluate strategies for improvement of wastewater irrigation to maintain groundwater quality.

Results

- ⇒ León currently has a population of about 1.2 million. A volume of some 2600 l/s of wastewater from the city is used to irrigate 3000 ha close to the city. Because the city is the centre of Mexico's leather industry, the industrial component of the wastewater is highly polluting. As a result of rapid growth of groundwater usage in the valley of León for agriculture, potable supply and industry, groundwater abstraction has been about double the recharge for the last 20 years. Groundwater levels in the area of greatest abstraction are falling by up to 5 m/yr, with a total decline of some 90 m between 1959 and 1995. The need for improved management of these vital groundwater resources is urgent. In contrast, within the area of wastewater irrigation, levels are within 5-10 m of the ground surface and stable with time; and infiltration from the wastewater has been an important source of recharge.
- ⇒ As a result, poor-quality groundwater has penetrated to significant depths, as confirmed by the geophysics and drilling. Public supply boreholes located within the wastewater area showed steep rises in chloride concentration during the project, suggesting that salinity was likely to be the most mobile and persistent of the contaminants in the wastewater. In contrast, nitrate, organic carbon loading, heavy metals and bacteria were observed to be much less likely to reach the deeper groundwater. The metals, including chromium from the leather industry, were held in the soils and storage lagoons of the distribution system. The modelling showed that, under the present irrigation regime, groundwater quality in the aquifer would continue to deteriorate with respect to salinity for many years to come. Various management scenarios were modelled, including separate collection and treatment of the most-polluting industrial effluents and increasing abstraction from the upper part of the aquifer sequence. Both of these could have significant benefits in terms of quality of the underlying groundwater.

Follow-up

- In order to disseminate the results of the project to interested professionals within Mexico, a national seminar on this and other BGS/CNA projects in Mexico was held at Queretaro in March 1996. This was combined with one of a series of regular courses on groundwater protection organised by BGS in the Latin American region. 73 national professionals attended the course, and an additional 51 the one day seminar on project methods and results.
- The recommendations made in the final report concerning the need for monitoring have been implemented by the León municipal water undertaking, and the results have been sent on to BGS for continuing review and advice.

Selected publications

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**DETERMINATION OF ATMOSPHERE METHANOL AND ETHANOL DERIVED
FROM THE USE OF ALCOHOL AS VEHICLE FUEL**

Co-ordinator : Institut für Spektrochemie und Angewandte Spektroskopie (ISAS),
Dortmund, Germany (Dieter Klockow)

Objectives

The goal of this project was the development of a methodology for the quantitative determination of methanol and ethanol in ambient air in the ppbv range in different locations in Brazil.

Activities

In the first phase of this project, different methods were tested and optimized for the determination of methanol and ethanol in aqueous solutions. The basis of all of them is the enzymatic oxidation of the alcohols. ISAS, Dortmund, investigated the luminometric detection after chromatographic separation and enzymatic conversion of methanol and ethanol, while UFBA, Salvador, tested the electrochemical detection. Based on these procedures, methodologies were developed and compared for the determination of methanol and ethanol in ambient air. For sampling, ISAS, Dortmund, used a scrubber coil, where the alcohols are transferred from air into an aqueous solution continuously. UFBA, Salvador, applied a cryo sampler, in which the alcohols are frozen out together with the atmospheric humidity. Finally, both groups tested the developed methods during field campaigns in Brazil at different locations.

Results

- ⇒ For monitoring purposes, a sampling system was constructed, which allows the continuous preconcentration of methanol and ethanol from air. The influence of gas flow rate, scrubber solution flow rate and temperature on the absorption efficiency was tested, and a device for field measurements was built. The detection limits achieved with this system are 4,6 ppbv for methanol and 17,8 ppbv for ethanol when combining it with the HPLC/enzyme (alcohol oxidase) determination method, and 2 ppbv for ethanol alone when applying the alcohol dehydrogenase/NAD method.
- ⇒ For the continuous determination of ethanol a monitor was built which consists of the scrubber coil connected directly to the ADH/NAD⁺ analytical system. The scrubber solution contains ADH and NAD⁺ and is directly transferred into a fluorescence detector behind the scrubber coil. Possible ozone interferences can be avoided by placing a KI coated filter in front of the scrubber inlet. The detection limit of this system is 1ppbv ethanol.
- ⇒ The applicability of the developed methods was demonstrated during field campaigns in Sao Paulo and Salvador/Bahia, Brazil. As expected high concentrations up to 500 ppbv for ethanol and 150 ppbv for methanol were found, dependent on the different locations and the meteorological conditions.

Publications

M. Schilling, G. Voigt, D. Klockow, M. Lourdes Botelho, T. Tavares, 1996. "Determination of Methanol and Ethanol in the Atmosphere at ppb level, derived by the use of alcohol as vehicle fuel". Presentation at "26th International Symposium on Environmental Analytical Chemistry", April, 9.-12., 1996, Wien, Austria.

M. Schilling, G. Voigt, D. Klockow. Determination of Ethanol in ambient air. (in preparation)

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Period: February 1993 to January 1996

REMOVAL OF VOLATILE ORGANIC CONTAMINANTS FROM GROUND AND WASTE WATERS BY PERVAPORATION

Co-ordinator: University of Twente, Enschede, Netherlands (Marcel Mulder)

Objectives

The aim of the project was to develop capillary composite membranes that were used for a transverse flow module. This new module concept gives an improved mass transfer which is beneficial in the removal of volatile organics from water.

Activities

The activities were clearly divided among the two partners : the Federal University of Rio de Janeiro and the University of Twente. The membrane development was focused on Twente. Here hollow fibre membranes based on polyetherimide were spun. Different supports were used for a coating procedure and various coating procedures were studied. In the coating process a thin layer of a second polymer, ethylene-propylenedimer (EPDM) was deposited by means of a dip-coating technique. The second part of the project was carried out in Rio de Janeiro and aimed at developing transverse flow modules for a small pilot-plant scale system. The efficiency of the system was compared with traditional hollow fibre, spiral wound and plate-and-frame modules. It was found that the transverse flow module showed best performance.

Results

The project resulted in various achievements. First of all composite hollow fibres were developed, with very high selectivities in VOC removal. Furthermore, various transverse flow modules were constructed and this know-how can be used directly for further up-scaling in industry. Finally an economic evaluation was performed, which gives a very clear insight in the various costs of this separation problem for module concepts and competing separation systems.

Follow-up

The know-how generated in this project can directly be used for further industrial scale-up. The partners in the project are considering new possibilities and opportunities for further cooperation on a similar project.

Selected publications

Borges C.P., Futselaar H., Habert A.C., Nobrega R., Meuleman E.M.M., and Mulder M.H.V., 1994. Removal of VOC's from ground and waste water by pervaporation. Proceedings of the Seminario Internacional del Agua, Mazatlan, Mexico.

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Meuleman E.M.M., Bosch B., Mulder M.H.V. and Strathmann H., 1998. Modelling of liquid/liquid separation by pervaporation- toluene from water. Submitted to *Ind. Eng. Chem.*

Meuleman E.M.M., Willemsen J.H.A., Mulder M.H.V. and Strathmann H., 1998. EPDM as barrier material for pervaporation. Submitted to *J. Appl. Pol. Sci.*

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**STUDY OF THE COMPOSITION OF BACKGROUND AND BIOMASS BURNING
ATMOSPHERIC AEROSOLS IN THE AMAZON BASIN**

Co-ordinator: Universiteit Gent, Gent, Belgium (Willy Maenhaut)

Objectives

- ◆ Perform a physico-chemical characterization of the atmospheric aerosol in the Amazon basin,
- ◆ Identify the important aerosol sources,
- ◆ Assess the contribution of each source to the atmospheric concentration of the particulate mass and of the aerosol constituents.

Activities

Continuous long-term aerosol collections were performed at three sites in the Amazon Basin, i.e., Serra do Navio (01°N, 52°W), Alta Floresta (09°S, 56°W), and Cuiabá (16°S, 56°W). The sampling device was a stacked filter unit (SFU) sampler, which separates the aerosol in a coarse (2-10 µm) and fine (<2 µm) size fraction. Besides the long-term SFU collections, intensive campaign samplings with a wide variety of instrumentation were conducted in 1993, 1994, and especially 1995. The 1995 intensive samplings took place within the framework of the Smoke Clouds and Radiation - Brazil (SCAR-B) experiment. The samples were analyzed for the particulate mass (PM), black carbon, and over 40 elements. Source identification and apportionment were obtained by subjecting the multi-species data sets to receptor modeling.

Results

All results presented here pertain to the Alta Floresta and Cuiabá sites. At these sites there was a marked seasonality in the atmospheric concentrations of the coarse and fine PM, with elevated levels during the dry (biomass burning) season and much lower levels during the wet season. Three main types of aerosol sources were identified: biomass burning, natural biogenic emissions, and soil dust resuspension. During the dry season, biomass burning was, on average, responsible for 70% of the fine PM, while soil dust was the major contributor to the coarse PM. Biogenic aerosols were the dominant particle type during the wet season and also the main source of atmospheric phosphorus in the region. The crustal elements exhibited a unimodal coarse mode size distribution, while the pyrogenic elements showed a clear submicrometer mode. Black carbon had a submicrometer mode centered at 0.18 µm. High aerosol optical thickness values were observed in the dry season. They suggest important implications for the atmospheric radiative balance.

Follow-up

The long-term SFU collections are being continued. Besides, new intensive campaign samplings and measurements are being done, especially within the framework of the Large-Scale Biosphere-Atmosphere Experiment in Amazonia (LBA).

Selected publications

Artaxo P., Gerab F., Yamasoe M.A., and J.V. Martins. 1994. Fine mode aerosol composition in three long term atmospheric monitoring sampling stations in the Amazon Basin. *J. Geophys. Res.*, **99**, 22857-22868.

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**DEVELOPMENT AND APPLICATION OF COST-EFFECTIVE METHODS FOR
BIOLOGICAL MONITORING OF RIVERS IN COSTA RICA**

Co-ordinator: University of Ghent, Gent, Belgium (Niels De Pauw)

Objectives

The general goal was to test the applicability of several cost-effective methods for biological monitoring of rivers in Costa Rica. These included bio-assessments based on the analysis of the benthic macroinvertebrate communities and bio-assays performed by means of 'toxkits' on effluents from different domestic and industrial activities and in certain cases also on river water and river sediment (pore water) possibly contaminated by toxic pollutants.

Activities

During four years, biological and physical-chemical samples were taken in two of the major river basins in Costa Rica, the Grande de Tarcoles and the Reventazon. Sampling campaigns covered the rainy and dry seasons as well as the dry-wet and wet-dry transition periods. Sampling sites were selected in function of the river type (fast and slow flowing), altitude (mountains, plains), land use (e.g., pastures, greenhouses, production of coffee, bananas) and degree of impact from point source (domestic, agricultural, industrial) and diffuse pollution (mainly intensive agriculture).

Results

Two diversity and three biotic indices have been calculated on the collected macroinvertebrate taxa. All of these indices proved, in one or another way, to be appropriate to describe differences in biological water quality, be it that the biotic indices tested apparently underestimated the real water quality when compared to the physical-chemical data and the chemical indices. For this reason, it was attempted to modify one of the indices: the Belgian Biotic Index (BBI), and increase its sensitivity by including other indicator groups and modifying the scores of the indicator groups. However, this newly proposed Costa Rican Biotic Index (CRBI) needs to be further refined and validated in relation to reference communities of unaffected sites. It was also shown that the presence of acute toxicity of industrial effluents and sediment pore water can be demonstrated easily by means of a battery of cost-effective toxkits like the Thamnotoxkit, the Rotokit F and the Algaltoxkit. Toxic stress in river sediments could also be demonstrated by the occurrence of buccal deformities of chironomid larvae. It is assumed that these deformities are the result of contamination with heavy metals and pesticides.

Follow up

Several publications are being prepared on the results obtained so far. They cover taxonomic and systematic aspects of the benthic macroinvertebrate communities as well as the physical-chemical, biological and ecotoxicological assessments obtained for the two major river basins

in Costa Rica. It is hoped to continue the above project which laid the basis for the development of a biomonitoring programme in Costa Rica. A further exploration of better reference situations (unaffected rivers) is however needed before a workable biotic index can be proposed. The establishment of a river invertebrate prediction and classification system (RIVPACS) for Costa Rica would be the ultimate goal. Possibilities are explored to cooperate with other countries in Central America. However, the right opportunity still has to be found.

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MARINE RESOURCES AT THE BEAGLE CHANNEL PRIOR TO THE INDUSTRIAL EXPLOITATION: AN ARCHAEOLOGICAL EVALUATION.

Co-ordinator : Consejo Superior de Investigaciones Científicas, Barcelona, Spain
(Assumpció Vila Mitjà)

Objectives

The global objective of the project was to reconstruct the evolutionary dynamics of the paleoecosystems of marine resources during the last 6000/6500 years. This meant:

- ◆ Evaluate paleosystem dynamics of the Beagle-Channel marine biological communities through time by studying:
 - a) the period 6000-6500 to ± 300 years BP through the zooarchaeological record
 - b) the period ± 300 to ± 100 years BP by means of both zooarchaeological and historical data
 - c) the period ± 100 years BP to the present through historical data and biological research;
- ◆ Evaluate the abiotic factors of the past marine environment by using several indicators from the archaeological record by:
 - a) determining the paleotemperature/paleosalinity by $^{18}\text{O}/^{16}\text{O}$ analysis on *Mytilus* shells sampled from archaeological sites;
 - b) analysing the Reservoir Effect through the isotopic (^{14}C) differential ageing between absolutely synchronic charcoal and shell samples;
- ◆ Validate the results of the investigation on abiotic conditions with paleoclimatic information already obtained by pollen and/or dendrochronological analysis;
- ◆ Verify whether fluctuations of abiotic conditions are correlated to marine paleo-ecosystem dynamics or not.

Activities

- ★ The Argentinean coast of the Beagle Channel was surveyed. One hundred and thirty one archaeological sites were pit-test sampled for absolutely synchronic charcoal and *Mytilus* shells for isotopic analyses and also for other associated faunal remains. Large samples at two archaeological sites (Lanashawaia and Alashauwaia) were collected through two campaigns with 15 people during two months each. In order to have a comparative sample for isotopic analysis over the last quinquennium, inter-tidal *Mytilus* shells were collected and selected.
- ★ The north coast and islands of the Beagle Channel were surveyed to evaluate the present-day marine resources of the archaeological sites. Surveys of resource aggregations (as sea-bird and pinniped breeding colonies) were carried out seasonally (on year-round basis) to assess the temporal dynamic occurrence of marine resources in the Beagle Channel and the role of the different resource aggregations.
- ★ To date the faunal samples and detect marine resource presence and changes through time, radiocarbon and laboratory studies were made on the collected samples: 180 analyses were performed to complete paleotemperature curves and to assess the influence of abiotic conditions on the changes of the marine resources observed.

Results

New paleoenvironmental and environmental knowledge was acquired through this project, allowing conclusions about recent and dramatic ecological changes in this area. Long-term biotic and abiotic variations over a period of 6000 years were obtained by analysing faunal, floral and isotopic fine oscillations registered in archaeological midden deposits and the situation today; for instance the first detection in sub-Antarctic sea waters of a warming period comparable - in age - to the Mediaeval Optimum Climaticum, the first data of SST related to the Little Ice Age, the high abiotic stability of the Beagle Channel SST over the last 6000 years, the findings that today's SST is higher than any reached in this period, the common presence of the *T. atun* up to 100 years ago in the Beagle Channel waters, the presence and large distribution of the *Maytenus magellanicus* up to the same antiquity, etc. All this advance was possible thanks to the base data obtained from archaeological sites. Neither present-day biological studies, nor long-term geological research would have achieved the objective.

Selected publications

Schiavini A.C.M. and Yorio P. 1995. Distribution and abundance of seabird colonies in the Argentine sector of the Beagle Channel, Tierra del Fuego. *Marine Ornithology* **23(2)**: 39-46.

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Estevez Escalera J. and Vila Mitja A. (Co-ordinators). 1995. Encuentros en los conchales fueguinos. Universitat Autònoma de Barcelona and Consejo Superior de Investigaciones Científicas (Eds): 337 pp. Presented in the Third Annual Report.

Obelic B., Alvarez A., Argullos J. and Piana E. Determination of the Paleotemperature in Beagle Channel (Argentina) through stable isotope composition of *Mytilus edulis* shells. Accepted for publication in: Quaternary of South America and Antarctic Peninsula. Jorge Rabassa (ed). Balkema Publ. Rotterdam.

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Contract number: CII*CT930030

Period: March 1994 to March 1998

**ELIMINATION OF AROMATIC NITROSO AND NITRO COMPOUNDS FROM
WASTE WATER BY ELECTROCHEMICAL REDUCTION AND SUBSEQUENT
CHEMICAL TREATMENT**

Co-ordinator: University of Erlangen-Nürnberg, Erlangen, Germany (Ulrich Nickel)

Objectives

Removal of harmful organic substances, in particular aromatic nitroso and nitro compounds, from the effluents of industrial processes. The organic compounds should be converted into harmless or biodegradable substances.

Activities

- * Fundamental studies on electrochemical behaviour of aromatic nitro compounds have been carried out. The most suitable conditions for the complete reduction to the corresponding amines were determined. Moreover, a procedure was developed to convert the amines anodically to harmless products like carbonic acid. A variety of small laboratory electrochemical cells as well as some medium-sized devices have been constructed.
- * The German partner was primarily involved in the cathodic process, the Mexican partner in the anodic process. Some of electrochemical cells constructed in Germany were transferred to Mexico. The degradation of several nitro compounds was studied as well as the purification of industrial waste water. The design of the cell and the electrodes, the kind of electrodes, the voltage, the movement of the solution as well as other parameters were optimised in order to obtain data for the construction of a pilot plant.

Results so far

- ⇒ For the most important nitro phenols and nitro resorcines electrochemical data were determined and a procedure for the rapid chemical degradation of these compounds was developed. The design of the electrochemical cell was optimised. A quasi-continuous flow treatment of industrial effluents was developed. Based on the results obtained with laboratory scaled cells a device for treatment of 250 litres waste water was constructed. In this device up to 250 g Tricine in 250 l can be degraded completely in two hours. A pilot plant for the treatment of 20 m³/per day will now be constructed. Usually the cause of degradation is followed photometrically. Additionally a procedure was developed to determine the organic compounds electrochemically.
- ⇒ For the procedure, a patent has been applied for.

Partners

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Contract number: CII*CT930055

Period: January 1994 to April 1997

THE FATE OF ORGANOCHLORINE MICROPOLLUTANTS IN A TROPICAL RIVER SYSTEM; PARAIBA DO SUL (BRAZIL CASE STUDY)

Co-ordinator: DLO-Instituut voor Agrobiologie en Bodemvruchtbaarheid, Haren, The Netherlands (Jan Japenga)

Objectives

To carry out an extensive sampling and analysis program in the Paraiba do Sul - Guandu river system (PSG) to monitor organic micropollutants, mainly focusing on evaluating the effects of intensive industrial activities along the PSG on drinking water quality in the Rio de Janeiro Metropolitan Area. To develop a model describing the fate of organic micropollutants in the PSG.

Activities

The project started with the installation of laboratory facilities at the Federal University of Rio de Janeiro, aiming at the determination of organic micropollutants including PAH's, PCB's and chlorinated pesticide residues in environmental samples. The installation was accompanied by a thorough transfer of know-how between partners. After laboratory installation, a series of sampling campaigns was undertaken along the river and in the resulting sediment and suspended matter samples, target pollutants were determined together with parameters necessary for model validation. An existing model description has then been adapted to adequately describe the fate of organic micropollutants. The project ended with a successful international workshop held in Rio de Janeiro.

Results

- ⇒ It could be clearly shown that the huge steel factory at the town of Volta Redonda is the main input source of PAH's and other organic micropollutants in the Paraiba do Sul river: almost no pollutants were detected in sediments upstream but moderately high concentrations were detected downstream. High concentrations then persist downstream along the river, indicating high transport rates, until 70% the river water is deviated to a system of sedimentation lakes before entering the Guandu river. After passing the sedimentation area concentrations dramatically decrease, showing that the organic micropollutants precipitate there, together with suspended matter. This means that the second biggest water treatment plant in the world at Guandu receives water which is almost uncontaminated by organic micropollutants. Water quality for Rio de Janeiro citizens is therefore not affected.
- ⇒ The model is based on the distribution of organic micropollutants over three mobile phases ((i) freely dissolved, (ii) bound to the organic matter in the suspended matter matrix and (iii) associated with dissolved organic matter and one solid phase (organic matter in sediment).

⇒ Model calculations indicate that under conditions prevalent in the river, transport of more than 70% of the pollutants takes place adsorbed onto suspended matter particles. This result agrees with the experimental finding: efficient pollutant precipitation in the sedimentation lakes.

Follow-up

- A new project has been started to determine the fate of DDT, introduced at point sources in remote villages in the Amazon rain forest. To be able to extend monitoring work to risk assessment for aquatic ecosystems taking into account biodegradation patterns under tropical wetland conditions, a new joint research proposal has been submitted to the EC, counting with the participation of the Technical University of Berlin and the University of Para (Brazil) and partners of this project.
- The existence of specialized laboratory facilities at the Federal University of Rio de Janeiro has had its spin off, stimulating other environmental research including a study on the biodegradation of lindane at a dump site near Rio de Janeiro.

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Period: April 1994 to March 1998

NITROGEN OXIDES AND HYDROCARBON ABATMENT IN EXHAUSTION GASSES

Coordinator: Université Catholique de Louvain, Louvain La Neuve, Belgium
(Jean Marie Dereppe)

Objectives

The overall goal of this research is to provide new insight into the nature of phenomena occurring inside the zeolite network when NO is reduced by hydrocarbons in the presence of excess oxygen. To this end, a set of instrumental techniques were used in combination with thorough catalytic tests. The role of gaseous and adsorbed intermediates is being investigated.

Activities

- * The project focused on the study of zeolitic systems for the SCR of NO with methane in the presence of oxygen excess, likewise in the kinetics and the reaction mechanism. In this field, we have investigated zeolitic structures through ^{27}Al MAS NMR and ^{129}Xe NMR of physisorbed Xenon.
- * We have also performed an extensive characterization of acid and oxide sites through spectroscopic techniques and their relationships with possible deactivation mechanisms.
- * Kinetic studies and modelling of reaction schemes were also carried out, together with studies of the surface species from NO, NO + O₂, and NO + CH₄ adsorbed on metal exchanged zeolites through *in-situ* FTIR.

Results so far.

- ⇒ A series of H-mordenites with varying Si/Al ratios (5.9-16.9) were prepared by acid leaching and they were thoroughly characterized before and after reaction.
- ⇒ The distribution of Al was determined through ^{27}Al MAS NMR. All the samples presented three signals: one at 54 ppm corresponding to lattice Al^{IV}, another at 0 ppm associated with octahedrally coordinated Al, and a broad band, BB (ca. 100 ppm wide), assigned to aluminum-containing species. As the spinning rate increased up to 11.3 kHz, a decrease of the BB intensity and an increase of the Al^{IV} signal took place, while the Al^{VI} slightly increased. The best estimate of lattice aluminum was obtained from the Al^{IV} peak intensity. Despite the high spinning rate employed, it was possible to observe only between 70-80% of the total Al present in the samples.
- ⇒ The samples were also analyzed by XRD, FTIR and ^{129}Xe NMR of physisorbed Xenon. By correlating the variation of the cell constant with Al/u.c., only qualitative information was obtained. The IR band shift at ca. 572 and 588 cm⁻¹ at higher wave lengths, and the decrease of the bands intensity at 650 and 730 cm⁻¹ with decreasing Al content were examined. These changes in the IR spectra are a clear indication of the dealumination process carried out in the

samples, thus supplementing the ^{27}Al MAS NMR results and supplying information on the dealumination mechanism as well. ^{129}Xe NMR results show that non-lattice Aluminum may interrupt the free exchange of molecules between main channels and side pockets.

⇒ A linear correlation was found between the turnover frequency of the NO disappearance and the lattice aluminum content. The catalysts were partially deactivated after being on stream at 650°C due to the additional de-alumination occurring at high temperatures in the reacting stream. Both in new and used catalysts, only the sites related with lattice aluminum were active in the reaction under study. The non-lattice, polymeric species, generated during de-alumination hinder the access of the reactant molecules to the active sites.

Follow-up

Questions the answers to which are being sought are: What is the nature of the cation species in the operating catalyst? Do cations move around the lattice in the reacting atmosphere?, and how does this affect the catalytic behaviour? What is the role of oxygenated species as possible intermediates? How do transport properties affect the overall performance and particularly the balance between the two competing reactions, namely the oxidation of the hydrocarbon with either O_2 or NO_x ?

Publications

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NOVEL TECHNOLOGIES FOR THE TREATMENT OF INDUSTRIAL EFFLUENTS USING IMMOBILISED MICROALGAE AND AQUATIC PLANTS

Co-ordinator : King's College London, London, U.K. (David Hall)

Objectives

The main objective of the research programme was to develop a novel integrated system for the treatment of agro-industrial effluents using immobilised micro algae and aquatic plants. To achieve this objective the experiences of the three institutions participating in algal bioreactor construction, cyanobacterial biochemistry and physiology, cell immobilisation technology, and wastewater treatment biotechnology were pooled.

Activities

- ★ The King's College research group focused their studies on cell immobilisation of *Phormidium* and on the design, assembly, and optimisation of helical indoor bioreactors for the mass production of *Arthrospira (Spirulina)*. Both cyanobacteria have the capacity to assimilate heavy metals from contaminated waters.
- ★ The UPV, Bilbao, team conducted detailed studies on the feasibility of using cyanobacterial cells (*Phormidium* Spp, *Scenedesmus*, *Arthrospira*, etc.) immobilised in different polymers and resins for the removal and subsequent recovery of metals or inorganic ions from simulated agro-industrial effluents. The performance of 'live' and 'dead' cells were tested as depollution agents.
- ★ The Institute of Ecology, Xalapa, Mexico concentrated their efforts on *Lemna* and *Salvinia* (aquatic plants) and *Arthrospira* culturing in outdoor ponds using, wherever possible, natural farm wastes as nutrients for the plants and cyanobacteria so that algal growth technology could be combined with environmental depollution.

Results

- ⇒ At KCL, a tubular 'airlift' bench top reactor, of 14l volume, was constructed for the mass production of *Arthrospira platensis* which was operated in the batch, semi-continuous or continuous mode. In the batch-culture mode this bioreactor produced approximately 26g of *Arthrospira*/d/reactor volume at a photosynthetic efficiency (PAR) of 11.0%.
- ⇒ At UPV, microalgae were immobilised in polyurethane foam, and epoxy and polysulfone resins, and then tested for their capacity for biosorption of Fe(II), Cr(III), Cu(II), Ni(II) and Pt. After determining the absorption parameters for various metal-matrix combinations the rate of metal desorption (by HCl wash) was determined.
- ⇒ The activities at the Institute of Ecology culminated in the construction of an outdoor pilot plant (high-rate oxidation pond) for the production of *Spirulina* and aquatic plants at "La Mancha", Veracruz. The conditions for optimal production of *Arthrospira* using pig waste were identified.

Throughout the Contract Period, the partners were in constant contact, meeting annually, and also at major International conferences on Algal Technology, and waste water bioremediation.

Selected publications

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**DETERMINATION AND DYNAMICS OF HEAVY METALS AND ORGANO-
CHLORINES IN THE VALDIVIA RIVER ESTUARY IN SOUTHERN CHILE**

Co-ordinator: Biologische Anstalt Helgoland, Hamburg, Germany (K.-R. Sperling)

Objectives

The project aimed at investigating the pollution status of the Valdivia River and its estuary in Chile. The key task of the programme involved environmental analyses at the Universidad Austral de Chile.

Activities

- ★ The project started with the purchase of appropriate equipment: a graphite-furnace/atomic-absorption spectrometer (CF-AAS) for the determination of heavy-metal contents, and a gas chromatograph (GC) for the determination of organohalogen contents.
- ★ The Sülldorf Laboratorium of the Biologische Anstalt Helgoland (BAH) in Hamburg, Germany, following successful participation in several certification rounds of the Bureau de Référence of the EC, gave valuable advice on the preparation of ultratrace analyses at the University of Valdivia, on the setting up of a sampling and monitoring programme and organized training for colleagues and laboratory personnel.

Results

- ⇒ The first part of the project was dedicated to setting up and training issues,
- ⇒ In the second part, analytical techniques were applied for the assessment of the pollution status of the Valdivia River and its estuary. For this purpose, aqueous organisms and sediments were analyzed, with a view to determining their heavy metals and organohalogen contents.

Follow-up

- The analysis methods applied were highly sophisticated. Consequently, the Universidad Austral de Chile found itself in the role of a pacemaker and training centre for such analyses.
- A monitoring plan was worked out for further surveillance studies on the pollution status of the Valdivia estuary and adjacent regions. The number of relevant compounds and target organisms will be expanded. Due consideration will also be given to the natural geochemical cycles of heavy metals such as Hg in volcanic and Cd in upwelling areas.

Selected publications

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Period: January 1994 to January 1997

USE OF BIOMARKERS FOR EVALUATING THE PRESENCE AND EFFECTS OF PENTACHLOROPHENOLS IN THE BIOBIO RIVER BASIN

Co-ordinator: Università degli Studi di Siena, Siena, Italy (Silvano Focardi)

Objectives

The two major goals of this research were to define the ecotoxicological characterization of pentachlorophenols (PCPs) by studying its environmental diffusion and the effects it has on biological systems, and to transfer new and appropriate methodologies from the Dipartimento di Biologia Ambientale (University of Siena) to the Centro EULA (University of Concepcion).

Activities

For the analysis of biomarkers, three sampling areas with different human impact were chosen in the basin: the Santa Barbara area, upstream the Biobio with reduced farming and industry input; the Nacimiento area with industrial, farm and forestry pollution; the mouth of the Biobio river with urban and industrial pollution.

In the second and in the third year of the project, all the analysis were conducted in the laboratories of the Centro EULA-Chile.

Results

- ⇒ The use of biomarkers has revealed the Biobio river as a highly polluted area compared to other fluvial systems of the same region.
- ⇒ The EROD induction, AChE inhibition and the porphyrin levels appear to be the most powerful biomarkers for the identification of the toxicological risk related to the presence of xenobiotics compounds in the Biobio river.
- ⇒ *Mugil cephalus* and *Eleginus maclovinus* in fish and, *Larus dominicanus* and *Phalacrocorax olivaceus* in birds could be good bio-indicator species to assess the environmental quality of this aquatic system.
- ⇒ As PCP is used as salt, and under the environmental conditions (pH of Biobio river waters: 7-7,5), it is almost completely dissociated in the anion form. A part remains in the water column (with a loss of 42% by degradation reactions) and in a large part is transferred to sediments.
- ⇒ EROD induction in *Mugil cephalus* and *Eleginus maclovinus* was particularly alarming (about 7 times higher than that found in strongly induced Mediterranean fish). These results appear as an early warning signal of the high potential toxicological risk of this river ecosystem, considering its use as drinking water source for a large human population.

⇒ The people of Concepción and Talcahuano use the Biobio river waters for drinking and fishing. Therefore, inhabitants in this area may be exposed to PCPs present in the potable water, as well as to aromatic and chlorinated hydrocarbons they absorb with food. In the future it will be necessary to evaluate the overall genotoxic effects of this mixture of contaminants present in the river, so vital to the surrounding populations.

Selected publications

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COASTAL ZONE MANAGEMENT IN SANTA CATARINA ISLAND (BRAZIL)

Co-ordinator : Université de Bordeaux 1, Talence, France (André Klingebiel)

Objectives

This contract involved research activities on marine sciences, located in the Santa Catarina Island (Brazil) coastal zone, which is now subjected to the pressure of urbanization tourism.

Activities and results

Activities were aimed at obtaining a holistic and detailed knowledge of the various ecosystems producing renewable resources, in order to design coastal-zone management programmes compatible with the conservation or restoration of the exceptional environmental qualities of this island.

Use and optimization of new remote sensing methodologies from satellite SPOT data:

This technology was used to complement field observations and measurements to evaluate and map the rapid changes occurring in the mangrove ecosystems. Another methodology was used to identify coastal water masses around the Santa Catarina Island and characterize coastal-wave patterns by reflectance measurements of the water surface

Evaluation of sand fluxes in beach arenosystems

Operations involved two approaches:

- a theoretical one using formulae to calculate the longshore transport, taking into account sand-grain size and wave characteristics, based on field measurements and on remote-sensing data (specifically SPOT, giving good wave patterns);
- an experimental one using a fluorescent tracer technique.

Pollution of coastal waters

Domestic (mainly faecal) and inorganic pollutants (heavy metals) are located around urban zones: levels of cadmium and aluminium are increasing in organisms, by bioaccumulation processes, while selenium appears in high concentrations in sediments from an unpolluted zone.

Renewable resources of the island:

Mariculture techniques applied for the production of Mitylidae and Ostreidae were developed, studies on the quantification of migratory fluxes of Mugilidae and Gerreidae were carried out; the permanence and abundance of extremely productive constant species in coastal and lagoon waters was assessed.

Social and economic development of the island:

At the final colloquium participants emphasized the need for basic information about the economic and social condition of the Santa Catarina Island, in order to provide a common reference enabling the integration of research results into synthetic conclusions on the management of the coastal zone.

Selected publications

Blanca Sierra de Ledo. 1997. Subsídios ecológicos para un plano de gestão integrado na zona costeira da ilha de Santa Catarina. Actes du colloque franco-brésilien Aquitaine-Océan. n°3, pp. 9-34.

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Contract number: CI1*CT930336

Period: May 1994 to June 1997

**DENDROCLIMATIC RECONSTRUCTION FROM SOUTHERN SOUTH
AMERICAN TEMPERATE FORESTS**

**Co-ordinator: Climatic Research Unit, University of East Anglia, Norwich, UK
(P.D. Jones)**

Objectives

To develop millennial-long tree-ring chronologies from temperate forest species living between 32° and 55°S in Chile and Argentina. The developed and existing chronologies will then be used to reconstruct features of the climate in this region such as summer temperature and precipitation.

Activities

The project has focussed on two species, *Fitzroya cupressoides* and *Austrocedrus chilensis*. The first species was studied using samples taken from Andean parts of Argentina between the towns of Bariloche and Esquel. The species is the longest lived in South America, with some specimens up to 3000 years old, and has considerable climatic potential, but there are problems with standardization (removal of growth trends) and in defining the relationship between ring growth and climate. The second species has been studied in Chile in the zone 30-40°S. Several sites were sampled in Andean parts of Chile with some samples living up to 1000 years.

Results

A tentative reconstruction has been developed from the *Fitzroya cupressoides* work combining the samples taken during the project with earlier chronologies of the same species constructed in Chile and Argentina. The reconstruction explains about 35% of the summer (December-March) temperature variance. The *Austrocedrus chilensis* chronologies have been used to develop summer precipitation reconstructions, complementing related work undertaken on the other side of the Andes in Argentina. In some regions of Chile the species has been severely affected by fires and a history of this has been documented.

Follow up

Several papers have been submitted for publication and all groups are pursuing various avenues for extending the collaborative work.

Selected publications

Aravena J-C., Lequesne, C. and Boninsegna, J.A. 1998: Dendroclimatic reconstruction for northernmost populations of *Austrocedrus Chilensis* (*Cupressaceae*) in central Chile. (Manuscript in preparation.)

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Period: July 1994 to June 1997

**BEHAVIOURAL ECOLOGY OF INVERTEBRATES ON CHILEAN SHORES
EXHIBITING DIFFERENT DEGREES OF HUMAN DISTURBANCE**

Co-ordinator: University of Wales-Bangor, Menai Bridge, U.K. (Ernest Naylor)

Objectives

Primarily to study behavioural adaptations of selected Chilean invertebrates in coastal marine ecosystems endangered by human exploitation and disturbance, and to propose measures for the conservation and sustainable management of such intertidal assemblages.

Activities

Experiments in behavioural ecology were carried out in the field and the laboratory, based on local background knowledge and using actograph recording techniques introduced from Europe to Chile. European partners, research associates and Ph.D. students worked for extended periods in Chilean laboratories, and reciprocal visits to Europe were made by Chilean partners. A Chilean research associate worked closely with fishermen in a co-operative (caleta), and several European and Chilean students obtained postgraduate training.

Results

For commercially exploited rocky-shore molluscs, detailed studies of foraging and migratory behaviour were carried out and published. Findings provided background data for developing new management practices in a fishing co-operative: these have yielded increased catches per unit effort of the 'loco' *Concholepas*. Related improved management practices are being developed for commercially exploited sea urchins (*Loxechinus*) and octopus. Behavioural ecology studies of sandy-shore invertebrates have been carried out over longer time scales than hitherto and have demonstrated species differences in rhythmic migratory behaviour, sometimes under internal biological-clock control. High species diversity and abundance have also been demonstrated near an upwelling area.

Follow-up

Practical recommendations concerning management of coastal fisheries and environmental resources have been communicated to the Subsecretary of Fisheries, Chile, and to CONICYT. Based on project findings, one Chilean partner has received new local funding to carry out a further project entitled: 'The diversity, biogeography, and dynamics of nearshore ecosystems in Chile: foundations for marine conservation ecology'. The other Chilean partner has been appointed by the Chilean government to direct a major national research initiative in marine science. EU-Chilean links continue with other funding.

Selected publications

Castilla J.C., 1997. Chilean resources of benthic invertebrates: fishery, collapses, stock rebuilding, and the role of coastal management areas and national parks'. Second World Fishery Congress, Brisbane, Australia, pp. 119-124.

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FATE, CYCLING AND ENVIRONMENTAL EFFECTS OF AGROCHEMICAL RESIDUES FROM COTTON CULTURE IN COASTAL LAGOON ENVIRONMENTS OF NICARAGUA

Co-ordinator : Danish Institute of Agricultural Sciences, Slagelse, Denmark (Arne Helweg)

Objectives

- ◆ Investigate pesticide usage,
- ◆ Monitor pesticide residue distributions in the aquatic environment of the coastal lagoon systems of the Atoya River
- ◆ Study the fate of pesticide residues and the pesticide distribution and cycling in aquatic ecosystems.

Activities

- * Analysis of the use of pesticide chemicals in Nicaragua,
- * Sampling of sediments and biota in the lagoon environment,
- * Analysis of the samples for a number of different organochlorine and organophosphorous pesticides.
- * The participants have also been involved in the congress: "*Congreso nacional: impacto de plaguicidas en ambiente, salud, trabajo y agricultura*", held in Managua, Nicaragua 27–31 October 1997.

Results so far

- ⇒ Samples from the lagoon environment were analysed for 16 different organochlorine pesticidal compounds. Levels of contamination of organochlorine pesticides, especially toxaphene and Σ DDT-products; demonstrate extensive use of these chemicals in the past. These chemicals run off from cultivated soils to the coastal lagoons. The reservoirs of toxaphene and Σ DDT present in certain soils of the region will probably continue to contaminate lagoons for many years. Determination of organophosphorous pesticides in water from the lagoon system indicate widespread use of dichlorvos and chlorpyrifos on the surrounding fields. Contamination came primarily from surface run-off and river discharge.
- ⇒ The most important agricultural activity has been cotton cultivation.
- ⇒ Degradation studies show very slow degradation of DDT in the sediments, whereas the fungicide maneb does not seem to cause problems with accumulation of an important degradation product (ETU).
- ⇒ The coastal lagoon is surrounded by mangrove forest that seems to play an important role in retaining pesticide residues transported by surface run-off.
- ⇒ Analyses, particularly of clams from the area, which are consumed, have shown high concentrations of toxaphene and Σ DDT.

Follow up

- Discharge of pesticide residues entering the lagoon should be reduced.
- Exposure of humans to toxaphene through ingestion of clams should be reduced.
- Contamination trends should be followed annually.
- Protected locations for shrimp farming areas should be selected.
- The total exposure of the population to pesticides through drinking water, food residues, exposure of workers should be reduced.

Partners

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THE EFFECT OF NUTRIENT BALANCE AND PHYSICAL FACTORS ON THE OCCURRENCE, TOXICITY AND CONTROL OF CYANOBACTERIAL BLOOMS IN THE PATOS LAGOON, BRAZIL: A LABORATORY AND FIELD STUDY

Co-ordinator: University of Dundee, Dundee, United Kingdom (G.A. Codd)

Objectives

- ◆ Establish enabling mechanisms and methods at the Fundação Universidade do Rio Grande, FURG, Rio Grande do Sul, Brazil, to investigate the occurrence and toxicity of cyanobacterial (blue-green algal) blooms in the Patos Lagoon
- ◆ Facilitate the development of experience in toxic cyanobacterial bloom research and the development of a centre for such experience, with international contacts, and to serve as a reference centre in Brazil and Latin America in particular.

Activities

The project was centred on the Patos (the largest lagoon system in South America), which is subjected to intensive human use (drinking water, fisheries, industry, agriculture, leisure and navigation) and produces massive nuisance blooms of scum-forming cyanobacteria with the potential to produce potent, tumour-promoting hepatotoxins (microcystins). Environmental monitoring, using the University's research vessel *Larus*, ranged from the upper freshwater regions of the lagoon to the Atlantic coast outside of the lagoon mouth. Analysis included: physicochemical measurements on board; nutrient and pigment analyses at FURG; toxicity assessments of cyanobacterial bloom samples at FURG; further toxicity assessments and chemical analyses for toxins at the University of Dundee. Dundee provided cyanobacterial toxin data, techniques for toxin extraction and preservation from environmental samples, and reference purified toxin. Joint sampling programmes in the lagoon included the collection and isolation of new strains and cultures of cyanobacteria from the lagoon and associated waters. Contacts were developed between Dundee and FURG with postgraduate students and academic staff.

Results

Blooms of cyanobacteria (mainly *Microcystis aeruginosa*) occurred throughout extensive stretches of the lagoon in 1994, 1995 and 1996, with toxic scums and deposits in areas of fisheries, recreational waters, and along beaches. Toxicities and levels of microcystins ranged from non-detectable to as high as found in nutrient-enriched European waters and laboratory cultures at Dundee. Microcystins in the Patos Lagoon occurred both in particulate form (associated with the cyanobacterial cells), in dissolved form in the water, and in the lagoon sediment. Conditions favouring cyanobacterial bloom development (high summer temperatures, episodes of freshwater flow from the rivers feeding into the north of the lagoon (Porto Alegre area) and nutrient inputs) were characterized. A major role of salinity was identified in influencing cyanobacterial development and distribution was found, *Microcystis* growth mainly occurring in the upper freshwater regions before being transported to lower

estuarine areas with deposition on beaches. Several single strains of *Microcystis* were established in laboratory culture at FURG from lagoon samples, and investigations started on toxin analysis, and the regulation of growth and toxin production by environmental factors.

Follow-up

The project has established records of cyanobacterial and hepatotoxin levels in the Patos Lagoon over a three-year period and a valuable level of expertise in cyanobacterial ecotoxicology at FURG for the benefit of Rio Grande do Sul and environments elsewhere in Brazil and other Latin American countries. Evidence of the value of the project includes a principal role of FURG in the investigation of major fish deaths associated with cyanobacterial blooms in 1996 and developing links between FURG and cyanobacterial laboratories in Uruguay and Chile. It is hoped to extend collaboration to the development of policies for cyanobacterial toxin management and control.

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Period: August 1996 to August 1998

ANAEROBIC DIGESTION CONTROL STRATEGIES FOR WASTES CONTAINING XENOBIOTICS AND RECALCITRANTS

Co-ordinator: Loughborough University, Loughborough, Leicestershire, UK
(A.D. Wheatley)

Objectives

The aim of this research was to develop strategies to enable the anaerobic digestion of waste streams containing xenobiotics and recalcitrant compounds.

Activities

The detailed objectives are concerned with developing, monitoring and control procedures for the anaerobic digestion of industrial (Loughborough) and domestic (UAM) wastewater. ORSTOM (France) have investigated the use of biocells and bioassay to determine the efficiency of anaerobic biodegradation of common aromatics found in the wastewaters, specifically terephthalic acid (PTA) (plastics industry) and detergents. The project also investigated the effect of refractory chemicals on the structure of anaerobic biomass (UNAM) by feeding activated sludge as exogenous VSS to a steady-state bioreactor. UNAM also further investigated automatic alkalinity-monitoring and tested it at both laboratory and then at industrial scale. A technique first developed in previous EC programmes.

Results

The control strategies were tested by Loughborough. These were adaptive control, expert systems and neural networks. Several different types of on-line sensors were also investigated. These were hydrogen, hydrogen sulphide, and methane in the off gas, plus conductivity, turbidity and pH in the liquid phase. The hydrogen monitoring, coupled with an adaptive control algorithm, was shown to give better control during toxic loading (Loughborough). UAM also investigated the use of hydrogen as a control parameter to identify problems with waste streams containing detergents and terephthalic acid. UNAM results from the use of alkalinity ratio as a control parameter were also successful. The automatic titration system was further developed to be incorporated in an 'expert system'. ORSTOM determined the precautionary inhibitory thresholds of several detergents (SLS, SDBB and TMAC). The work also established that the PTA was anaerobically biodegradable and non-inhibitory to methane production from acetate but did inhibit methane production from hydrogen. This was a novel and valuable fundamental result.

Follow-up

The experimental research is now finished and a final report is being compiled. Loughborough is continuing to investigate the breakdown of recalcitrant wastes, and has a current research project on the toxicity of by-products of anaerobically treated dye-house waste. A second follow-on project based on an expert system has been proposed, to

incorporate and disseminate information and data on the anaerobic treatment of refractory wastewaters gathered during this research.

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THE USE OF NON-DESTRUCTIVE BIOMARKERS TO ASSESS THE HEALTH STATUS OF ENDANGERED SPECIES OF MARINE MAMMALS IN THE SOUTHWEST ATLANTIC

Co-ordinator : University of Siena, Siena, Italy (Cristina Fossi)

Objectives

The aim of this study was to develop the basis for a new methodology of investigation, based on non-destructive biomarkers and residue analysis, for the evaluation of the ecotoxicological risk of endangered species of Southwest Atlantic marine mammals.

Activities

The project focused on the development and validation of a non-destructive approach in stranded and free-ranging specimens of marine mammals. In the different non-destructive materials (skin biopsies, blood, faeces and fur for the free-ranging specimens, and organs and tissues for the stranded specimens) we measured biomarker responses (MFO, DNA damage, esterases, clinical biochemistry, porphyrins) and performed residue analysis (heavy metals, PAHs and OCs). The sampling of the marine mammal specimens was carried out by Buenos Aires partners, with the participation of the Siena researchers. The biomarker and the residue analysis were carried out in Siena; the latter in collaboration with Buenos Aires.

Results so far

The biomarker approach and the residue analysis were applied to the study of marine mammals in two different kinds of samples: stranded and free-ranging animals. Cetaceans and pinnipeds were collected for residue analysis in polluted coastal areas of the Brazil and the Argentina. Blood, skin, subcutaneous blubber, fur and faeces were sampled in the sea lions (*Otaria flavescens*) of two colonies in Argentina: Mar del Plata, an heavily polluted harbor, and Punta Bermeja, a relatively clean environment of Patagonia. The MFO data of stranded sea lions confirmed the metabolic imbalance between high toxifying and low detoxifying potential in pinnipeds. This biochemical vulnerability reveals the severe risk to which these animals are exposed in environments heavily contaminated with xenobiotics such as the Mar del Plata harbor. Non-destructive biomarkers evaluated in samples of faeces, blood and skin were found to be useful for evaluating the toxicological risk to which the sea lion population of Mar de Plata is exposed. Faecal porphyrins, certain blood metabolites and enzyme activities, and especially monooxygenase induction in skin biopsy samples suggest that the sea lions of this colony are subjected to chemical stress. The dramatic induction of BPMP activity in the skin biopsy combined with high levels of PAHs revealed the high toxicological risk to which this population is exposed. This data is a warning sign for the Argentine Regional Authorities that stricter control of pollution in Mar del Plata harbor is necessary. The high levels of PAHs found in several biological materials of sea lions from Punta Bermeja in May and September 1996 are a warning signal for the "pristine" environment of Patagonia. The dramatic increase in PAH contamination may be related to the oil spill of March 1996 in

Rio Colorado. In conclusion, these preliminary results confirm that the non destructive biomarker approach combined with residue analysis of free ranging and stranded animals is a useful tool for assessing the health status of endangered species of marine mammals in the southwest Atlantic.

Follow up

On the basis of the interpretation of the results obtained from the biomarker and the residue analysis, efforts are being made by the two partners to carry on investigations on the status of marine mammals populations living in Patagonia. Other non-destructive biomarkers in marine mammals samples are presently being developed at Siena University (e. g. cellular cultures of marine mammals fibroblast).

Selected publications:

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**THE STUDY OF THE GEOMORPHOLOGY, HYDROGRAPHY AND CIRCULATION
OF ARGENTINIAN ESTUARIES**

Co-ordinator: University of Plymouth, Plymouth, United Kingdom (Keith Dyer)

Objectives

- ◆ Survey the geomorphology and estuarine dynamics of the Quequen Grande and Gallegos estuaries;
- ◆ Establish the general hydrography, circulation and air-sea interaction processes during tidal, lunar and seasonal timescales;
- ◆ Measure the sediment transport, deposition and erosion rates to establish sediment budgets for each estuary;
- ◆ Adapt existing one and two-dimensional mathematical models using the measured data for calibration;
- ◆ Provide predictive results for the impacts of anthropomorphic and natural changes.

Activities

- * A research fellow from Plymouth has joined staff from the Instituto Argentino de Oceanografia in two extensive field surveys of the Rio Gallegos estuary, and three of the Quequen Grande.
- * Measurements have included hydrographic surveys, current and salinity measurements, collection and analysis of bed sediment samples.
- * Weather stations and tide gauges have been established for monitoring of weather conditions in relation to sea level variation.
- * Mathematical models have been used to test their applicability to the observed characteristics.

Results

- ⇒ Basic bathymetric charts have been produced for the estuaries, which have very contrasting tidal ranges and sedimentary regimes.
- ⇒ Measurements have established their salinity structure and the longitudinal distributions of salinity, temperature and currents. By comparison with other estuaries, the Rio Gallegos estuary is well mixed and macrotidal, whereas the Quequen Grande is highly stratified and microtidal.
- ⇒ Sufficient data has been obtained to provide a basic description of estuaries and their response to tides and to weather.
- ⇒ Papers are in preparation for publication of this information.

⇒ In the Quequen Grande additional measurements have been taken which are being analysed for the dynamics of the mixing regime which will aid specification of the exchange coefficients in modelling.

Follow-up

- The data will be made available to local authorities, and the results prepared for presentation at conferences and for publication in refereed journals.
- The IADO have responsibility for the basic geomorphological and estuarine descriptions, and Plymouth for the dynamical aspects.
- Several PhD theses are being prepared, based on the project.

Selected publications

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PLANT FUNCTIONAL TYPES AND COMMUNITY RESPONSES TO GLOBAL CLIMATE CHANGE. A COMPARISON BETWEEN TAXONOMICALLY DISTINCT FLORAS

Co-ordinator : University of Sheffield, Sheffield, United Kingdom (J. Philip Grime)

Objectives

- ◆ Identify plant functional types of broad application, testing their validity in two taxonomically distinct herbaceous floras: central Argentina and Northern England.
- ◆ Understand how these functional types interact at the community level under contrasted scenarios in terms of disturbance and resource availability.
- ◆ Predict vegetation responses to changes in global climate and land-use regime.
- ◆ Develop a functional interpretation of the flora of Argentina, applicable to other geographical areas in Latin America.

Activities

- ★ Develop databases of attributes for the British and Argentina floras, which will allow prediction of responses to changes in land use and climate. The emphasis is on selection of traits, which have maximum predictive power, yet are relatively simple to measure, if possible on herbarium material. Such 'soft' traits are calibrated against the biochemical and physiological characterization of species within the northern European flora, conducted as part of the Integrated Screening Programme (ISP). In addition a reduced version of the ISP, including tests of decomposition and palatability has been conducted on a subset of the Argentine species under investigation.
- ★ Bring the two databases together in a search for a general system of plant functional types.
- ★ Joint experimental and modelling work.

Results

- ⇒ Measurement of 44 traits on 342 species is largely complete and the data are organized in two fully compatible databases.
- ⇒ The first analysis of the Argentine database has already revealed that the major axis of variation in the Argentina flora (from fast-growing species of fertile habitats to slow-growing species of infertile habitats) agrees closely with the corresponding axis in the British flora.
- ⇒ Joint analysis of experimental work on decomposition and palatability has also revealed strikingly similar patterns in the two floras.
- ⇒ Modelling work at Sheffield, with the assistance of visiting Argentine workers, has made good progress on the development of new software for modelling plant and community progresses by means of cellular automata.
- ⇒ A new model of plant growth, which is general, deterministic and non-stationary, is designed to investigate the extent to which the whole plant's morphology and function b-

can be determined by resource (light and mineral nutrients) acquisition and utilization on the part of its component modules.

Follow-up

- Analysis of the combined databases is expected to lead to generalizations about recurrent patterns of specialization in different floras, with emphasis on responses to global change, and predictions of the role, at the community level, of the major functional types under different global change scenarios.
- Bids to continue various parts of the work have already been made to several Argentine and UK sources.

Selected publications

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Period: February 1995 to January 1998

PROJECT PARAT : A STUDY OF THE TRANSFER OF PARTICULATE AND DISSOLVED PHASES SOUTHERN SOUTH AMERICA TO THE SW ATLANTIC OCEAN

Co-ordinator: CNRS/Université Louis Pasteur, Strasbourg, France (Jean Luc Probst)

Objectives

The purpose of PARAT is to probe into the transport dynamics, through aeolian and riverine paths, of dissolved and particulate substances from southern continental South America (south of 35°S) to the SW Atlantic Ocean. It also aims to assess the different sources of land-derived materials.

Activities

Waters, suspended matter and bottom sediments are periodically collected from the Parana and Uruguay rivers and from 7 major Patagonian rivers (Colorado, Negro, Chubut, Deseado, Chico, Santa Cruz, Coyle and Gallegos). Since 1995 6 field sampling trips were made, mainly to the Patagonian river basins. Four dust sampling collectors have been installed along the Atlantic coast (Bahia Blanca, Puerto Madryn, Puerto San Julian and Rio Gallegos). In connection with the aeolian dust aspect, a set of top-soil samples were also collected 300-400 km apart. Cordoba (Argentina) is in charge of the organisation of the sampling trips and of the determination of physico-chemical parameters in the field. Cordoba and Strasbourg (France) are in charge of the major element chemistry of river water and of the mineralogical and chemical compositions of soil particles, river sediments and aeolian dust. Darmstadt (Germany) determines the nitrogen, carbon, sulphur and biogenic silica contents in the river sediments. Strasbourg also analyses trace elements, rare earth, radiogenic strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) and stable carbon isotopes ($^{13}\text{C}/^{12}\text{C}$). Strasbourg and Cordoba do most of the research concerning inorganic geochemistry : aeolian dust and river-suspended sediment transport, heavy metal speciation in dissolved and particulate phases in Cordoba and surface geochemical processes, rock weathering and atmospheric/soil CO_2 flux in Strasbourg. Darmstadt concentrates on organic geochemistry and N, C, and S biogeochemical cycles in the river systems.

Results

For the first time, the chemical composition of waters and sediments transported by Patagonian rivers have been characterised. An intercalibration exercise allowed us to check the quality of partner analytical results. The river-suspended and dissolved materials present more radiogenic strontium and less radiogenic neodymium isotopic ratios in the Parana than in the Uruguay in relation to more abundant Jurassic-Cretaceous basalts in the Uruguay basin. The first carbon ($^{13}\text{C}/^{12}\text{C}$) and strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotopic ratios measured in the Patagonian River waters show a wide range of values going respectively from -3.44 to -12.83 and from 0.704 to 0.711. The isotopic signatures of riverine material will be used to determine the contribution of the different continental sources (carbonate and silicate rock weathering,

atmospheric/soil CO₂, organic matter, pollution...) and to evaluate the riverine contribution to the Brazil/Malvinas Confluence. Chemical sequential extraction procedures allowed us to determine the speciation (soluble, exchangeable, bound to carbonates, bound to manganese oxides, bound to iron oxides, bound to organic matter and residual fraction) of particulate trace elements (Mn, Fe, Co, Ni, Cu, Rb, Sr, Y, Sb, Cs, Pb, U, Th and lanthanides) in Patagonian river-suspended matters and in bottom sediments.

Follow-up

During the next year, the two last sampling trips will be completed and two more dust sampling collectors will be installed. The geochemical data will allow us to calculate, within the two next years, the aeolian and riverine transports into the South Atlantic Ocean and to determine the different sources of materials. An effort will be made to model the river transport as a function of hydroclimatic parameters (as already done for the Parana) but also in relation to environmental factors and anthropogenic activities.

Selected publications

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INFORMAL SETTLEMENTS IN SANTIAGO DE CHILE: THE ROLE OF SPATIAL CONFIGURATION IN NEIGHBOURHOOD, HOUSING AND COMMUNITY CONSOLIDATION

Co-ordinator: University College London, London, United Kingdom (Bill Hillier)

Objective

Rapid urbanisation in Santiago has led to a large number of informal settlements on the periphery of the city which, from an initial common origin, now exhibit very different degrees of economic and social consolidation. The objective of the project is to measure the extent to which the spatial configuration of informal settlements has an effect on the level of household, neighbourhood and community consolidation.

Activities

- ◆ A configurational model of the street network of the entire Santiago metropolitan area has been built using computer modelling software developed in London. These have been used to quantify differences in the spatial configuration between urban areas of Santiago and between individual peripheral settlements.
- ◆ In Chile, a methodology for measuring housing, neighbourhood and community consolidation on informal settlements has been developed. A sample of 17 informal settlements legalised through the 'sites and services' programme have been analysed using detailed questionnaire and site survey data and multiple indices of consolidation have been calculated for the sample. Pedestrian and vehicular movement observation studies have also been carried out on the sites.
- ◆ A methodology for linking the computer model to both economic and social indices at the district level, consolidation indices and movement observation data at the site level has been developed and implemented.
- ◆ Analysis and write up of the project are being undertaken at present.

Results

- ⇒ At the macro level of the city as a whole, the spatial model has been correlated with social and demographic data from the 34 metropolitan districts. Within the poorer areas, suggestive relations between spatial integration and income indices have been found. The influence of time in consolidation has been investigated in relation to the higher consolidation of the older outlying towns.
- ⇒ Experimental work on analysing parts of the city individually has explored the spatial correlates with the well known division of Santiago into two areas of rich and poor, especially in the relation of land use to integration in the richer area of Oriente.
- ⇒ At the micro level, the spatial model has been related to the observation data on movement in the settlements. Preliminary results from a pilot study have shown a strong relationship between the spatial structure of the settlement and pedestrian movement patterns. The analysis of movement data for all sites is being completed at present. The

relationship of spatial integration to economic and social consolidation of the settlements is being explored at house, street and settlement levels.

- ⇒ At the settlement level results to date show a positive relationship between the spatial integration of the settlement, the amount of movement and the level of commercial activity on the site seen in informal shops. The pattern of movement and commercial activity is further related to the level of housing consolidation and social well-being on the site.
- ⇒ These findings suggest a mechanism whereby the configuration of the site can be seen to have an influence on the level of consolidation through the importance of movement patterns and commercial activity.

Follow Up

Once the project is completed it is foreseen that the mechanisms whereby spatial structure has an effect on consolidation in these settlements will have been identified. This will be of assistance in formulating planning policy for such settlements to ensure that the layout of sites best serves the inhabitants in consolidating their neighbourhoods. Given the importance of informal settlements in rapid urbanisation throughout the developing world, the possibility of extending this research with a further comparative study is being explored.

Selected Publications

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STUDIES OF PHOTOCATALYTIC REACTIONS AND REACTORS FOR WATER POLLUTION ABATEMENT

Co-ordinator: Centre National de la Recherche Scientifique, Nancy, France
(Michel Bouchy)

Objectives

Heterogeneous photocatalysis on TiO_2 is a promising process for eliminating toxic and bioresistant organic compounds from waste water through their oxidation into harmless species. The objectives were to study the reaction and the catalyst in order to design photocatalytic reactors.

Activities

Cooperative activities of the research groups were carried out on

- * reaction kinetics (effect of various physicochemical parameters)
- * development of novel catalysts (doping, immobilisation)
- * optical properties (light absorption and scattering)
- * radiation field (modelling of light distribution). This has permitted to model reactors.
- * In addition, catalyst ageing (stability and reuse) has been assessed.

Results

- ⇒ The kinetics of the photocatalytic degradation of various pollutants have been studied using suspended and immobilised catalyst. The effect of various parameters has been investigated and the complex reaction mechanism has been elucidated. These studies have proved the importance of choosing optimum conditions for an efficient waste-water treatment.
- ⇒ Colloidal Ti(IV)/Fe(III) mixed oxides and photoplatinised commercial TiO_2 have been prepared and tested but they did not exhibit a better activity than the commercial catalyst. The techniques of deposition of commercial TiO_2 or of synthesis by the sol-gel method have been investigated. They are good techniques of immobilised catalyst preparation but a compromise has to be found between adhesive strength and photocatalytic activity.
- ⇒ Special spectrophotometric experiments have been developed to measure the absorption and scattering coefficients of catalyst suspensions. An experimental set-up has also been designed which permits to record the scattering distribution of immobilised catalyst films.
- ⇒ The radiation field has been determined by solving the Radiative Transfer Equation. This has been successfully carried out using the Discrete Ordinate Method. This method has been applied to simulations of lamp/reactor systems of various geometries and of an experimental example of photocatalytic degradation.
- ⇒ The preceding results have permitted to model reactors for photocatalytic degradation, in systems of various geometries, using suspended or immobilised catalyst, with artificial or solar irradiation. These simulations have been corroborated with some experiments.

⇒ The adhesive strength of deposited catalysts has been assessed by various techniques that have shown that the lack of adhesion was found mainly in top layers of particles. After an initial partial loss, the deposits remain stable.

Follow-up

It has been shown that the process of photocatalytic degradation on suspended catalyst was feasible but of complex nature and that the catalyst could be successfully immobilised. Pilot reactors are presently under study.

Selected publications

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ESTIMATION OF ACTUAL EVAPORATION FOR IRRIGATED CROPS AND SEMI-ARID RANGELAND IN NORTHWEST MEXICO FROM SATELLITE DATA

Coordinator: Institute of Hydrology, Wallingford, Oxon, UK (John Stewart)

Objectives

- ◆ Assess the accuracy of estimates of evaporation from irrigated crops using formulae that could run on data derived from satellite measurements.
- ◆ Test the success of using measurements of surface temperature to estimate components of the energy budget using published relationships.

Activities

- ★ Evaporation measurements were made over irrigated cotton and wheat within the Yaqui Valley Irrigation Scheme in Sonora State, Northwest Mexico, and compared to estimates of reference crop evaporation by different formulae.
- ★ Evaporation measurements were also made from a tower erected in a natural area of grass, bushes and trees in the Sonoran Desert 30 km from Hermosillo, Sonora State.

Results

⇒ ***Measurements of evaporation from irrigated crops***

The comparison of reference crop evaporation against measured actual evaporation showed that while the crops were fully developed the agreement was good. One of the formulae for estimating reference crop evaporation only requires measurements of incoming solar radiation and climatological values of temperature. A previous EC project had shown that reliable estimates of daily incoming solar radiation could be obtained from geostationary satellite data. Therefore, the reference crop evaporation could be successfully derived from satellite data. However, it was found that the measured actual evaporation was less than the reference crop evaporation, when the crop was in the early development and senescing stages. By using published crop factors the over-estimation of the measured actual evaporation was successfully corrected.

⇒ ***Measurements of energy components of semi-arid rangeland***

Measurements of the heat lost into the atmosphere showed that estimates derived from measurements of surface temperature and published relationships were significantly in error. However, the use of scintillometer measurements could be used to calibrate the relationship, allowing use of satellite data to determine the heat loss and hence the evaporation. The ten months of continuous measurements also showed how the evaporation varied during the dry and wet seasons.

Follow up

The results for the irrigated crops show that an operational scheme could be set up using satellite data to provide irrigation managers with daily or monthly regional values of reference crop evaporation and actual evaporation for irrigated crops.

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**EXPERIMENTAL AND NUMERICAL STUDY OF PRECIPITATION FORMATION
IN NORTHEAST BRAZIL**

Co-ordinator: Météo-France, Centre National de Recherches Météorologiques, Toulouse,
France (J.L. Brenguier).

Objectives

The general goal was to conduct experimental studies in North-East Brazil to document the microphysical properties of clouds in relation to the characteristics of the air masses, for improving cloud and rain parameterization in the forecast models.

Activities

- ★ The initial phase of the project involved staff training in cloud microphysics and in-cloud microphysical measurements using an aircraft equipped with measuring instruments. This included operating airborne instruments to study the dynamics, thermodynamics and microphysics of droplet nuclei, cloud droplets and drops; the methods for processing airborne measurements and analyzing data, and finally, numerical modelling of clouds with Large Eddy Simulation models (LES).
- ★ The second phase consisting of field campaigns in North-East Brazil, focused on the differences between clouds developing in marine air masses and air masses contaminated by natural continental aerosols, bio-mass burning and industrial pollution.

Results

- ⇒ A scientist was trained at Météo-France in the maintenance, calibration, operation and data analysis of a droplet spectrometer especially built for the project. Detailed analysis of the microphysical data collected in 1994 with an instrumented aircraft in Ceara clearly shows the effect of urban pollution around Fortaleza on cloud microphysics.
- ⇒ Two students were trained at Météo-France and at the University of Manchester on the use of a non-hydrostatic LES model for small-scale simulation of cloud dynamics and microphysics. A 3-D research model developed at Météo-France (Meso-NH) was installed on the University of Fortaleza computer and the code adapted to the orography of North-East Brazil.
- ⇒ Technical studies were performed at the Centre d'Aviation Météorologique of Météo-France for the development of an integrated system for thermodynamics and turbulence measurements on aircraft. The system was especially designed for installation on the Brazilian instrumented aircraft (Bandeirante).

Follow-up

The Cearense group is now ready for conducting a field experiment on cloud microphysics and the experimental study on precipitation formation. However, the integration of the instrumentation on the aircraft has been delayed for logistical reasons and the campaign has been postponed to the winter 1998-1999.

Selected publications

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STUDY OF THE FATE AND IMPACT OF POLLUTANTS ON THE COSTA RICAN COASTAL ZONE

Co-ordinator: Vrije Universiteit Brussel, Brussel, Belgium (P. Polk)

Objectives

The central theme of this project was a better understanding of the behaviour and impact of pollutants in tropical areas.

- ◆ The first objective was to evaluate the influence of water exposure concentrations, environmental variables and biological variables on the accumulation of organic and inorganic pollutants in different organisms, representative for different trophic levels. This information could then be used to optimize normalization procedures for pollutant data, allowing for reduction in variability of data and to develop accumulation models, allowing for a better understanding of the remaining variabilities.
- ◆ The project furthermore intended to gather basic information on the state of the Costa Rican marine environment and, if necessary, to formulate suggestions towards improved sustainable development.

Activities

Environmental samples (water, sediments and biological organisms of different trophic levels) were collected in different areas of the Costa Rican coastal zones over a two year period. The concentrations of locally used pesticides (such as 3,4-dichloroanilide, methamidophos, cadusafos, chlorpyrifos, diazinon, ethoprophos, fenamiphos, monocrotophos, parathion methyl, terbufos, edifenphos, tiobencarb, pendimetalin, oxadiazon, carbofuran, chlorthalonil, ametryn, imazalil, thiabendazole and propiconazole and organochlorine pesticides), PCBs as well as ten transition and post-transition metals were analyzed in the samples. The relations between the pollutant concentrations and environmental (DOC, TOC, temperature, salinity, pH) and biological data (lipids, physiology) were evaluated and whenever possible, normalization procedures were deducted. Short-term pollutant uptake experiments were conducted under different levels of POC/DOC and the uptake rates of different pollutants were calculated. Results were compared with predictions of an accumulation model.

Results

Pesticides residues in the analyzed samples were generally below or close to the quantification limits. Carbofuran (0.20-6.27 µg/L), propiconazol (0.06-1.5 µg/L), ethoprophos (0.09-0.28 µg/L), cadusafos (0.04-0.07 µg/L), diazinon (0.24-0.31 µg/L) and fenamiphos (0.12-0.40 µg/L) were detected mainly in the water samples, some of them at the Tortuguero channel outlet to Caribbean Sea; propiconazol (6-19 µg/K dw) was the only pesticide detected in sediments. In the samples of organisms, no pesticide residues were found. Lipids were the most important co-variable for stable apolar organic pollutant levels in biological organisms. Normalization of PCB levels on lipids reduces data variability among biological samples of

different trophic levels. The data indicated that, in order to compare data internationally, lipid and lipid class extraction and analyzing procedures should be standardized. For metals, no or only a poor relationship between exposure and accumulation of metals by aquatic organisms was observed. The accumulation levels of metals in some organisms were higher than expected, most likely due to high uptake efficiencies and/or low elimination rates. The comparison of the results from the laboratory uptake and accumulation studies revealed interesting relations between the physico-chemical characteristics (K_{ow} , solubility and vapour pressure) of the organic pollutants (PCBs and locally used pesticides) and their fate in the tropical aquatic and biological compartments.

Follow up

A last sampling campaign has been performed. The samples were analyzed. The last six months (extension of the project) essentially aimed at compiling and synthesizing all results. The results were compared to predictions of a bioenergetics based accumulation model, using estimates of uptake and elimination rates as well as environmental and biological variables.

Publications

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**NUMERICAL SIMULATION OF GAS FLOW IN POROUS MEDIA WITH
APPLICATION TO LANDFILL**

Co-ordinator: Wessex Institute of Technology, Southampton, UK (Henry Power)

Objectives

The objective of this project was to develop a scheme for the numerical solution of the convection-diffusion equation for flow of a mixture of gases in multi-layer porous media to be applied for the prediction of the dynamic behaviour of landfill systems.

Activities

- ★ The main activities included development of a boundary element method (BEM) scheme for the solution of the problem of convection-diffusion flow of a mixture of gases in multi-layer porous media using the dual reciprocity method (DRM) to take the domain integrals to the boundary. The model was applied to the problem of emission of gases from a landfill.

Results so far

- ⇒ Two different DRM formulations were developed, where the first one treats the whole problem domain as a single one, and the second formulation divides the initial domain into a large number of sub-domains. The main advantage of the first kind of boundary only formulation is the reduction in data preparation, as only surface elements are necessary. The main advantages of the second scheme are: no numerical integration is required (surface of domain), there is no major restriction on the arrangements and forms of the sub-regions, and the final matrix system is band-diagonal which is suitable for solution of large problems. Three different DRM approximating functions were compared, regarding the accuracy of the predicted potential and its normal derivative on the boundaries, so the most appropriate one can be chosen for future work.
- ⇒ The numerical model developed was applied to the problem of the design of venting trenches in multi-layer landfills. Several different types of venting trenches were analysed which could be divided in two major groups: a) venting trenches crossing the whole depth of the landfill, and b) venting trenches crossing the cover layer only. It was found that the trenches b) are marginally less efficient than the trenches a) which are commonly used in landfill gas control strategies. It is common practice in landfill engineering to install a low permeability layer of small thickness (separation layer) between waste layers. If the venting trenches b) are implemented in such conditions, it is necessary to provide a pathway at the separation layers for a free flow of the gases between waste layers in order to avoid pressure build up at the waste layers. The numerical results show that the efficiency of the trenches b) is not significantly affected by the presence of separation

layer between waste layers, as long as flow pathways are provided within the separation layer.

Follow-up

The results obtained, though giving some guidance in designing landfills, are based on the assumption of two-dimensional isotropic landfill. It is hoped that research can be continued on the development of a fully three-dimensional, anisotropic model in order to obtain more realistic results about the efficiency of the landfill gas-emission control structures.

Selected publications

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ASSESSMENT OF THE IMPACT CAUSED BY THE INTRODUCTION OF A *PINUS CARIBAEA* FOREST ON THE ARTHROPOD COMMUNITY IN A VENEZUELAN NATURAL SAVANNA.

Co-ordinator: Autonomous University of Barcelona, Barcelona, Spain (Carmen Bach)

Objectives

- ◆ The general aim was to determine the composition of arthropod fauna in a Venezuelan savanna and then to determine the origin and composition of the arthropod fauna in the introduced pine forest.
- ◆ The final objective was to define the effect of the introduction of the pine forest on the arthropod fauna of the savanna.

Activities

The first task was sampling; so we developed sampling methods in field plots. There are seven field plots, each of them representative of a given set of conditions. Sampling began in June 1996. It was done monthly until June 1997. In July-September 1997 a more extensive sampling was carried out in order to provide a more detailed picture of the successional process.

Results so far

In the course of 1997 the primary separation of the samples was begun. It was a hard task because the material was very heterogeneous (samples mixed with soil and remains of vegetation, etc.) so it was necessary to undertake a cleaning operation which was accomplished simultaneously with a primary division of taxonomic nature. Labelling and storage was done at the same time.

Follow-up

- During the last year an analysis of the vegetation of the six savannas was done by Venezuelan team. They analyzed the vegetation on two sampling dates for comparative purposes. The analysis was centred on the differences and similarities between the six savannas; it was concluded that this is a group of rather homogeneous savannas with respect to green and dead biomass (green/dead ratios close to one) and that it displays a discrete increase of biomass and cover at the end of the rainy season.
- Now, efforts are being made to finish the primary separation of the samples. Because of the time necessary to work the separation of the samples, any publications have not yet been prepared.

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ALLOCHTHONOUS ORGANIC MATTER AS A RESOURCE FOR STREAM COMMUNITIES: COMPARISON OF KEY PROCESSES AMONG DIFFERENT GEOGRAPHICAL AREAS

Co-ordinator: Universidade de Coimbra, Coimbra, Portugal (Manuel A.S. Graça)

Objectives

This project addressed functional aspects of organic matter dynamics in three contrasting biomes at three levels:

- ◆ energetic bases,
- ◆ fungi - detritus interactions and
- ◆ invertebrates - fungi + leaves interactions.

Therefore, we intended to test the following hypothesis: System functioning of temperate and tropical low-order streams is identical. Specifically, we hypothesised that:

- ◆ Temperature corrected breakdown rates of a given leaf species are similar across latitude.
- ◆ There are not differences between temperate and tropical streams in fungal biomass accumulation on decomposing leaf litter.
- ◆ Patterns of fungus - shredders interactions follow identical principles in these streams.

Activities

- ★ Measurement of leaf litter production in Mediterranean (Portugal) and Tropical (Venezuela) forests and the transport and accumulation of organic matter in streams running through these forests.
- ★ Measurement of breakdown rates of key leaf types in 3 systems: Northern Europe (Germany), Mediterranean area and a Tropical zone.
- ★ Measurements of associated macroinvertebrates, fungal biomass, nitrogen content, chemical changes in the leaves and diversity of aquatic hyphomycetes on decomposing leaves.
- ★ Determination of the importance of leaf conditioning on growth and survivorship on key detritivores.

Results

The annual litterfall at the Venezuelan site was twice as much as in Portugal. Decomposition was fastest in the tropical and slowest in the German streams. Temperature differences can only explain part of the differences. Dry mass loss was significantly higher in coarse mesh bags than in fine mesh bags. In all climatic zones, invertebrate colonisation was apparent by one week of leaf submersion. Maximum ergosterol contents were reached by day 42 in the temperate German streams and day 14-28 in the tropical stream. Total ergosterol amount was similar in the three systems. Consistently, invertebrate growth was significantly higher on the conditioned food. However, a similar difference was not apparent in the case of the caddisflies (temperate and tropical) fed conditioned and non-conditioned *Hura* leaves.

Detritivores from both temperate and tropical streams discriminated between leaves of different conditioning status.

Follow-up

We are now analysing the data and drafting the papers. New proposals are being prepared to give continuity to the work and collaboration among the three partners.

Selected publications

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Contract number: CI1*CT940111

Period: April 1995 to August 1998

**MODEL VALIDATION, ATMOSPHERIC VARIABILITY AND CLIMATE
SENSITIVITY OVER SOUTH-AMERICA**

Co-ordinator: Laboratoire de Météorologie Dynamique du CNRS, Paris, France
(H. Le Treut)

Objectives

The climate of South America is affected by a number of specific features : (i) presence of the Andes, (ii) contrast between the forested regions of Amazonia and the deserts of Southern Argentina, (iii) influence of both the Pacific (El Niño) and the Atlantic ocean.

The objective of the project was to evaluate the impact of those processes on the regional climate, as simulated by the models, using available observations. During the course of the project it has been decided to shift the emphasis from higher latitude regions to the meteorological processes linking tropical and extratropical regions (South Atlantic Convergence Zone, Low Level Jet).

Activities

- * The work has been organized on the basis a yearly meeting, and more specific visits (from a few weeks to a few months).
- * The European modeling group have carried out long inter-annual simulations of the climate, at the global scale.
- * The South American partners run models at a shorter time scale, but with a higher spatial resolution over South-America.
- * The CIMA group also used the LMD General Circulation Model to embed its regional model.
- * The visits were defined to permit the sharing of these tools and results, and the development of specific diagnostic methods.

Expected outcome and results so far

The region of Northern Argentina and Southern Brazil (which roughly corresponds to the Parana basin) has a very strong economic importance, and very much depends on hydrological resources and therefore on climatic fluctuations. The present project will improve the assessment of regional climate changes in response to global changes (natural, such as those due to El Niño, or anthropogenic).

Follow-up

This project requires a follow-up at two levels:

- Regular visits between present partners are required to ensure the continuity of the diagnostic work. These visits are also necessary to assist the use of the LMD GCM by the CIMA/CONICET;

- the nature of the project implies its extension to new partners in order to address not only the possible occurrence of local climate changes, but also their potential impacts.

Selected publications

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Period: March 1995 to December 1997

REACTION DYNAMICS AND PHOTOCHEMISTRY WITH APPLICATIONS IN ATMOSPHERIC CHEMISTRY AND COMBUSTION

Co-ordinator: University College, London, UK (David C. Clary)

Objectives

The aim was to combine the expertise of the European and Argentinian groups in theoretical and experimental studies of elementary chemical reactions important in atmospheric and combustion chemistry.

Activities

The project is concerned with chemical reaction dynamics, including radical and recombination reactions over a wide variety of conditions. Experimental techniques include crossed molecular beams and laser. Theoretical methods developed in the project include quantum scattering algorithms, classical trajectory computations and statistical theories. Potential energy surfaces for chemical reactions have also been calculated. Collisional energy transfer between molecules is also being studied including relaxation of highly excited molecules and temperature dependence. Photofragmentation of molecules is also a major topic include detailed quantum mechanical calculations and analytical approximations on atmospherically important molecules such as ozone and ClNO.

Results so far

A new quantum theory for four-atom reactive scattering has been developed jointly by the groups in London and Quilmes and applied to the OH + H₂ reaction, important in combustion. Similar theory has also been jointly extended to the Cl + CH₃Cl reaction. The groups in Madrid, Quilmes and London have also performed calculations on the O₂ + O₂ → O₃ + O reaction with one O₂ highly vibrationally excited and have shown that this is not a source of ozone in the stratosphere. The groups in Göttingen and La Plata have studied energy transfer in azulene and also the OH + CO reaction which is a key reaction in combustion. The groups in Toulouse, Madrid and Buenos Aires have developed new computational and analytical methods for studying the photodissociation of molecules solvated by rare gases. The teams in Madrid (Complutense) and Cordoba have studied the Ca + CH₃I reaction. The group in Cordoba has also done extensive work on the kinetics and mechanisms of CF₃ radicals, a key species for atmospheric chemistry.

Selected publications

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Health and Biomedical Sciences

**PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTIONS IN WOMEN WITH
CERVICAL DYSPLASIAS, CARCINOMA OF THE CERVIX AND IN A NORMAL
HONDURAN POPULATION**

Co-ordinator : Katholieke Universiteit Nijmegen, Nijmegen, The Netherlands
(Willem JG Melchers)

Objectives

The general goal is to verify the value of a molecular test for the detection of human papillomavirus (HPV) to prevent deaths from cervical cancer.

Activities

Carcinoma of the cervix is the most common type of cancer in the developing world and the leading cause of death from cancer among women in developing countries. The project focused on studying the relationship between HPV infections and the development of cervical disorders and on obtaining epidemiological data to assess the importance of co-factors in the pathogenesis of cervical cancer. Cervical samples from women with the whole spectrum of cervical abnormalities were selected in a case-control definition in Tegucigalpa (Honduras). The DNA from all samples was extracted in Tegucigalpa and sent for further processing to Nijmegen (The Netherlands). Detection and subsequent HPV typing was carried out in Nijmegen. The HPV outcome was coupled to a wide range of possible co-factors, collected with a questionnaire from all cases and controls during sampling in Tegucigalpa. All data were statistically analysed to identify risk-factors for the development of cervical cancer in Tegucigalpa and Nijmegen.

Results

A molecular biological laboratory was built and fully equipped in Tegucigalpa to train scientists in Honduras. From the case-control study, it is evident that there is a close correlation between HPV infections and the subsequent development of cervical dysplasias and cervical cancer. As in most other parts of the world, HPV types 16 and 18 are the most prevalent oncogenic HPV types in Honduras although HPV 31 is also frequently found. One of the most striking findings was the large number of "rare" HPV types (HPV types other than HPV 6,11,16,18,31, and 33). In fact, 25% of the HPV positive samples from women with cervical cancer contained an "HPV X". Further analysis revealed a high number of mainly HPV 58 among the HPV X samples, which means that next to HPV 16, HPV 58 is the most prevalent oncogenic HPV type in this geographical region, and in fact in Latin America. Another striking result was the remarkably high number of positive women who had no cervical abnormalities (up to 40%), another common feature in Latin America as it seems from the literature. A number of risk factors (co-factors) were identified from the statistical analysis of the total number of data-sets analysed. Apart from HPV infection, early age of first intercourse, early age of first pregnancy, increasing parity and parity at a young age were significantly related to an increased risk of cervical dysplasia and cancer, also exposure of women to wood smoke although cigarette smoking was not associated.

Follow-up

Now the project as described has ended, our attention has focused on possible associations of genetic factors in susceptibility/resistance to HPV infection-related regression or progression of dysplasia to cervical cancer (collaboration with Mexico). This could identify markers for future therapeutic vaccination. In another study we use an engineered poliovirus as a vector system for the expression of HPV antigens (L1 and L2) (in collaboration with Colombia).

Selected publications

- Melchers W., Ferrera A., Willemsse D., et al., 1994. Human papillomavirus and cervical cancer in Honduran women. *Am. J. Trop. Med. Hyg.* **50**: 137-142.
- Ferrera A., Baay M., Herbrink P., Figueroa M., Velema J., Melchers W., 1997. A sero-epidemiological study to the relationship between sexually transmitted agents and cervical cancer in Honduras. *Int. J. Cancer* **73**: 781-785.
- Ferrera A., Melchers W., Velema J., Figueroa M., 1997. Association of infections with human immunodeficiency virus and human papillomavirus in Honduras. *Am. J. Trop. Med. Hyg.* **52**: 138-141.

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Period: September 1993 to August 1994

**IMMUNOPATHOLOGY OF LESIONS IN PARASITIC AND AUTOIMMUNE DISEASE
- ROLE OF EXTRACELLULAR MATRIX**

Co-ordinator : Hôpital Necker - CNRS URA 1461 - Paris, France (Mireille Dardenne)

Objectives

Extracellular matrix (ECM) influences the entrance of lymphoid precursors into the thymus anlagen and lymphocyte binding to ECM is higher in activated T cells. Interactions mediated by ECM ligands and receptors might intervene in migration and/or activation of effector cells. Our project aimed to investigate the putative role of ECM on the immunopathology of lesions in distinct models, including autoimmune and parasitic diseases, as well as a physiological model of intrathymic T cell migration and differentiation.

Activities

- ★ ECM components perform key functions in several biological processes including cell migration and differentiation, from early ontogeny to ageing. The choice of the various models mentioned above was related to expertise of the three research teams involved in the project, and converging results from such independent studies were expected to pen a rather wide concept of the physiology of the immune system.
- ★ The studies on thymocyte migration and differentiation were made jointly by French and Brazilian groups using immunohistochemical methods. The Brazilian team set up *in vitro* models to study the role of ECM ligands and receptors in the interactions between thymic epithelial cells (TEC) and thymocytes. Using foetal thymus organ culture they also determined the role of some ECM proteins such as fibronectin in the regulation of the T-cell repertoire.
- ★ Autoimmune and parasitic diseases were studied in the three groups: the French group studied the NOD mouse, a model of type I diabetes, the Brazilian group conducted studies on experimental Chagas' disease, and the German team focused on human and murine myasthenia gravis.
- ★ Work on this project also directly encouraged the development of various doctoral theses, thus contributing to stimulate of young students to do research. This was particularly important in Brazil, where the need for active scientists is specially high.

Results

⇒ Several specific objectives of the project have been completed in both physiological and pathological situations and the concept stating that ECM is relevant to T cell physiology at different lesion sites, in both autoimmune and parasitic diseases, has been consistently established. Regarding thymocyte migration and differentiation, we made a phylogenetic

study of the distribution of ECM ligands (fibronectin, laminin, tenascin) and their respective receptors in the mammalian thymus; we demonstrated that TEC/thymocyte interactions were mediated by these molecules and could be modulated by neuroendocrine circuits.

⇒ The studies conducted on experimental Chagas' disease focused on two target organs, the thymus and the heart, and the mechanisms involved in triggering autoimmune myocarditis were analyzed. They led to therapeutic approaches to block heart rejection in chagasic animals. In the NOD mouse, our studies led to evidence of thymic abnormalities involving ECM molecules, and in myasthenia gravis, we evidenced a positive correlation between TEC-mediated ECM expression and malignancy.

Selected publications

Ribeiro dos Santos R., Laus J.L., Silva J.S., Savino W., Rossi M., Mengel J.O. 1992. Anti-CD4 abrogates rejection and reestablishes long-term tolerance of syngeneic newborn hearts grafted in mice chronically infected with *Trypanosoma cruzi*. *J. Exp. Med.*, **175**: 29-39.

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Colomb E., Savino W., Wicker L., Peterson L., Dardenne M., Carnaud C. 1996. Genetic control of giant perivascular space formation in the thymus of NOD mice. *Diabetes.* **45**: 1535-1540.

Silva-Barbosa S.D., Cotta de-Almeida V., Riederer I., De Meis J., Dardenne M., Bonomo A.C., Savino W. 1997. Involvement of laminin and its receptor in abrogation of heart graft rejection by autoreactive T cells from *Trypanosoma cruzi*-infected mice. *J. Immunol.*, **159**: 997-1003.

De Mello-Coelho V., Villa-Verde D.M.S., Dardenne M., Savino W. 1997. Pituitary hormones modulate extracellular matrix-mediated interactions between thymocytes and thymic epithelial cells. *J. Neuroimmunol.*, **76**: 39-49.

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Period: November 1992 to April 1996

**LONDON-SAO PAULO COLLABORATIVE PROJECT ON THE MOLECULAR
GENETICS OF THE MAJOR PSYCHOSES.**

Co-ordinator : Institute of Psychiatry, London, United Kingdom (Michael Gill)

Objectives

Identify large multigenerational Brazilian pedigrees with a high density of schizophrenia or manic-depressive psychosis. Brazil is an ideal country because of its cultural, religious and demographic factors to obtain such multigenerational pedigrees with large sibships. Cases will be diagnosed according to the methods of the European Science Foundation Collaboration, and immortal cell lines will be generated from all members of the pedigrees. DNA from these cell lines will be used in a linkage analysis program underway at the Institute of Psychiatry, and will be made available to other researchers on a fully collaborative basis.

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Period: November 1992 to November 1995

**MOLECULAR APPROACHES TO THE STUDY OF THE SECRETORY PATHWAY
IN *ENTAMOEBIA HISTOLYTICA***

Co-ordinator : Consejo Superior de Investigaciones Científicas, Granada, Spain
(Antonio González)

Selected publications

A. Alagón, A. Cortés, F. Overa, A. Olvera, A. González, P. Lizardi. 1996. Identification and analysis of the u6 small nuclear RNA gene from *Entamoeba histolytica*. Gac. Med. Mex. (Mexico). Diseño y construcción de vectores para la transformación estable de *entamoeba histolytica*.

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**MECHANISMS OF ANTIGEN-PROCESSING STUDIES IN T-CELL TOLERANCE
AND ESTABLISHMENT OF A MODEL OF B-CELL-MEDIATED SYSTEMIC
DISEASE INDUCED IN TRANSGENIC MICE BY PASSIVE TRANSFER OF T-CELL
SPECIFIC AGAINST A B-CELL-SPECIFIC ENDOGENOUS ANTIGEN**

Co-ordinator : German Cancer Research Center, Heidelberg, Germany
(Günter J. Hämmerling)

Objectives

The project focuses on the processing of endogenous antigen, the immune recognition of endogenous antigens, and the conditions leading to either tolerance or autoimmunity. To achieve this we have kinetically modified the model antigen hen egg lysozyme (HEL) and expressed it in distinct intracellular compartments of antigen presenting cells, namely in the cytosol on the plasma membrane, and in the endoplasmatic reticulum (ER). Presentation by MHC class II molecules was assessed using a panel of T cell hybridomas specific for different HEL epitopes.

Results

The most important site for processing and loading of endogenous antigen proved to be the endosomal/lysosomal compartment, regardless of the precise location of intracellular expression, implying that the antigen must somehow reach the endosomal compartments. This was even too when expression was confined to the ER as no significant loading onto MHC class II molecules could be observed in the ER. However, distinct HEL epitopes were differently presented depending on the intracellular sight of expression suggesting that they were processed and loaded in different compartments of the endosomal/lysosomal system. These studies on the processing pathway were complemented by studies on tolerance and autoimmunity using transgenic mice in which a model antigen (K^b) was selectively expressed outside the thymus. Such extra-thymic expression, e.g. in the liver, resulted in non-deletional tolerance. Interestingly, although tolerance could be successfully broken in vivo by application of antigen in combination with Il-2, no autoimmunity was observed. However, when the mice were subsequently infected with *Listeria monocytogenes* autoimmunity occurred in the liver. These data suggest the presence of autoactive T cells does not necessarily cause autoaggression, but that additional factors generated during an inflammatory response enable the autoreactive cells to become tissue-destructive.

Follow-up

The findings achieved in this joint project have important implications for our understanding of antigen processing and recognition, and the pathogenic events leading to autoimmunity. The mechanistic details for autoimmunity mediated by B and T cells are presently being investigated.

Selected publications

Bonifaz I., Hämmerling G.J., Garcia Hinojosa A.P. and Moreno J. Cryptic determinants derived from different intracellular forms of the same protein (to be submitted).

Bonifaz I., Hämmerling G.J. and Moreno J. Role of the endoplasmic reticulum in antigen processing for MHC class II presentation (to be submitted).

Arnold B., Alferink J., Limmer A., Tafuri A. and Hämmerling G.J., 1997. Reversal of peripheral T cell tolerance: Implications for autoimmunity. HLA and Disease - the Molecular Basis, Alfred Benzon Symposium 40. Eds. A. Svejgaard, S. Buus, L. Fugger, Munksgaard, p. 272-278.

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**ANALYSIS OF CYTOKINE PRODUCTION IN SYSTEMIC LUPUS
ERYTHEMATOSUS AND RELATED DISORDERS**

Coordinator: INSERM U131, Clamart, France (Pierre Galanaud)

Objectives

The objective of the project was to improve the definition of cytokine production abnormalities in systemic lupus erythematosus (SLE), and to determine their contribution to immunological abnormalities of the disease.

Activities

The project focused on interleukin-10 (IL-10), which appeared to be the best candidate to explain both the auto-antibody production and the decreased cell-mediated immunity of the disease. All samples studied were collected by the Mexican partner, in view of the frequency of SLE in this country as well as on the possibility to evaluate relatively large numbers of newly discovered, and therefore untreated, patients. Immunological evaluations were performed both in Mexico and in France, with the techniques developed in the laboratory of the French partner.

Results

We demonstrated an increased production of IL-10 in SLE patients, as well as in some other B lymphocyte-mediated autoimmune disorders (Sjögren's syndrome, rheumatoid arthritis). We showed the B lymphocyte and monocyte origin of IL-10 hyperproduction in these disorders. Using SCID mice engrafted with patients' mononuclear cells, we showed that the production of auto-antibodies is totally dependent on the production of human IL-10. We also showed that healthy relatives of SLE patients, who are known to display frequent immunological abnormalities (impaired cell-mediated immunity, presence of non pathogenic auto-antibodies), display in 70% of cases the same IL-10 hyperproduction as patients.

Follow-up

We have planned two extensions of this work. The first one, to test genetically patients and their relatives in order to determine whether the recently described polymorphism of IL-10 gene regulatory sequences explain IL-10 dysregulation in SLE. The second one, to set up a therapeutical trial in refractory SLE with an anti-IL-10 mAb provided by a French company, in Mexican patients.

Selected publications

L. Llorente, Y. Richaud-Patin, J. Wijdenes, J. Alcocer-Varela, M.C. Maillot, I. Durand-Gasselin, B. Morel-Fourrier, P. Galanaud and D. Emilie, 1993. Spontaneous production of Interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. *Eur. Cytokine Netw.* **4**: 421-430.

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Period: February 1993 to February 1995

**CHARACTERIZATION OF LEISHMANIA PARASITES AND THEIR VECTORS
FROM CENTRAL AMERICA USING MOLECULAR TECHNIQUES**

Co-ordinator : University Liverpool, Liverpool, United Kingdom (R. Ward)

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RABIES AND VACCINATION IN VAMPIRE BATS

Co-ordinator : Université de Liège, Liège, Belgium (Paul-Pierre Pastoret)

Objectives

- ◆ Describe the genetic variability of rabies viruses in Mexico and Latin America by using molecular biology tools.
- ◆ Define (by genomic sequencing) a viral isolate representative of vampire lineage for challenge experiments.
- ◆ Learn more about rabies virus infection in vampire bats, using a challenge rabies strain representative of vampire lineage.
- ◆ Evaluate the efficacy of oral vaccination of vampire bats against experimental challenge (homologous challenge virus), using a recombinant *vaccinia* virus that expresses the immunizing glycoprotein of rabies virus (VRG vaccine, currently used for sylvatic rabies control in Europe).

Activities

- * The project focused on the molecular analysis of rabies variants in Mexico and on the study of a specific rabies infection and oral vaccination in the most important sylvatic vector of rabies in Mexico and Latin America: vampire bats (*Desmodus rotundus*).
- * Concerning the molecular analysis of rabies variants, the isolation of different strains of rabies was carried out in Mexico on different animal species and covering a wide geographical area of the country. Molecular analyses were carried out in France, including the selection of a representative vampire challenge strain (strain CASS-88).
- * The studies on rabies infection and oral vaccination in vampire bats, capture of adequate animals, adaptation to captivity and experimental trials including challenge, oral vaccination, serological sampling, virus isolation, rabies diagnosis, etc., were carried out in Mexico with the help of the partners from Belgium. Serological analyses and statistical studies were carried out in Belgium.

Results

- ⇒ The molecular analysis of rabies variants in Mexico allowed us to find important facts on the epidemiology of rabies virus in Mexico. As previously indicated, in the whole Latin America two rabies epidemiological cycles prevail: one is terrestrial (the main vectors of which are dogs, coyotes, racoons, etc.) and the other one is aerial (the main vector of which is the vampire bat). In spite of these two well-known cycles, a new cycle, the main vector of which is probably skunk (*Mephitis mephitis*), was found in Mexico during this project. On the other hand, we found that the virus that circulates in foxes is shared by wild cats.
- ⇒ Experimental infection in vampire bats with aerial virus strain CASS88, allowed us to find that this specific infection produces a paralytic non-aggressive disease in these species. Some vampire bats could excrete infectious virus without clinical signs. These results could partially explain the high mortality rates (20- 30%) in paralytic rabies outbreaks of

cattle in Mexican environments. Vaccination of vampire bat with VRG recombinant vaccine could protect these chiroptera species against lethal homologous aerial virus challenge. These results could provide some new strategies on the control of wild rabies in Latin American countries.

Follow-up

- Epidemiological and antigenic characteristics of the new virus (coming from skunks) reported in this project are now being studied by the Mexicans with the help of the partners from Belgium and France.
- New work on the excretion of rabies virus in the saliva by vampire bats, with different degrees of immunity has begun, as well as the study of new DNA vaccines.

Selected publications

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- Bahloul Ch., Badrane H., Sacramento D., Morgeaux S., Loza Rubio E., Brochier B., Perrin P., Gavilán-Salinas A., Aguilar-Setién A., Pastoret P.P., Tordo N. 1997. Molecular Epidemiology of Rabies and cross-protection studies in Latin America (submitted).
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- Loza Rubio E., Aguilar-Setién A., Bahloul Ch., Brochier B., Pastoret P.P., Tordo N. Discrimination between aerial and terrestrial epidemiological cycles of rabies virus in Mexico, using RFLP. Arch. Invest. Med. (submitted)

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Period: May 1993 to April 1996

**ANALYSIS OF THE T-CELL RECEPTOR SPECIFICITY IN LYMPHOCYTES
INFILTRATING THE INTESTINAL MUCOSA IN CELIAC DISEASE CHILDREN
OF HISPANIC ORIGIN**

Co-ordinator: Universitat Autònoma de Barcelona, Bellaterra, Spain
(D. Jaraquemada)

Objectives

Characterization of the T-cell repertoire of lymphocytes infiltrating the intestinal mucosa of CD patients. Generation of T-cell clones from lymphocytes infiltrating intestinal mucosa from CD patients. Identification of the HLA molecule(s) involved in antigen presentation and correlation with T-cell receptor usage.

Activities

Characterization of the T-cell repertoire associated with CD in the intestinal mucosa was carried out in Barcelona with samples from CD and normal biopsies from Buenos Aires. The technique was set up and transferred to the Buenos Aires laboratory. *In vitro* culture of biopsy material was started in Buenos Aires, and the resulting cultured cells were cryopreserved and transferred to Barcelona where the T-cell cloning was performed. Cytokine expression was examined by RT-PCR, using cytokine-specific primers, and was performed in parallel in both laboratories.

Results

Characterization of the T-cell repertoire associated with CD in the intestinal mucosa was only partially achieved. TCR was analyzed in few samples due to limited material, but the data showed normal distribution of Vbeta and alpha TCR families. Cloning T cells from the biopsies gave several T-cell clones with epithelial specificity and a classical intra-epithelial phenotype. Cytokine analysis was performed in a series of biopsies from CD patients of grades I, II, III and IV as well as normal biopsies, and the results have shown a largely heterogeneous pattern in the cells infiltrating the intestinal epithelium in the early stages of the disease, with high, increased expression of both IL-4 and IFN- γ which, in the later stages of the disease, results in a deviation in favour of a predominant Th1 (IFN- γ) pattern of response.

Follow-up

This work is being continued in Barcelona by a scientist from the Argentinian laboratory. His project is based on the study of the ligand(s) recognized by T-cell clones from infiltrated intestinal mucosa in the epithelium, and the relationship of this/these ligand(s) with the CD1 molecules, which are recognized by the T-cell clones in a series of transfectants.

Selected publications

Rubio A.E., Cherniavsky A.C., De Rosa S., Roura-Mir C., Jaraquemada D., Fainboim L., and Satz L. High levels of IL-4 followed by a switch to Th1 dominance correlate to disease progression in celiac disease. Submitted for publication.

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Period: March 1993 to February 1996

**INCIDENCE AND PATHOGENESIS OF HTLV-I INDUCED ADULT T-CELL
LEUKAEMIA/LYMPHOMA IN BRAZIL**

Co-ordinator : The Royal Marsden Hospital, London, UK (D. Catovsky)

Objectives

To ascertain the incidence and role of the human retrovirus HTLV-I in the pathogenesis of adult T-cell leukaemia/lymphoma (ATLL) in Brazil.

Activities

- ★ Assessment of the incidence of ATLL in Brazil and establishment of a national register of cases and a network of centres to collect information and material for the study which was centralised at the National Cancer Institute of Rio de Janeiro. This aim was achieved in the first two years.
- ★ Development of an international cooperation between groups in Brazil, Chile, Japan and the UK. This involved Japanese groups with experience in the serological and molecular techniques for diagnosis of HTLV-I and in the possible genetic diversity in relation to the retroviruses HTLV-I and TT.
- ★ Development of sensitive techniques for detecting HTLV-I and search for the retrovirus in other T-cell malignancies.
- ★ Use of novel therapies for the treatment of ATLL.

Results so far

- ⇒ A register of cases has been set up as well as the organisation of annual meetings in Brazil on HTLV-I induced malignancy. This series of international symposia culminated, in 1997, with hosting the Eighth International Conference on HTLV-I in Rio de Janeiro. Development of new techniques such as PCR have been applied to samples received from Brazil and studied in the UK. A reference laboratory has also been set up in Rio for diagnosis of ATLL. The first estimates of the incidence of the disease in Brazil have been established in large studies and these range from 0.5-1% in blood donors to 8-10% in patients with haematological malignancies, a third of which are of T-cell nature. In those who had received multiple transfusions the incidence was determined as 15%.
- ⇒ Progress towards the therapy of ATLL was initiated with cooperative studies in Europe with a common protocol using the combination of interferon and zidovudine.

Follow-up

Since completion of the project in 1996, there has been great impetus in the studies in Brazil and Chile and some improvements in treatment have taken place. Further studies on new therapies in ATLL are developing in Europe under the auspices of the HTLV-I European Research Network (HERN) which provides a forum which includes investigators from Latin American countries and, through its workshops, communicates advances in this field.

Selected Publications

Matutes E., Schulz T., Andrada Serpa M.J., de Queiroz-Campos-Araujo A., Pombo de Oliveira M.S., 1994. Report of the Second International Symposium on HTLV in Brazil. Meeting Report. *Leukemia* **8** : 1092-1094.

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Period: January 1993 to January 1996.

**EFFECT OF DIETARY LONG-CHAIN POLYUNSATURATED FATTY ACIDS
ON INTESTINAL REPAIR IN CHRONIC DIARRHEA AND MALNUTRITION**

Co-ordinator: Universidad de Granada, Granada, Spain (Angel Gil)

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Period: April 1993 to April 1996

**THE ROLE OF NEUROTRANSMITTERS IN THE REGULATION OF
GONADOTROPHIN SECRETION DURING PREPUBERTAL PERIOD AND IN THE
ONSET OF PUBERTY IN THE FEMALE RAT**

Co-ordinator: Georg-August-Universität, Göttingen, Germany (W. Wuttke)

Objectives

The aim of this project was to clarify the neuroendocrinological basis of sexual maturation and the onset of puberty in the female rats. For this purpose, the interactions of ovarian hormones with the hypothalamic neurotransmitters, the interrelation between neurotransmitter systems involved in the LHRH hypothalamic control and the resulting effects of LHRH and LH and FSH release were studied in rats during sexual maturation and the onset of puberty.

Selected publications

Feleder C., Jarry H., Leonhardt S., Wuttke W., Moguilevsky J.A., 1996. The GABAergic control of gonadotropin-releasing hormone secretion in male rats during sexual maturation involves effects on hypothalamic excitatory and inhibitory amino acid systems. *Neuroendocrinology* **64**:305-312.

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Roth C, Jung H., Kim K., Arias P., Moguilevsky J., Jarry H., Leonhardt S., Wuttke W., 1997. Involvement of gamma amino butyric acid (GABA) in the postnatal function of the GnRH pulse generator as determined on the basis of GnRH and GnRH-receptor gene expression in the hypothalamus and the pituitary. *Exp. Clin. Endocrinol Diabetes*. **105**:353-358.

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**BIOSYSTEMATIC STUDIES ON MEDICALLY IMPORTANT SIMULIDAE
SPECIES COMPLEXES IN CENTRES OF ENDEMISM IN THE BRAZILIAN
AMAZON**

Co-ordinator : Natural History Museum, London, United Kingdom (Anthony Shelley)

Objectives

Elucidate the biosystematics and distribution of the anthropophilic simuliid species (*Simulium oyapockense*, *S.roraimense*, *S.guianense*, *S.exiguum*, *S.incrustatum*) in three centres of endemism (Central Amazonian, South east Amazonian, Guianan) as a part of the onchocerciasis research and control programme of Brazil.

Activities

The above cited simuliid species are known or suspected vectors of human onchocerciasis (river blindness) in Brazil. An accurate assessment of their distribution and the reasons for this are of paramount importance in predicting dispersal of the disease from the Amazonia focus to other parts of Brazil in conjunction with the migration patterns of gold miners infected in the Amazon focus. The finding of a new focus of onchocerciasis by our team during the course of the project resulted in greater emphasis being placed on the two centres of endemism (Central Amazonian and South East Amazonian) in which the two foci of onchocerciasis occur. Our work concentrated on species elucidation in previously poorly prospected parts of Brazil and species distribution in relation to various physical factors. The team at Fiocruz in Rio de Janeiro were largely responsible for field collections and morphological identifications and distributional studies, while the UK team at the Natural History Museum (London) specialised in integrated morphotaxonomic, cytotoxic and molecular studies on the vector species, their image analysis and archiving and coordination of the project with other researchers. Of particular importance was the use of a new serodiagnostic test for detecting low densities of onchocerciasis, which resulted in the discovery of a new focus of the disease in central Brazil.

Results

- ⇒ Morphological differences between *S.oyapockense* and *S.roraimense* have now been interpreted through cytological and DNA studies and show that a highly complex species group exists, ranging from Venezuela to Argentina, in which markers can be used to identify different biologies of populations. *Simulium guianense* was shown to be a complex of five cytotypes, which now explains differing behaviour patterns that will limit the dispersal of onchocerciasis to other parts of Brazil. Clarification of the taxonomy of the other two vector species was less successful due to the failure to obtain polytene chromosomes from the numerous preparations made. Simuliid distribution was found to be related to river type, altitude, rainfall and vegetation but did not correspond to the centres of endemism established for other animals.
- ⇒ Vector species distribution is a major limiting factor in the dispersal of human onchocerciasis from its Amazonia focus as infected people move to other parts of Brazil.
- ⇒ A Brazilian biologist spent two years in London where he received the appropriate training in cytological and molecular methods to be able to set up a laboratory on his return to Brazil.

Follow-up

- The project provided a framework for simuliid research in the onchocerciasis programme of the Brazilian Ministry of Health. The Brazilian partner is now principal investigator in a major Brazilian on *simuliid* and *onchocerciasis* project which includes Dr Shelley as consultant.
- The main thrust of the project is monitoring of onchocerciasis control in the Amazonia focus of the disease. The British Council and CNPq have set up a three year exchange programme to study the effect of man-made environmental changes on human health using simuliids and mosquitoes as the study groups.
- A new project funded by the Royal Society and CONICET (Dr A.J. Shelley & Prof. S. Coscaron) has been set up in Argentina to discover whether *S.exiguum* is the vector of onchocerciasis and mansonelliasis.
- We are currently studying the feasibility of setting up a network of simuliid workers in Latin America to coordinate research on simuliids as vectors of onchocerciasis and mansoncliasis, as biting pests to man and cattle, as indicators of environmental pollution and as disease dispersal agents in conjunction with human migration patterns.

Selected publications

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ACTIVE SENSORY IMAGING AND THE CENTRAL REGULATION OF PERCEPTION: AN EXPERIMENTAL AND THEORETICAL STUDY OF ELECTROLOCATION IN PULSE-EMITTING ELECTRIC FISH

Co-ordinator: C.N.R.S., Gif-sur-Yvette, France (Kirsty Grant)

Objectives

- ◆ Explore biological neuronal networks involved in forming neuronal representations of the sensory environment,
- ◆ Understand how past memory traces may contribute to modulation of sensory perception.

Activities

- ★ The functional organization of sensory processing and sensorimotor coordination was compared in two species of electric fish: one South American species native to Uruguay and the other from West Africa, using *in vivo* and *in vitro* electrophysiology and anatomical tracing, mainly in Gif, and theoretical modelling based on the experimental data, directed from Montevideo. Uruguayan expertise in theoretical modelling has stimulated the formulation of predictive theories and the design of future experiments.
- ★ A student from Montevideo has spent 2 years in Gif, learning intracellular recording techniques and a similar laboratory for electrophysiological recording has been established in Montevideo.
- ★ Several articles have been published jointly, including two in Nature.

Results

- ⇒ Measurements of skin impedance, internal resistivity and body geometry were used to develop a theoretical "electric" model of the fish and to calculate electric images seen at the electroreceptive surface. Modelling predicted that the center/surround contrast of an object image would indicate the distance to that object. This was confirmed by direct measurement and tested in behavioural studies in collaboration with a new partner team in Germany.
- ⇒ The dynamic properties of motor-command-related descending signals that modulate sensory perception were studied *in vitro* and *in vivo*.
- ⇒ Associative pairing paradigms demonstrated "anti-Hebbian" synaptic plasticity within the sensory processing network and showed that potentiation or depression of synaptic strengths depends on the temporal order of pre- and postsynaptic events during the pairing of descending gating signals and incoming sensory signals.

Follow up

Strong links continue between the participating labs in Uruguay and France (support from ECOS) and with our new partner in Germany (support from PROCOPE). Present projects

center on a fuller understanding of electrosensory imaging and possible technological applications of electronic analogue systems, and on the theoretical modelling of biological control mechanisms in electrosensory and electromotor neuronal networks. A German doctoral student will work for 6 months in France and will later transfer to Montevideo for an extended study visit if funds can be found.

Selected Publications

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Period: September 1993 to February 1996

**STUDY OF VOLCANIC EMISSIONS AND THEIR EFFECTS ON HEALTH AT
POAS VOLCANO, COSTA RICA**

Co-ordinator: University of Cambridge, Cambridge, United Kingdom (Peter J. Baxter)

Objectives

Assessment of the impact of meteorological conditions, air pollution, volcano emissions, and acid rains with regard to populations' respiratory diseases in the Poas Volcano area.

Activities

- * Study of the effects of air pollution and acid rains on populations in areas affected by the volcanic emissions (Universidad Nacional Heredia)
- * Meteorological studies on Poas volcano to evaluate the role of weather as a determinant of population exposure to the volcanic emissions (National Meteorological Institute).
- * Survey of respiratory symptoms in the communities most exposed to the volcanic emissions to investigate the aetiological role of the emissions in the prevalence of asthma-related symptoms and the incidence of hyperactive airways disease (Ministry of Health.).
- * In addition, the project was supported by collaborating institutions including the British Geological Survey, (work on the geochemistry of the crater lake, local rainwater and rivers on the volcano) and the Department of Community Medicine, St. Thomas's Hospital, London (assistance in a study on the respiratory health of schoolchildren in the Poas area following the gas-emission crisis in 1994).

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**IDENTIFICATION, PURIFICATION AND CHARACTERIZATION OF SPECIFIC
PROTEINASES FROM *TRYPANOSOMA CRUZI* AND DRUG TARGETTING FOR
CHAGAS' DISEASE**

Coordinator: Muséum National d'Histoire Naturelle, Paris, France (Joseph Schrével)

Objectives

The long-term goal of the project was to design proteinase inhibitors against the Chagas agent, able to block selectively *Trypanosoma cruzi* proteinase(s) essential for parasite development without or, with reduced effects, on host proteinases. The selection of proteinases is dependent, first, on their identification by reliable procedures, then on their purification in order to produce selective (immunological or nucleotidic) probes and to undertake the study of their biological characters.

Activities

Taking advantage of fluorogenic peptides, specific substrates that provide sensitive assays of proteolytic activities, we have identified, characterized and purified novel proteases of *Trypanosoma cruzi*: the Tc80 and the Tc30 proteinases. Both laboratories were involved in the Tc80 proteinase purification, its biochemical characterization and the amino acid sequence determination, the production of specific antiserum and the characterization of the Tc80 gene. Specific inhibitors of the Tc80 proteinase were designed and synthesized in Paris, and their efficiency to inhibit *in vitro* the host cell invasion by trypanomastigotes was evaluated in Brasilia. Studies on Tc30 proteinase were mainly carried out in Brasilia.

Results

⇒ **The neutral Tc80 proteinase** is a 80-kDa serine-proteinase that shows a high specificity for the fluorescent substrate N-Suc-Gly-Pro-Leu-Gly-Pro-AMC derived from collagen, but also for human purified collagen types I and IV and native structure of collagens (e.g. rat mesentery). Tc80 proteinase possesses enzymatic characteristics distinct from the *bona fide* collagenases (mainly metallo-proteinases). By its specificity for proline, Tc80 can be classified as a prolyl endopeptidase (oligopeptidase EC 3.4.21.26). Tc80 is present in all the stages of *T. cruzi*, mainly associated to the infective trypomastigote form. The proteinase is secreted from the trypomastigote and not from the epimastigote forms. These data suggest that Tc80 could be involved in the *T. cruzi*-host cell infection by degradation of the extracellular matrix components required for the parasite access to the host cell. Internal amino acid sequences were obtained and the Tc80 proteinase gene is being cloned. Based on the specific sequence cleaved by the Tc80 proteinase, reversible and irreversible inhibitors were designed, which show reduced inhibitory activity on mammalian serine-proteinases and on other *T. cruzi* proteinases (Tc120 proteinase, cruzipain, Tc30 proteinase). These inhibitors inhibit the *in vitro* host cell invasion by the

infective trypomastigotes suggesting an essential role of the Tc80 proteinase in the host cell invasion process.

⇒ **The acidic Tc30 proteinase** is a 30-kDa cysteine-proteinase that hydrolyses the fluorescent peptidic substrate N-Suc-Leu-Leu-Val-Tyr-AMC. Tc30 proteinase has a broad substrate specificity, being capable of hydrolysing natural and non related substrates such as bovine serum albumin, collagens and gelatins. Molecular cloning was performed by screening of cDNA library with a specific antiserum and the translation products of positive clones were highly homologous to cathepsin B-like proteases. The *T. cruzi* cathepsin B-like gene is in single copy. The N-terminus sequence derived from the purified Tc30 protease is found within the protein translated from the cDNA sequence. These data demonstrate that the cathepsin B class of cysteine-proteinases does occur in *Trypanosoma cruzi*, besides the already known cathepsin L class represented by cruzipain.

Follow up

Efforts are made to obtain 1) active recombinant proteinases to optimize the design of inhibitors via the protein cristalization, 2) null mutants of the corresponding genes to demonstrate the essential roles of these proteinases in the *T. cruzi* life-cycle and to develop new chemotherapeutic approaches against Chagas' disease, based on proteinase inhibitors.

Selected publications

Santana J.M., Grellier P., Rodier M.H., Schr vel J. and Teixeira A.R.L. 1992. Purification and characterization of a new 120 kDa alkaline proteinase of *Trypanosoma cruzi*. *Biochem. Biophys. Res. Commun.* **187**: 1466-1473.

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Period: April 1994 to April 1997

IMMUNE RESPONSE IN HUMAN CYSTIC HYDATID DISEASE

Co-ordinator: University of Salford, Salford, United Kingdom (P. Craig)

Selected publications

R. Bonifacino, E. Dogiani, and P.S. Craig. 1997. Albendazole treatment and serological follow-up in hydatid disease of bone. *International Orthopaedics*. **21**: 127-132.

H. Cohen, E. Paolillo, R. Bonifacino et al. 1998. Human cystic echinococcosis in a Urugayan community: a sonographic serologic and epidemiological study. *American Journal of Tropical Medicine and Hygiene*. In press

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PATHOGENESIS OF PITUITARY TUMOURS: A MULTIDISCIPLINARY APPROACH

Co-ordinator: Vrije Universiteit Brussel, Brussels, Belgium (Elisabeth Hooghe-Peters)

Objectives

- ◆ Develop practical protocols for a refined characterization of pituitary adenoma
- ◆ Define factors possibly involved in the pathogenesis of pituitary adenoma.

Activities

The project focused on the morphological characterization of pituitary tumours using combined immunocytochemistry and non-radioactive *in situ* hybridization (the specimens were collected in all participating countries; Lyon and Buenos Aires concentrated on immunocytochemistry, Brussels on *in situ* hybridization) and the analysis of the expression of factors (growth factors, transcription factors, and their potential role), receptors for factors possibly involved in the pathogenesis of pituitary adenoma. Lyon was especially involved in the development of the animal model of tumours with the same characteristics as human ones, and tumour cell culture. With these new tools the behaviour of pituitary cells derived from tumours can be analyzed upon treatment with various drugs. Brussels concentrated on the cloning and characterization of the human Pit-1 gene encoding a transcription factor responsible for three pituitary cell types differentiation, proliferation and the regulation of their hormone secretion. This factor is also possibly involved in tumour formation. Buenos Aires was especially involved in the morphological characterization of the tumours. All the laboratories searched for factors that possibly control tumour formation.

Results

In the course of this project, the human Pit-1 gene was cloned and the regulatory mechanisms controlling its promoter and enhancer activity were analyzed. The results obtained underscore the importance of the interplay of various transcription factors to activate tissue-specific protein expression. Antibodies to human Pit-1 were developed and characterized. A variant of Pit-1, Pit-1 β , was identified. Tumours were transplanted under the kidney capsule, and could be also maintained *in situ* for months. In addition, adenomas were dissociated into single cells and could be maintained in culture up to one month, allowing a variety of biological studies. An important observation was the high expression of TGF β , IL-6 and oestrogen receptor in different adenomas.

Follow-up

Collaborative efforts continue to characterize further the factors involved in the pathogenesis of pituitary adenoma.

Selected publications

- Delhase M., Castrillo J.-L., de la Hoya M., Rajas F. & Hooghe-Peters E.L. 1996. AP-1 and OCT-1 transcription factors down-regulate the expression of the human PIT1/GHF1 gene. *J. Biol. Chem.* **271**: 32349-32358.
- Vertongen P., Velkeniers B., Hooghe-Peters E.L. & Robberecht P. 1995. Differential alternative splicing of PACAP receptor in pituitary cell subpopulations. *Mol. Cell. Endocrinol.* **113**: 131-13.
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- Delhase M., Vila V., Hooghe-Peters E.L. & Castrillo J.L. 1995. A novel pituitary transcription factor is produced by alternative splicing of human GHF1/Pit-1 gene. *Gene* **155**: 273-275.

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Period: January 1994 to January 1996

**CONSTRUCTION OF POTENTIAL ROTAVIRUS VACCINES AND EVALUATION
OF THEIR CAPACITY FOR INDUCING BOTH HUMORAL AND CELLULAR
IMMUNITY**

Co-ordinator: University of Bristol, Bristol, United Kingdom (Neil A. Williams)

Objectives

Defining a suitable protein from rotavirus, which may be used in the future to establish a vaccine.

Activities

- * Generation of recombinant rotaviral proteins in *E. coli* for studies of immune responses (m1, m2).
- * Study of immune responses to recombinant rotaviral proteins (m3, b1, b2, b3, b5).
- * Expression of rotaviral proteins in attenuated *Salmonella* (m5).
- * In vivo studies with rotaviral protein expressing *Salmonella* (m6, m7, b4).

Results

- ⇒ The work achieved clearly established that the rotaviral protein VP6 is a strong stimulator of T-cell reactivity in a murine model. Further, evidence suggested that the reaction to this protein is not strain-specific but primed T-cells to a murine VP6 response to the same protein from other virus strains, including human strains. These findings suggested that a sub-unit vaccine incorporating VP6 may be effective in this field.
- ⇒ The advances that the project has made make the prospects of an effective mucosal rotavirus vaccine a real possibility for the future.

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**EFFECTS OF CYTOCHROME P450 INHIBITION ON PULMONARY OEDEMA
FORMATION INDUCED BY OZONE AND PARATHION**

Co-ordinator: Université de Liège, Liège, Belgium (Pascal Gustin)

Objectives

The main objective was to study the role of the pulmonary CytP450 in the development of pulmonary disorders induced by parathion and ozone in different species, using alternative methods, with the long term goal to identify new therapeutic approaches and new toxicological mechanisms potentially useful as biomarkers.

Activities

The pulmonary effects of parathion and ozone were assessed by *in vivo* and *in vitro* experiments on guinea pigs and rabbits. In Mexico, airway obstruction and hyperresponsiveness were evaluated by using plethysmography, organ baths for electric field stimulation and bronchoalveolar lavages. Oedema was assessed by wet/dry weight ratio and Evans blue technique. In Liège, the isolated perfused rabbit lung model was used for the evaluation of microvascular permeability, airway mechanics and hemodynamics and for toxokinetic studies. Determination of the CytP450 activities was performed.

Results

Our data on organophosphates showed that : isolated lung preparation is a useful alternative method to study organophosphate toxicity; lung metabolic activity plays a determinant role in the activation of parathion into paraoxon in rabbits and guinea pigs and in the pulmonary effects of this drug; - pharmacokinetic parameters are not good tools to assess this metabolic activity so that a method to measure the CytP450-dependent enzyme activity responsible for this biotransformation (parathion oxidase) in lung microsomes was developed (possible biomarker); - parathion induces pulmonary oedema, airway obstruction and airway hyperreactivity, which depend on complex pathophysiological and pharmacological events, suggesting new therapeutical approaches. Related to ozone, original mechanisms strongly dependent on the animal species were identified: - spontaneous increase in vascular permeability, hypertension, airway obstruction and hyperreactivity but also activation of protective mechanisms involving neuropeptides against lung injuries; - characterization of ozone-induced subtle abnormalities in airway neurogenic transmission, leading to reduced adrenergic relaxation and enhanced non-cholinergic contraction; - disappearance of airway hyperresponsiveness induced by an acute exposure to ozone after chronic exposure. This tolerance phenomenon was not related to an increase in superoxide dismutase levels; - sexual dependent inhibition of CytP450 activity by ozone (possible biomarker) and demonstration of the possible protective effects of CytP450 inhibitors against ozone-induced lung injuries.

Follow-up

Taking advantage of the technology transfer that occurred during the fruitful interaction of both teams, research on organophosphates and ozone continues interactively in both laboratories, with special focus on the interactions between cytochrome P450 and ozone effects in rabbit lungs (in Liège) and suicide metabolism of parathion, as well as ozone toxicity in guinea pig lungs (in Mexico).

Selected publications

- Delaunois A., Segura P., Dessy-Doizé C., Ansay M., Montaña L.M., Vargas M.H., Gustin P., 1997. Ozone-induced stimulation of pulmonary sympathetic fibers: a protective mechanism against oedema. *Toxicol. Appl. Pharmacol.* **147**: 71-82.
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Period: April 1994 to September 1997

**INTERACTION AMONG CYTOKINES AND PROTEOGLYCAN IN
SCHISTOSOMIASIS**

**Co-ordinator: Paterson Institute, Manchester, United Kingdom
(John T. Gallagher)**

Objectives

To evaluate the structure and properties of heparan sulphate proteoglycans in cell culture systems that mimic the myelopoietic environment of schistosome effected tissues.

Activities

Schistosomiasis is a major medical problem in many third-world countries. The disease is highly debilitating and results in extensive fibrosis of the liver; the affected hepatic tissues create an environment that is conducive to haemopoiesis, with the myeloid lineage predominant. Visits were exchanged between laboratories and primary and established cell lines were acquired, that had been developed from mesenchymal cells from livers of schistosome-infected rodent hosts. These lines were cultured and their heparan sulphates analysed. We also examined the heparan sulphates produced in mouse bone-marrow cultures that represent a normal haemopoietic environment.

Results

The heparan sulphates derived from cell lines of schistosome-infected rodents had standard structural motifs that are common to most cell types - no atypical features were noted. However, our investigations were limited because the primary cultures were difficult to grow and the established cell lines may have drifted from the *in vivo* phenotype. We carried out more detailed work on the murine marrow stroma HS; this species was structurally distinct in that the polysaccharide chain length was very short (approx. ~ 50 sugar residues) but the degree of sulphation was unusually high, well into the upper range of sulphate densities seen in heparan sulphate. Analysis by Western blotting, Northern blotting and RT-PCR revealed new information on the expression of trans-membrane and GPI-anchored heparan sulphate proteoglycans core proteins in the inductive haemopoietic environment. At this stage of the work it is not clear whether the schistosome-induced haemopoiesis is mediated, at least in part, by effecting characteristic structural changes in heparan sulphate nor is it known whether heparan sulphate contributes to the pathogenesis of disease by binding specific cytokines.

Follow-up

Better comparisons are needed between the molecular structures and binding properties of heparan sulphate in livers damaged by schistosome infection, normal livers and normal

haemopoietic marrow stroma. New methods for sequence analysis of heparan sulphate are being developed which should aid this objective.

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**THE EPIDEMIOLOGY OF *LEISHMANIA BRAZILIENSIS* IN THE UNFORESTED
EASTERN ANDEAN HIGHLANDS OF HUANUCO DEPARTMENT, PERU.**

Co-ordinator: London School of Hygiene & Tropical Medicine, London, United Kingdom
(Clive Davies)

Objectives

Characterize the epidemiology of cutaneous and mucocutaneous leishmaniasis in the unforested highlands of Huanuco Department, Peru.

Activities

In 1994, a cross-sectional epidemiological survey was carried out in nine rural villages in Huanuco province and in four peri-urban sites in Huanuco City, recording full demographic and clinical details. The majority of the population were leishmania skin-tested (n = 3218). This population was then followed prospectively at 3-6 monthly intervals for changes in clinical status. A second skin test survey was made in 1996-7 on a sample of the rural study population (n = 1046). A total of 49 parasite isolates were made from patients with cutaneous (38) or mucocutaneous leishmaniasis (11) during these surveys, and most were characterised by isoenzyme analysis. A further 14 parasites isolated during a survey of 573 dogs (suspected reservoir hosts) from the same sites were also characterized. Sandflies were collected at intervals over 12 months in two peri-urban sites and eight villages, using CDC light traps and Shannon traps. Natural infections in sandflies were identified by dissection and PCR. Other entomological studies included investigations of (i) possible pheromone-mediated communication between sandflies, and (ii) determinants of blood-feeding success. All patients detected during the project were provided with free treatment. Health-promotion activities included the training of primary health care workers (in each village), nurses and physicians, and the holding of a series of health education meetings.

Results

At the first survey, 24% of the rural population and 6% of the peri-urban population were skin test positive, 83% of whom had a scar or lesion characteristic of cutaneous leishmaniasis. Active cutaneous lesions were detected in 2.6% and 0.6% of the rural and peri-urban population, respectively. Transmission appears to be largely domestic, with cumulative prevalence increasing immediately but there is some evidence of slight additional risk for adult males. According to a retrospective analysis, it appears that this region only became endemic in the early 1980s, since when the mean incidence rate in the rural villages from 1985-1994 was 4.4% per year. However, this rate (measured prospectively) was only 1.7% between 1994-7. The life-time risk of mucosal disease for cutaneous leishmaniasis patients appears to be ca. 20%. Much of the mucosal disease observed was of relatively low severity (e.g. lesions on the nasal septa), with the number of mucosal linings affected increasing with time since the primary cutaneous lesion. Parasites isolated from dogs and humans have been

characterised as *L. braziliensis*, *L. peruviana*, suspected *L. braziliensis*-*L. peruviana* hybrids and *L. lainsoni*. *Lutzomyia tejadai* is the suspected vector as it comprised 99% of ca. 10,000 sandflies collected in and around houses in this focus, and flagellate infections in *Lu. tejadai* were identified by PCR as members of the *L. braziliensis* complex.

Follow-up

Analysis of the various entomological and epidemiological data sets is on-going. The study of risk factors for infection and disease in Huanuco was not fully conclusive, as the incidence rate during the 2-year prospective survey was unexpectedly low; and so a further 2-year prospective survey is now underway in the same and additional villages. Parasites isolated during this new survey are also being characterized by a battery of molecular tools. In addition, we are also directly addressing the question whether dogs act as reservoir hosts for both *L. braziliensis* and *L. peruviana* in Huanuco; and a control trial targeting domestic dogs is planned.

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Contract number: CII*CT930049

Period: January 1993 to January 1996

**IDENTIFICATION OF THE INTERFACE OF ACTIN AND ACTIN-BINDING
PROTEINS, USING A SET OF BI-FUNCTIONAL REAGENTS**

Co-ordinator: University of Ghent, Gent, Belgium (Christopher Ampe)

Selected publications

M. Van Troys, D. Dewitte, M. Goethals, M.F. Carlier, J. Vandekerckhove, and Ch. Ampe 1996. The actin binding site of thymosin 4 mapped by mutational analysis. *EMBO J.* **15**: 201-210.

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A. Lambrechts, J.L. Verschelde, V. Jonckheere, M. Goethals, J. Vandekerckhove and C. Ampe. 1997. The mammalian profilin isoforms display complementary affinities for PIP₂ and proline-rich sequences. *EMBO J.* **16**: 484-495.

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Contract number: CI1*CT930051

Period: April 1994 to October 1996

**ROLE OF NUTRITIONAL STATUS IN CARCINOGENESIS AND MUTAGENESIS
BY AIR POLLUTION SAMPLES FROM SANTIAGO (CHILE)**

Co ordinator: University of Surrey, Guildford, United Kingdom (G. Gordon Gibson)

Objectives

- ◆ The objectives of this collaborative study between the Universities of Chile and Surrey were to chemically characterise the respirable, particulate matter derived from atmospheric pollution in downtown Santiago (Chile) and to assess the biological properties of the adsorbed chemicals.
- ◆ The overall objective of this study was to provide data in bacterial and animal models that may be extended to a risk assessment of environmental pollutants to human health.

Activities

Filters were used to trap PM10 airborne particles and organic solvent extracts were examined by HPLC analysis. These extracts were examined for their ability to cause chemical mutagenesis in the Ames test and for their ability to cause enzyme induction in the livers of rats treated with these extracts.

Results

- ⇒ Chemical analysis of filter extracts by HPLC identified a seasonal and site-specific variation in the total polycyclic aromatic hydrocarbons (PAH) adsorbed onto respirable particles, and several (13 in total) mutagenic PAHs were identified, including benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(a)anthracene, benzo(b)fluoranthene, and indeno(1,2,3,c,d)pyrene. These particles contained both direct and indirect-acting mutagens when tested in the Ames test and the seasonal variation in mutagenicity of the extracts.
- ⇒ When the organic extracts were administered to male Wistar rats, they produced a significant induction of the hepatic cytochrome P450 (CYP) 1A1 enzyme as assessed by substantial increases in the cognate enzyme activity and by Western blotting analysis using mono-specific antibodies. The nutritional status (protein malnutrition) of the rats influenced both their basal and particle-inducible complement of CYPs in that the CYP4A sub-family was induced by the malnourished state and altered the response to induction by particle treatment.

Outcomes

The overall conclusions reached by this study are the presence of mutagenic/carcinogenic PAHs in respirable air, and that these chemical contaminants may well pose a hazard to human health. These results have substantially raised the public's awareness of pollution in Santiago, partly due to several radio (15) and TV (13) interviews given on this topic, in addition to local newspaper articles (14). Moreover, we believe that this study has

commanded the attention of the Chilean Government, and recent events have resulted in the Government lowering the permissible levels of environmental pollution derived from both industrial sources and from automobile exhaust emissions in particular.

Selected publications

Adonis M., Quinones L., Gil L., and Gibson G.G. 1997. Hepatic enzyme induction and mutagenicity of airborne particulate matter from Santiago (Chile) in the nourished and malnourished rat. *Xenobiotica*. 27: 527-536.

Air pollution in Santiago (Chile): polycyclic aromatic hydrocarbon levels and mutagenic activities of organic extracts from airborne particles. ISBN 3-906470-05-9. In: *Organic Volatile Compounds in the Environment*. R. Perry and J.J. Knight Eds. Pp. 139-154..

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Contract number: CI1*CT930056

Period: February 1994 to February 1997

**ONTOGENY AND GENETIC CONTROL OF THE NATURAL ANTIBODY
REPertoire**

Co-ordinator: Institut Pasteur, Paris, France (Antonio Coutinho)

Objectives

- ◆ Study the natural antibody repertoire modifications in the mouse during ontogeny and aging. The antibody repertoire was studied using a recently developed quantitative immunoblot assay that allows to screen for reactivity on many hundreds different antigens individually.
- ◆ Study the genetic controls operating on the natural antibody repertoire.
- ◆ Analyze the repertoire of modifications of natural antibodies accompanying the development of spontaneous and/or induced autoimmune diseases in mouse strains
- ◆ Establish a classification of natural antibody repertoires using multivariate statistical methods.

Selected publications

M. Haury, A. Sundblad, A. Grandien, C. Barreau, A. Coutinho, A. Noriega. 1997. The repertoire of serum IgM in normal mice is largely independent of external antigen contact. *Eur. J. Immunol.* **27**: 1557-1563.

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Contract number: CII*CT930092

Period: April 1994 to March 1997

**INTEGRATIVE STUDY ON THE ROLE OF INTERLEUKINS IN THE
REGULATION OF PITUITARY ACTIVITY, IMMUNE FUNCTION AND
CELLULAR GROWTH.**

Co-ordinator: Max-Planck Institute for Psychiatry, Munich, Germany (G.K. Stalla)

Objectives

Our aim is to study the significance of interactions between the HPA and immune systems for neuroendocrine regulation, immune function and cellular growth. In particular, to understand the role of interleukin production by the pituitary and the spleen in the regulation of pituitary function and growth.

Activities

A subtle interaction between cytokines, pituitary hormones and HPA axis hormones, takes place at the pituitary and splenocyte-monocyte level. The mechanisms of regulation of pituitary growth by auto-paracrine cytokines expressed in the pituitary and their role in pituitary adenoma development was analyzed both at Munich and Buenos Aires. We have established an animal model for the neuroendocrine characterization of immune challenges. This model is different to all previous studies in which T-cell independent antigens were used. Samples obtained at either Institute were analyzed in the other. Discussions were permanently performed and protocols exchanged in order to continue the experiments and to integrate the results.

Results

In the course of this project we have characterized the regulation of the IL-1/L-1 ra system by glucocorticoids, CRH and ACTH. We have described the enhancement of glucocorticoids transcriptional activity and biological action by cytokines, an important mechanism aimed to control the overexpression of the immune system by an interaction of cytokines and glucocorticoids at their target level. We have shown that the IL-1 autocrine system is important for the control of pituitary growth. Using animal models we have established the instrumental role of the TRH-Prolactin response during the specific T-cell antigen response, and that the blockade of these genes provides a useful tool for blocking these type of responses. This model seems to be very useful for future design of pharmacological treatments in many immune diseases in which a neuroendocrine alteration is involved". In conclusion, confirming our working hypothesis, a) cytokines are important auto/paracrine regulators of pituitary function and growth, b) HPA hormones (glucocorticoids and CRH) control the IL-1 system, also acting on the endogenous antagonist, c) cytokines regulate the sensitivity to glucocorticoids at their target cell level, d) the neuroendocrine changes of the T-cell specific antigen response are instrumental for the correct response to take place, e) the mechanisms and models established constitute very useful tools for further designs of pharmacological strategies for both immune and neuroendocrine diseases in which neuroendocrine-immune circuits alterations are involved.

Selected publications

Páez Pereda, M, Sauer, J, Perez Castro, C., Finkielman, S., Stalla, G.K., Holsboer, F., Arzt, E. 1995. Corticotropin-releasing hormone differentially modulates the interleukin-1 system according to the level of monocyte activation by endotoxin. *Endocrinology* **136**: 5504-5510.

Costas, M., Trapp, T., Páez Pereda, M., Sauer, J, Rupprecht, R., Nahmod, V., Reul, J., Holsboer, F., Arzt, E, 1996. Molecular and functional evidence for in vitro cytokine enhancement of human and murine target cell sensitivity to glucocorticoids: TNF- α priming increases glucocorticoid inhibition of TNF- α -induced cytotoxicity/apoptosis *J Clin. Invest.* **98**: 1409-1416.

Páez Pereda, M., Goidberg V., Chervín A, Carrizo, G., Molina, A., Andrada J., Sauer, J., Renner, U., Stalla, G.K, Arzt, E., 1996. Interleukin-2 (IL-2) and IL-6 regulate c-fos protooncogene expression in human pituitary adenoma explants. *Mol. Cell. Endocrinol.* **124**: 33-42.

Arzt, E., Stalla, G.K., 1996. Cytokines: autocrine and paracrine roles in the anterior pituitary. *Neuroimmunomodulation* **3**: 28-34 (review per invitation).

Pérez Castro, C, Peñalva, R, Páez Pereda, M., Reul, J., Holsboer, F., Renner, U., Stalla, G., Arzt, E., 1997, (submitted). The enhancement of TRH and prolactin is instrumental during the T-cell dependent immune response.

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**MOLECULAR AND FUNCTIONAL ASPECTS OF DYSTROPHINS IN THE
ANIMAL AND HUMAN NERVOUS SYSTEMS**

Co-ordinator: Université Louis Pasteur, Strasbourg, France (Alvaro Rendón)

Objectives

Duchenne/Becker dystrophy (DMD/BMD) affects the nervous system as well as the muscles. The general and long-term goal of the work was to develop appropriate cell models of dystrophin expression and to apply this knowledge to understanding the molecular basis of Central Nervous System (CNS) troubles in DMD/BMD-affected patients.

Activities

There are seven transcripts from the dystrophin gene, with independent promoters, all expressed in the CNS. Their relative importance in the CNS function still is an open question. The expression of dystrophins in PC12 cells induced by the Nerve Growth Factor (NGF) was studied in Mexico (Genetic and Molecular Biology Department) in close collaboration with Strasbourg. The Neurosciences and Cell Biology Departments in Mexico focused on the study of membrane and cytoskeleton interactions of native dystrophins. Both departments were involved in comparative studies of Dystrophin protein 71 (main DMD protein product of the DMD gene expressed in the brain), isoform expressions in cultured hippocampal neurones and astroblasts (Neuroscience), and brain regional distribution (Cell Biology). Strasbourg concentrated on the study of dystrophin expression in retina, with special emphasis on the correlation of visual phenotype on DMD animal models and retina-cells expression of DMD-gene products. All laboratories developed multidisciplinary approaches and shared common tools in molecular and cell biology.

Results so far

In the course of two-year's work, various tissue and neural cells were validated as models to reveal dystrophin function(s) in the nervous system.

Follow-up

- After completion of the contract, a long-range collaborative network was set up. Recently, efforts were made to define the CNS function of dystrophins. The Department of Genetics and Molecular Biology continues to concentrate on the study of the regulatory mechanisms of DMD gene expression.
- The Neurosciences and Cell Biology departments concentrate on membrane and cytoskeleton interactions of dystrophins *in situ* and *in vitro*; and Strasbourg is pursuing the definition of the molecular basis of the DMD visual phenotype.

Selected publications

- Vaillend C., Rendón A., Misslin R. and Ungerer A. 1995. Influence of dystrophin-gene mutation on behaviour in mdx mice I. Acquisition and retention of delayed alternation and bar-pressing tasks, and emotional reactivity. *Behaviour Genetics*, **25**, 569-579.
- Cisneros B., Rendón A., Genty V., Aranda G., Marquez F., Mornet D., and Montanez C. 1996. Expression of dystrophin Dp71 during PC12 cell differentiation. *Neuroscience Letters*, **213**, 107-110.
- Coral R., Vasquez D., Arenas D., Cisneros B., Penaloza L. and Montanez C. 1997. Pattern of deletions of the dystrophin gene in DMD-BMD Mexican patients. *American J. of Med. Gen.* **70**, 240-246.
- Rodius F., Claudepierre T., Rosas-Vargas H., Cisneros B., Montanez C., Dreyfus H., Mornet D., and Rendón A. 1997. Dystrophins in developing retina : expression correlates with synaptic maturation. *NeuroReport*, **8**, 2383-2387.

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DIFFERENTIATION OF HYPOTHALAMIC NEURONS IN PRESENCE OF THEIR TARGET CELLS IN CULTURE SYSTEMS

Co-ordinator: INSERM U159, Paris, France (Annie Faivre-Bauman)

Objectives

The aim of the project was to study the cellular and molecular interactions between a developing central neuron and a target cell, using an *in vitro* model: foetal hypothalamic neurons co-cultivated with two possible targets, the intermediate lobe (IL) of the pituitary or the median eminence.

Activities

Several neuronal populations were studied: peptidergic (TRH) in Mexico and dopaminergic neurons (DA) in Paris. The serum-free culture of foetal hypothalamic neurons was settled in Mexico, and a Mexican student was trained for microdissection techniques in Paris. Morphological and biochemical analyses were performed by techniques used routinely in both laboratories.

Results

- ⇒ The presence of IL cells exerts a global trophic effect on hypothalamic neurons, and a specific acceleration of DA neurons maturation, as attested by increased levels of tyrosine hydroxylase (TH) and morphological evidences of higher differentiation. The use of conditioned medium from IL cells, IL membrane extracts and transwell chambers demonstrate that both diffusible and contact factors are implied.
- ⇒ Among the four DA-neurons hypothalamic sub-populations, only two are susceptible to innervate IL cells *in vivo*, namely those from the arcuate and the periventricular areas. We therefore established separate cultures of these areas sampled from E17 embryos. The presence of intermediate lobe cells increased tyrosine hydroxylase levels in both dopaminergic neuron subsets, but morphological differentiation was only accelerated in dopaminergic neurons originating from the arcuate nucleus. Removal of polysialic acid on NCAM by an endoneuraminidase N treatment completely abolished this effect, showing that polysialic acid-NCAMs are required for the accelerated morphological maturation of dopaminergic arcuate neurons. The role of extracellular matrix proteins (ECMP) was also studied for peptidergic neurons. The strongest effect was observed for laminin, which differentially increased the early development of TRH biosynthesis (pro-TRH levels) without modifying THR neuron survival.
- ⇒ We then investigated the possibility that hypothalamic neurons also respond to trophic diffusible factors, such as neurotrophins. Addition of neurotrophin 3 (NT3), but not brain-derived neurotrophic factor (BDNF), enhanced neuritic growth and branching and tyrosine hydroxylase (TH) levels of cultured arcuate DA neurons. Conversely, BDNF, but not NT3, affected the same parameters in cultured periventricular DA neurons. Addition of specific antibodies directed against neurotrophins or their respective receptors TrkB or TrkC inhibited development of DA neurons below that of control cultures, suggesting involvement of endogenous neurotrophins. Regarding TRH activity, BDNF strikingly

increased TRH mRNA levels several folds from DIV0 to DIV4 in a time-dependent manner. This effect was seen only at low cellular densities consistent with the existence of endogenous BDNF masking exogenous effects at higher densities.

⇒ BDNF and NT3 were indeed found in hypothalamic cultures and in the intermediate lobe, both by western-blot and by immunocytochemistry.

Follow-up

Now that the contract has ended, our attention has focused on the role of neurotrophins and growth factors selectively expressed either by targets (intermediate lobe, median eminence) or by the hypothalamic cells (neurons or glial cells) themselves. Depending on their cellular origin, these differentiation factors could act through distinct cellular mechanisms.

Selected publications

Charli J.-L., Faivre-Bauman A., Loudes C. and Kordon C. 1993. Coculture of rat melanotrophs with hypothalamic cells enhances differentiation of dopaminergic neurons. *Molecular and Cellular Neurosciences* **4**: 55-63.

Charli J-L, Cruz C., Redondo J.L. Guerra C, .and Joseph-Bravo P. 1995. Homologous conditioned medium enhances expression of TRH in hypothalamic neurons in primary culture. *Develop. Brain Res* **89**, 155-160.

Loudes C, Rougon G., Kordon C. and Faivre-Bauman A. 1997. Polysialylated neural cell adhesion is involved in target-induced morphological differentiation of arcuate dopaminergic neurons. *Eur. J. Neurosci.* **9**, 2323-2333.

Loudes C., Petit F., Kordon C. and Faivre-Bauman A. Distinct populations of hypothalamic dopaminergic neurons exhibit differential responses to Brain-Derived Neurotrophic factor (BDNF) and Neurotrophin-3 (NT3). Submitted to *Eur. J. Neurosci.*

Manuscripts in preparation:

Pituitary intermediate lobe membranes enhance hypothalamic differentiation in primary cultures, by J. Niquet, C. Loudes, R. Ubieta, C. Kordon, A. Faivre-Bauman and J-L Charli

Extracellular matrix protein influence the early expression of pro-TRH in hypothalamic neurons in vitro, by J. Niquet, D. Grouselle; P. Joseph-Bravo and J-L Charli.

BDNF increases TRH mRNA expression in primary hypothalamic cell cultures, by M. Guerra-Crespo, F. Romero-Arteaga; M. Zacarias-Soto, E. Sanchez-Jaramillo, P. Joseph-Bravo, J-L Charli and L. Pérez-Martínez.

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THE EPIDEMIOLOGY OF MALARIA AND CONTROL STRATEGIES IN NICARAGUA

Co-ordinator: School of Tropical Medicine, Liverpool, United Kingdom (A. Kroeger)

Objectives

- ◆ Characterize the epidemiological situation of malaria.
- ◆ Determine the protective efficacy and effectiveness of bed net impregnation against malaria.
- ◆ Analyze the results of an educational programme.
- ◆ Analyze 6-GPD deficiency in the population in order to predict certain risks of antimalarial drug treatment.
- ◆ Evaluate the efficacy of interventions in breeding places in four selected communities.

Activities

A baseline survey including formal interviews, blood smears and malaria antibody tests was conducted in the study communities of El Viejo, Chinandega and complemented by a longitudinal in-depth study in 4 communities and extensive entomological work in the area:

- * A randomised community trial was undertaken with bed-net impregnation (lambdacyhalotrin) in the intervention communities and general health education in the control communities.
- * A randomized community trial (overlapping with the previous one) was undertaken with malaria health education in the intervention group and general health education in the control group.
- * 70 persons were examined for 6-GPD deficiency.
- * Mosquito density in communities with and without clearing of breeding places was examined.

Results

- ⇒ The study populations showed the characteristics of rural poverty. Although illiteracy was high, knowledge of malaria symptoms and mosquito transmission was good. The incidence of malaria attacks was 6.3% during 3 months of the wet season; particularly children and adolescents were affected. The prevalence of a symptomatic parasitemia varied between 0% and 27%. *A. albimanus* was the main malaria vector.
- ⇒ The protective efficacy of bed net impregnation with lambdacyhalotrin against clinical malaria episodes was 66%.
- ⇒ No 6-GPD deficiency was detected.
- ⇒ Due to the short intervention period, no impact of the promotional activities could be measured.

Selected publications

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Kroeger A, Mancheno M, González M, Meyer R., 1996. Health education for community-based malaria control: An intervention study in Ecuador, Colombia and Nicaragua. Trop. Med. Int. Hlth. **1**: 836-846.

Kroeger, Meyer R, Mancheno M, Gonzalez M, Pesse K., 1997. Operational aspects of bednet impregnation for community-based malaria control in Nicaragua, Ecuador, Peru and Colombia. Trop. Med. Int. Hlth. **2** : 589-602.

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Contract number: CII*CT930305

Period: May 1994 to May 1997

**CYTOGENETIC, TOXICOLOGICAL, AND EPIDEMIOLOGICAL STUDIES IN
POPULATIONS CHRONICALLY EXPOSED TO INORGANIC ARSENIC IN
ARGENTINA**

Co-ordinator: Leiden University, Leiden, The Netherlands (A.T. Natarajan)

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STRUCTURAL ELEMENTS DETERMINING INTERFERON SENSITIVITY OF GENE EXPRESSION

Co-ordinator: University of Würzburg, Würzburg, Germany (Christoph Jungwirth)

Objectives

- ◆ Vaccinia-virus specific early protein synthesis is regulated in the IFN type-I treated chicken cell on the level of translation. A large part of the early mRNA is degraded. Synthesis of the majority of chicken host cell proteins is not inhibited. It is an enigma why in all interferon type-I treated mammalian cells studied so far, this selective inhibition of vaccinia-virus specific protein synthesis is not found. It is postulated that the antiviral effect induced by IFN type I is compensated in mammalian cells by the strategies the virus has developed to counteract the host defense system.
- ◆ The question we addressed specifically in our studies was how the expression of a cellular gene integrated into the vaccinia-virus chromosome is affected in the IFN type-I treated cell. The sensitivity of expression of these inserted genes under the control of vaccinia-virus early promoters was compared to that of authentic early viral genes. Observations indicated that flanking vaccinia-virus regions can control IFN type-I sensitivity. The most provocative observation was that histone H5/H10 gene expression from a vaccinia-virus recombinant was only marginally IFN type I sensitive. We postulated an “interferon action silencer domain”.

Activities

- ★ Our work concentrated on trying to identify the critical minimal DNA region of the histone genes which downregulates the IFN type-I sensitivity and whether the expression of other cellular genes inserted into the vaccinia-virus genome would also show a reduced IFN type I sensitivity.
- ★ In connection with our studies on the expression of foreign cellular genes inserted into vaccinia virus, we also studied the interaction of different vaccinia-virus recombinants with IFN type-I treated chicken and mammalian cells. The role of the K3L and E3L gene thought to modulate IFN type-I sensitivity of vaccinia-virus replication is being studied. (These studies are mainly being carried out in Belo Horizonte).

Results so far

IFN type-I sensitivity of the expression of the various chimeric histone/Tk genes was influenced by the relative length of the histone sequence. If the fusion gene contained more than 45 % cellular sequence the expression of the chimeric gene showed clearly a reduced IFN type-I sensitivity. A fusion gene with 32 % cellular gene showed an IFN type-I sensitivity indistinguishable from vaccinia-virus Tk expression. To generalize these observations we constructed vaccinia-virus recombinants carrying partially deleted or histidine-tagged glyceraldehyd-3-phosphate-dehydrogenase genes. These studies were mainly carried out in Würzburg.

Follow-up

- Both laboratories, in Würzburg and Belo Horizonte, further attempted to identify elements affecting the sensitivity of vaccinia virus to IFN type I.
- It appeared equally important to study the mechanism of cytotoxicity enhancement by which vaccinia virus may interact with the IFN treated cell and may hinder spread of viral infection.
-

Selected publications

Eisert V., Jungwirth C., Feirer N. and Kroon E., 1997. The interaction of vacciniavirus WR, K3L and E3L mutants with recombinant interferon type I (CH-IFN-I) treated primary and permanent chicken fibroblasts (CEF). Abstract ISICR Meeting, San Diego.

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THE IMPACT OF COMMUNITY TREATMENT WITH IVERMECTIN ON THE TRANSMISSION OF *ONCHOCERCA VOLVULUS* IN GUATEMALA AS ESTABLISHED BY CONVENTIONAL AND MODERN TECHNIQUES

Co-ordinator: Liverpool School of Tropical Medicine, Liverpool, United Kingdom.
(John B. Davies)

Objectives

- ◆ Study the effect of on-going mass ivermectin treatments in an endemic onchocerciasis area of Guatemala to determine whether the reduction in skin microfilarial loads in a cohort of the community would be progressive, and what would be its effect on the levels of infection in the vectors.
- ◆ Study the effect on the incidence of new infections as determined by immunological and molecular techniques.
- ◆ Try less invasive and more sensitive survey techniques as a more acceptable alternative to skin-snips.
- ◆ Try to predict the long-term outcome of the treatments and how long elimination might take.
- ◆ Train two Guatemalan scientists and technicians in molecular and immunological, and entomological techniques.

Activities

- ★ The project was based on field studies carried out in Guatemala (vector identification, biting and microfilarial uptake; skin-snips surveys for prevalences and skin microfilarial loads, collection of sera) by UVG, Guatemala.
- ★ The field entomology was supervised during visits by the Coordinator who also did a proportion of dissections and data analysis back in Liverpool. Isolation of *Onchocerca* DNA and its detection by PCR on specimens of vectors and skin-snips was done in Amsterdam, while all serodiagnosis and antibody response studies using Ov33 and Ov25 were done by Hohenheim.
- ★ The computer model of Onchocerciasis transmission SIMON-o was developed in Liverpool. Training of the scientist took place in Amsterdam while the entomological technicians were trained in Guatemala.

Results

- ⇒ We intended to follow the progress of 7 communities receiving mass ivermectin at 6-monthly and annual intervals. Even though the treatment campaign broke down after one year (change of government, reorganisation of health systems) interrupting the longitudinal studies, we were able to monitor the rate of recovery after ivermectin for over 2 years.
- ⇒ Uptake of mf. by flies recovered more slowly than skin-snip density would suggest.
- ⇒ A method of using engorged vectors (VmfU) to replace skin-snips to estimate mf. skin densities has proved acceptable to the communities.
- ⇒ The isolation of *O. volvulus* DNA from alcohol preserved flies is, after amplification by PCR, extremely sensitive (1 mf. detectable in 40 flies) and has shown that when treatment coverage was 97 % of eligible persons infection in wild *S. ochraceum* was reduced by 60 %.
- ⇒ With skin-snips, PCR detected mf in 50 % of snips scored negative by microscopy. Serodiagnosis based on Ov33 was more sensitive than PCR detection of mf in skin-snips.

- ⇒ Antibody responses against Ov25 showed that Guatemalans with or without infections did not respond, whereas infected Africans did.
- ⇒ A preliminary run of the SIMON-o model suggested that even at constant high coverage mass treatment with ivermectin alone would take more than 30 years to eliminate the parasite.

Follow-up

The current project is completed, but much remains to be done, especially a monitoring of the re-established treatment campaign. It is sad that the excellent Center for Health Studies at UVG, Guatemala, has been closed down, and its staff who were specially trained by this project, have been dismissed by the University. Therefore, there is little chance of any of this work being continued at present.

Selected publications

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- Oskam L., Schoone G.J., Kroon C.C.M., Luján R. and Davies J.B. 1996. Polymerase chain reaction for detecting *Onchocerca volvulus* in pools of blackflies. *Tropical Medicine and International Health*, 1 (4): 522-527.

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**CERVICAL CANCER IN DEVELOPING COUNTRIES : A PROSPECTIVE
POPULATION-BASED STUDY OF THE ETIOLOGIC ROLE OF HUMAN
PAPILLOMA VIRUSES, THEIR MOLECULAR CHARACTERISTICS AND THE
POTENTIAL OF PCR TESTING AND HYDROLYSED DNA ASSAY TO IMPROVE
THE COST-EFFECTIVENESS OF SCREENING PROGRAMMES**

Co-ordinator: Liverpool School of Tropical Medicine, Liverpool, United Kingdom
(Dave Haran)

Objectives

- ◆ Improve our understanding of the risk factors, particularly HPV, in the development of cancer of the cervix;
- ◆ Study ways of improving the cost effectiveness of cervical screening through the introduction of technologies that are appropriate to Nicaragua and other similar developing countries;
- ◆ Increase knowledge of the epidemiology and molecular biology of HPV in Nicaragua.

Activities

- ★ The study of screening for cancer of the cervix in Nicaragua began in March 1994, funded by ODA and EEC. The study was conducted with the assistance of a non-profit organisation (Ixchen) that operates women's clinics in Managua. Approximately 2,000 sexually active women were screened at 6-month intervals for the detection of cervical abnormalities and human papillomavirus (HPV) infection. Treatment was undertaken according to an internationally accepted protocol.
- ★ All screening, laboratory processing and treatment was undertaken by the Ixchen clinic under contract to the project. Quality control of the Ixchen HPV laboratory work took place through Dr Maingon's laboratories in the UK.
- ★ Successful completion of all follow-up visits, analysis of smears and completion of HPV detection work.
- ★ QA introduced into the Ixchen cytology laboratory.

Results so far

- ⇒ 2028 patients attended for the first visit between August 1994 and January 1995. 1455 women were still attending for repeat smears at visit 5. 65 smears were identified as having Cervical Intraepithelial Neoplasia (CIN). All CIN reports have been followed up by colposcopy and lesions treated. Nicaraguan staff members have been intensively trained in sample processing and the PCR-hybridisation method of HPV typing. External quality assurance has been introduced to the Ixchen cervical screening laboratory.
- ⇒ The presence of HPV has been determined in 1,861 samples out of the 2,028 recorded samples in the first visit and in 1,434 samples of the fifth last visit. The PCR method implemented was that described in the protocol for the project (Ting and Manos, 1989),

with approximately 80 % reproducibility for the first visit (6.5 % of the samples were subjected to separate determinations) and 99 % for the last visit (20 % of the samples were repeated and 5 % were tested three separate times). There were 84 HPV positives in the first visit, which constitutes a minimum prevalence of 8 % HPV infection. Of these, a high proportion (54 %) of women had high-risk HPV types. In the last visit there was 9,3% HPV infection. Of these 25 % had high-risk HPV. Only 3 women identified in the high-risk HPV group of the first visit were also identified as high-risk HPV in the last visit.

Follow-up

During the last year of the project, cost effectiveness was assessed and final specimen taken from women remaining in the study. Quality of screening at the Nicaraguan laboratory was improved.

Selected publications

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**ANALYSIS OF HUMAN CUTANEOUS LEISHMANIASIS IN MEXICO :
MECHANISMS FOR RECRUITMENT OF HOST CELLS AND INDUCTION OF
IMMUN EFFECTOR FUNCTIONS**

Co-ordinator: Universität Würzburg, Würzburg, Germany (Reinhard Gillitzer)

Objectives

- ◆ The overall objective of the research project is to analyze the local immune response mechanisms in the skin of patients suffering from cutaneous American Leishmaniasis at various stages of infection. Of particular interest are events regulating recruitment of host cells (tissue macrophages, Langerhans cells) and effector cells (T lymphocytes, macrophages); the role of chemoattractant cytokines (chemokines) being of primary interest.
- ◆ Furthermore, the research project intends to analyze the processes responsible for intracellular killing of parasites with particular emphasis on chemokines as cell-activating cytokines.
- ◆ A third aspect of the project is the identification and classification of *Leishmania* parasites with respect to the clinical course of leishmaniasis (localized cutaneous, sporotrichoid type and diffuse cutaneous leishmaniasis).

Activities

- ★ The project focusses on the identification of chemokines involved in the recruitment of host cells and effector cells during infection with *Leishmania* parasites. To this end, several field trips were made to Campeche, Yucatan region of Mexico where biopsies of patients suffering from leishmaniasis were taken before treatment. Pathohistological diagnosis and immunohistological evaluation of the composition of the inflammatory infiltrate were made by the partners in Mexico City. Since *Leishmania* lesions do not always show parasites at a microanatomic level, the diagnosis was further confirmed by PCR analysis (University of Erlangen/Nürnberg). The expression of chemokines on the mRNA and at the protein level, using the techniques of *in situ* hybridization, and immunohistochemistry were performed at the Department of Dermatology, University of Würzburg. Evaluation of the chemokine expression profile was done cooperatively by Research Center of Infectious Diseases, University of Würzburg.
- ★ In a second set of experiments, macrophages were cultured and infected by *Leishmania*; major parasites *in vitro*. These infected macrophages were incubated with those chemokines which had been detected *in vivo*, in particular chemokines which are specific to monocytes/macrophages. The capacity for stimulating intracellular killing was evaluated and biochemical processes analyzed at the Research Centre of Infectious Diseases.
- ★ The parasitological surveillance of *Leishmania* species was carried out in Mexico City (UNAM). Parasites were isolated and a genotypical analysis was carried out, with restriction endonucleases, by means of RAPD (random amplified polymorphic DNA).

Results so far

In localized cutaneous American Leishmaniasis (LCL), the dominating chemokine is MCP-1 (monocyte chemoattractant protein-1) which is co-localized with infiltrating macrophages. In contrast, in non-healing diffuse cutaneous Leishmaniasis (DCL), MCP-1 expression is significantly reduced and expression of MIP-1 α (macrophage inflammatory protein 1 α) is strongly upregulated. More recently, we could demonstrate, that the T-cell attractant chemokines MIG (monokine induced by interferony) and IP-10 (interferony –induced protein-10) are highly expressed in LCL but not in DCL. In conclusion, our data indicate that self-healing LCL and non-healing DCL are associated with a completely different chemokine profile.

In view of the *in vitro* effects of MCP-1, our data suggest that MCP-1 may also stimulate macrophage microbicidal mechanisms which was further investigated *in vitro*. *In vitro* studies have shown that MCP-1 is able to significantly enhance the antimicrobial activity against intracellular *Leishmania* parasites, whereas MIP-1 α is ineffective. Therefore, our *in vivo* and *in vitro* data suggest that MCP-1 expression in self-healing LCL stimulates recruitment of macrophages and intracellular killing of *Leishmania* parasites. This is further corroborated studying serial biopsies of two patients with DCL during treatment with IFN γ . The chemokine expression showed a switch in the chemokine profile towards the pattern typical for LCL lesions. Therefore, we conclude that effects of IFN γ treatment in DCL are partially exhibited through induction of chemokines and their partly beneficial anti-infectious activity.

Follow up

In recent years a series of chemokines have been discovered. The expression and the role of these chemokines in the two polar forms of the disease (LCL vs. DCL) will be studied to better understand the inflammatory network in *Leishmaniasis* and to develop strategies for chemokines as therapeutic agents using an animal model.

Selected publications

Ritter U, Moll H., Laskay T., Bröcker EB, Velzaco O., Becker I. and Gillitzer R., 1996. Differential expression of chemokines in patients with localized and diffuse cutaneous American leishmaniasis. *J. Infect Dis* **173**, 699-709.

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A STUDY OF THE MOLECULAR BASIS TO THE CHROMOSOMAL SIZE VARIATION IN LEISHMANIA PERUVIANA WITH PARTICULAR REFERENCE TO GP63 CONTAINING CHROMOSOMES

Co-ordinator: University of Cambridge, Cambridge, United Kingdom (James W. Ajioka)

Objectives

The overall objective of this work was to investigate the molecular basis for chromosomal size variation in *Leishmania* as it relates to loci important to parasite virulence, survival and consequent host disease pathology. This investigation required:

- ◆ To set up of a YAC library and physical map of the GP63 region.
- ◆ To use the contig and long-range restriction mapping to delimit the region of insertion/deletion/ rearrangement
- ◆ To show that most of the chromosomal size variation could be attributed to changes in this region for a geographic collection of natural isolates.

Activities

Our first survey of chromosomal size variation of the GP63 chromosome suggested that the GP63 region is highly variable as assessed by *EcoRI* RFLPs. Upon further analysis, it appeared as though the amount of RFLP variation associated with this region may not be due to polymorphic restriction sites but rather to insertion/deletion/rearrangement size variation in the region, so this was investigated. Parasites were collected by the Peruvian partner laboratory. Analyses were performed in Cambridge.

Results

Size variation could be the result of chromosomal insertion/deletion. To map the chromosome, a yeast artificial chromosome (YAC) library of *L. peruviana* (strain 1116c8) genomic DNA was set up. It consists of 2496 yeast colonies, giving a 6X genome coverage, with average size insert of about 70 kb. The relatively small variance between the numbers of positive clones identified by the various probes suggests that the library is not substantially biased and will be a reasonable representation of the *L. peruviana* genome. The combination of restriction mapping the YAC clones carrying the GP63 locus, long-range restriction mapping the *L. peruviana* clone 1116c8 map and densitometric analysis of the resulting autoradiograms, generated a map similar to those for other *Leishmania* species. Using the 1116c8 standard map, long-range restriction mapping and densitometric analysis of the GP63 chromosomal gene arrangement in other isolates showed substantial variation. The variation did not appear to be a result of the gain/loss of the flanking restriction, but rather a result of duplications and deletions within the region. The instability of the YACs is consistent with this notion, as both of these observations are likely due to mitotic recombination/repair processes. The analysis of the chromosome from a collection of geographic isolates showed

variation in size ranging from about 570 to 670 kb. Although this level of variation was observed in all the geographic regions, the average size of the chromosome differed between geographic regions. This suggests that there are sequences or sequence organisations (e.g. large tandem arrays) specific to particular chromosomes that will participate in large (i.e. 10-40 kb) rearrangements. Additionally, it is possible that selection may play a role in either maintaining or suppressing this kind of variation. These results suggest that the majority of the GP63-chromosomal size polymorphism observed in natural isolates of *L. peruviana* can be explained by size variation at the GP63 region. Moreover, the size variation is likely due to deletion/duplication events in the region. Since GP63 is a vital protein for parasite invasion and survival, it is possible that the variation seen in this chromosomal location may affect the fitness of the parasite and consequent disease pathology in the host.

Follow up

This work has led to a general geographic population study of *Leishmania peruviana*, using microsatellite markers. The geographic correlations in the new work appear to be consistent with the present reported results. Also, this work has led to a more general study of New World leishmaniasis and the important development of markers which show associations with disease pathology, in particular the mucocutaneous phenotype.

Selected publications

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Contract number: CII*CT930326

Period: January 1994 to December 1996

**RESEARCH PROJECT: DNA SUPERCOILING, REGULATION OF GENE
EXPRESSION AND VIRULENCE IN *SALMONELLA TYPHIMURIUM***

Co-ordinator: Imperial College School of Medicine, London, United Kingdom
(Christopher F. Higgins)

Objectives

Many genes which play a role in bacterial virulence are sensitive to changes in DNA supercoiling. Mutants in the *hns* gene alter DNA supercoiling and also the expression of many virulence factors. This has led to the suggestion that *hns* mutants might be severely attenuated in their virulence. The project was established to test this hypothesis, using a mouse model system and *Salmonella* to demonstrate whether *hns* mutants were indeed attenuated and might therefore be used (for example) in live-vaccine development. Furthermore, we aimed to develop an understanding of the mechanisms by which *hns* mutants cause attenuation, particularly whether they influence the initial processes of adherence to invasion of epithelial cells, or whether their effects are subsequent in terms of expression within the host.

Activities

- * Construction of appropriate strains of *S. typhimurium* for the proposed studies by introducing *hns* mutations into mouse-virulent strains of *Salmonella*.
- * Characterization of these mutant strains in terms of the presence of H-NS protein and gene expression.
- * Assessment of the effect of *hns* mutations on the expression of specific virulence-related genes.
- * Assessment of the effect of these *hns* mutations on the virulence of *S. typhimurium* strains in mice.
- * Study of the details of the initial stages of the infection process *in vitro*, measuring adherence and invasion of mammalian cells in culture.

Results

- ⇒ By Phase P22 transduction, several independent *hns* mutations were introduced into virulent strains of *Salmonella typhimurium*. Standard strains SL1344 (Falkow) and SB111 (Finlay) were used to allow comparison with data from other laboratories.
- ⇒ These mutants were characterized in terms of their phenotypes and genotypes. The *hns* gene was shown to be mutated in these constructed strains and to exert the expected phenotypes as seen in non-virulent strains; effects on DNA supercoiling, failure to express the H-NS protein, and defects in motility.

- ⇒ Using gene fusions to *invA* gene, which is known from the work of Galan and others to influence invasion of epithelial cells, it was shown that *hns* mutations significantly de-repress this gene (see publication).
- ⇒ The *hns* mutants of mouse-virulent *Salmonella* were shown to significantly alter the survival of Balb/c mice following oral infection. *hns* mutants were attenuated so that the mice survived, in contrast to the parental *hns*⁺ strains. Significantly, *invA* mutants were not attenuated, suggesting that, although the *invA* gene is required for invasion in tissue-culture cells, it is not necessarily essential for mouse virulence.
- ⇒ *In vitro* studies on mammalian cells (Hep-2) were conducted. It was demonstrated that *hns* mutants, despite de-repressing *invA* gene expression, had no significant effect on either invasion of or adherence to epithelial cells in culture. These data demonstrate that the effects of *hns* mutants on virulence are complex and that *hns* mutants influence stages in infection subsequent to adherence/invasion.

Selected publications

Harrison J.A., Pickard D., Higgins C.F., Khan A., Chatfield S.N., Dorman C.J., Hormaeche C.E. and Dougan G. 1994. Role of *hns* in the virulence type of pathogenic *salmonellae*. *Mol. Microbiol.* **13**:133-140 (1994).

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EFFECTS OF LIPOPROTEINS AND CHOLESTEROL ON "G" PROTEINS

Co-ordinator: Universidad de Alcalá, Alcalá de Henares, Spain (María José Toro Nozal)

Objectives

The general goal is to study relationships between variation in cell cholesterol content and possible changes in level and function of GTP-binding proteins and processes involve in signal transduction pathways mediated by these proteins.

Activities

In the existing literature, there are few and contradictory data regarding the effects of cholesterol and lipoproteins on enzymatic activities and ionic channels directly involved in hormonal action. Even less is known about the possible effect of cholesterol on both the levels and activity of the G transducer proteins and/or of its three sub-units (α , β , γ). These proteins act as mediators between specific receptors and effectors (AC, PLC, ionic channels, etc.). Data from our laboratory indicate that the increase in cellular cholesterol stimulates adenylyl cyclase (AC) in rat pituitary tumour cell line, and that the increase in cellular cholesterol stimulates adenylyl cyclase (AC) in rat pituitary tumour cell line, and that this increase is due to a greater presence of α s in the membrane. On the other hand, the cholesterol content also affects the secretion of GH and PRL in a differential manner. The project focused on studying the relationship between cholesterol cell content and levels of G proteins, mRNA levels of α , β and γ sub-units and different adenylyl cyclases.

We mainly used pituitary tumour cell from rats (GH4C1). These cells were cultured in the presence of lipoprotein deficient serum (LPDS) or LPDS plus cholesterol, 25 hydroxicholesterol or lovastatin in order to change cholesterol cell content. Under these conditions:

1. Measured levels of hormones secreted into the media (GH and PRL), levels of different sub-units of G proteins in plasma membrane, and AC activity (Spanish laboratory)
2. determined levels of mRNA by PCR mediated amplification of reverse transcribed RNA from α , β and γ sub-units of G proteins and different AC isoforms (Chilean laboratory).

Results

A culture unit was built in Concepción and fully equipped to train scientists in cell culture. We studied the effect of lovastatin, a cholesterol-lowering drug, on the basal state of G-proteins in GH4C1 cells. Our data show that the addition of lovastatin markedly decreased the amount of the α -sub-units of the Gs and Gi-proteins in the plasma membrane. The decrease of α s was correlated with a decrease in AC activity, and both effects were reverted by the presence of mevalonate. As the attachment of G-protein sub-units to the membrane is dependent on γ -subunit prenylation, we assume that the mechanism through which lovastatin exerts its effect on G-proteins is the lack of mevalonate for the synthesis of prenyl residues. On the other hand, we tried to verify whether that decrease in α s levels could affect PRL and GH secretion, as well as the expression of PRL and GH-CAT constructions. Since the regulation of these two genes is dependent on the pituitary specific transcription factor Pit-1,

the effect of lovastatin on the expression of Pit-1-CAT constructions was also studied. Ours result show that lovastatin decreased the basal expression of these three cAMP-responsive genes in GH4C1 cells, being partially reversed by the addition of mevalonate to the culture medium. This effect of lovastatin on the promoter activities of the transfected constructions was also observed in PRL and GH secretion to the medium, suggesting that this drug produces similar changes in the endogenous promoters of both hormones.

Follow up

Now our attention has focused on

- Studying possible changes on mRNA levels of different G protein sub-units and Ras protein after changes on cholesterol cell content.
- Studying function of G α sub-units : GTPase activity, nucleotide exchange and nucleotide binding.
- Studying changes in protein-protein association after the same treatments.

Selected publications

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URINARY TRACT INFECTIONS BY *PROTEUS MIRABILIS*: AN INVESTIGATION INTO THE ROLE OF FIMBRIAE AND FLAGELLAE AS VIRULENCE FACTORS AND IMMUNOGENS

Co-ordinator: University of Cambridge, Cambridge, UK (Duncan Maskell)

Objectives

The major objectives of this project are to achieve an understanding of the precise roles of fimbriae and flagellae in the pathogenesis of urinary tract infections caused by *Proteus mirabilis*.

Activities

The objectives of the project were to be attained through a strategy including: cloning of fimbrial and flagellar genes, generation of defined allelic replacement mutants of these genes in *P. mirabilis*, expression of *P. mirabilis* fimbrial and flagellar proteins in *E. coli* and *S. typhimurium*, assessment of virulence of the defined *P. mirabilis* mutants in mouse models of urinary tract infections, and assessment of the vaccine potential of purified native and recombinant proteins from *P. mirabilis* and attenuated multivalent *Salmonella typhimurium* vaccines expressing *P. mirabilis* proteins. The recombinant DNA work was mainly performed in Cambridge by a Uruguayan PhD student, while the work with native and recombinant proteins was mainly carried out in Montevideo. Infection studies were performed in both institutions.

Results

In the course of the project, a variety of *P. mirabilis* fimbrial genes have been cloned and sequenced. These genes have been used to generate mutants for over-expression in *E. coli*, with subsequent purification of the recombinant proteins. These proteins have been used to immunize rabbits for the generation of anti-sera against the proteins. To date, mutants lacking Mrp, Pmf and Atf fimbriae have been generated. We are currently in the process of constructing a Uca mutant. In addition, we are in the process of generating multiple mutants lacking more than one fimbriae. The mutants that have been generated are being tested in mouse models of urinary tract infection to attempt to define the precise role of each fimbria in infection. Previously, we have constructed a mutant lacking flagellae, and this has been tested in the mouse model of ascending urinary tract infection. It is as virulent as the wild-type bacterium in this assay, and thus we have not investigated this any further. Structural fimbrial proteins (MrpA, UcaA, PmfA and AtfA) have been over-expressed as recombinant proteins in *E. coli* and MrpA has also been expressed in a *S. typhimurium* aroA vaccine strain. Anti-sera have been generated against the proteins expressed in *E. coli*. Native fimbrial and flagellar preparations have also been obtained from several clinical isolates and anti-sera raised against these. The immune response to the recombinant and native proteins and to the multivalent *Salmonella* vaccine is being assessed in mouse models, to investigate whether the proteins

have any vaccine potential. All fimbrial extracts reacted with the polyclonal rabbit antiserum raised against the recombinant MrpA protein, and they also consistently contained common protein bands at 24 and 37 kD. Any of these conserved proteins could be considered as vaccine candidates. Sera obtained from rabbits immunized with the 24 and 37 kD proteins reacted with homologous and heterologous fimbriae in immuno-gold electronmicroscopy. Native flagellae have been isolated from *P. mirabilis* and used as immunogens in mice. These have provided a significant degree of protection against infection with *P. mirabilis* expressing the homologous flagellae, but not against those expressing heterologous flagellae.

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HEAT STRESS, SALT APPETITE AND NEUROGENIC HYPERTENSION

Co-ordinator: University of Leiden, Leiden, The Netherlands (E. Ronald de Kloet)

Objectives

The overall goal was to identify central mechanisms underlying disturbances in water and salt homeostasis in order to understand the initial events in the pathogenesis of neurogenic hypertension.

Activities

- * The project focused on the interaction between mineralocorticoid hormones secreted by the adrenal cortex and vasoactive neuropeptides synthesized in hypothalamic neurons. The mineralocorticoids readily enter the brain and bind to intracellular receptors, which act as transcription factors in control of neuropeptide gene expression. The latter comprise the natriuretic peptides ANP and CNP, which act in balance with angiotensine II and vasopressine.
- * As animal models were used : (i) rats receiving deoxycorticosterone acetate (DOCA)-salt which are exposed to chronic exogenous mineralocorticoid excess and (ii) rats exposed to discontinuous chronic heat and emotional stress, which develop hypersecretion of mineralocorticoids by the adrenal cortex. The project combined measurement of ingestion (appetite) of salt, blood pressure and heart rate with the analysis of transcripts of the mineralocorticoid receptor, the vasoactive neuropeptides and their receptors i.e. ANP, CNP, AngII and AVP, using in situ hybridization and Northern blots.

Results

- ⇒ Vasopressin (AVP) mRNA biosynthesis was increased after DOCA treatment. This increase occurs in the magnocellular PVN, a hypothalamic nucleus integrating responses to stress, fluid homeostasis and cardiovascular function.
- ⇒ DOCA salt treatment decreases AVP immunoreactivity and the number of AVP positive cells in the magnocellular PVN. Thus, depletion of the AVP peptide triggers AVP biosynthesis needed to replenish the stores depleted by the continuous action of salt and mineralocorticoid.
- ⇒ AVP mRNA expression differs between sexes under basal levels, after adrenalectomy, and after dexamethasone treatment suggesting a role of sex hormones in susceptibility to stress-induced hypertension.
- ⇒ Blockade of brain mineralocorticoid receptors attenuates the pressor response to stress in rats conditioned to daily exposure to elevated temperature. This suggests mineralocorticoid-sensitivity of sympathetic outflow significant in pathogenesis of hypertension.

Follow-up

Subsequent studies will focus on the interaction of mineralocorticoids and neuropeptides in the pathogenesis of hypertensive states, and changes in electrolytes and fluids leading to brain pathology.

Selected publications

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Period: March 1995 to February 1998

ANTIGENIC AND GENETIC ANALYSIS OF HUMAN RESPIRATORY SYNCYTIAL VIRUSES ISOLATED WORLD-WIDE

Co-ordinator: University of Warwick, Coventry, United Kingdom (Craig R. Pringle)

Objectives

The aim of the project was to create the first comprehensive network for surveillance of human respiratory syncytial virus (HRSV) infection in children on a world-wide basis. It was anticipated that this network would provide accurate information on the mode of spread of the A and B subgroups of HRSV in human communities. The data obtained would provide the first accurate estimate of the global circulation of this virus, and determine whether antigenic variation plays a significant role in the disease process. This information will be essential in designing strategies for control of this ubiquitous pathogen whenever any of the candidate vaccines now under development become available for clinical use.

Activities

The project involved collaboration between virologists in the UK, Spain and Uruguay. The UK group in Coventry collected viruses from Northern and Central Europe, Africa, Southeast Asia, Australia and North America, the group in Madrid collected viruses from Southern Europe and Central America, and the group in Montevideo collected viruses from South America. The genetic variability of the viruses was determined both by restriction endonuclease digestion analysis of the N (nucleoprotein) and partial nucleotide sequencing of the G (attachment) protein genes (in Coventry), and by Rnase/mismatch cleavage fingerprinting of the G protein gene (in Madrid). The Madrid laboratory also hosted and provided training in molecular virology for virologists from Montevideo. The antigenic properties of the isolates are being studied using panels of anti-G protein monoclonal antibodies already available in Madrid and others prepared there during the course of this project. The task of devising a simple Elisa test for rapid strain diagnosis was delegated to the Coventry laboratory.

Results so far

Significant progress has been made on all fronts. Isolates of subgroup A predominated and could be assigned to one or other of the six genotypes (or lineages) previously defined during a seven year study of the prevalence of HRSV in a single conurbation in the UK (Birmingham). These and other data indicate unexpectedly that HRSV exhibits temporal rather than geographical variation, suggestive of rapid global dissemination of new variants. Changes in the surface antigens are associated with this genetic variation, and current research is directed towards establishing whether this antigenic change represents a progressive response to immune selection. To facilitate this work a simple Elisa test has been developed which can detect virus in saliva samples. During the course of this project work was completed on sequencing of the genome of the attenuated ts1C candidate vaccine virus and its

virulent progenitor. Specific mutations in the L (polymerase) and F (fusion) protein genes have been implicated in determination of the attenuated phenotype. This information will be valuable in the development of additional vaccine viruses by reverse genetics should subgroup A vaccines currently under development prove inadequate in control of disease.

Follow-up

A proposal has been submitted to extend this work and in particular to examine the extent and characteristics of genetic and antigenic variation among subgroup B isolates of HRSV. A unique aspect of the disease situation in South American countries (and also in Scandinavia) is that B subgroup isolates are more frequent than elsewhere; this circumstance which will be addressed in the new project.

Selected publications

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**CENTRAL GABAERGIC MECHANISMS, MELATONIN AND AGEING.
EXPERIMENTAL CHRONOBIOLOGICAL STUDIES**

Co-ordinator: Université Louis Pasteur (CNRS-UMR 7518), Strasbourg, France (Paul Pévet)

Objectives

To examine ageing of the circadian apparatus in rodents by assessing behaviour related to g-aminobutyric acid (GABA), the principal neurotransmitter in the circadian clock, and by measuring the chronobiological organisation of the immune response.

Activities

Part of the project was focused on the identification of ageing-related changes in activity of GABA-containing neurons and of GABA-related behaviours, like anxiolysis and the resynchronization activity of melatonin in hamsters. These studies were carried out in Buenos Aires and Strasbourg. At Strasbourg, characterization of brain melatonin receptors by autoradiographic and *in situ* hybridization methods in hamsters of different ages were made. At Santander, the locomotor activity and the resynchronization capacity of the circadian system in a senescence-accelerated mouse were examined. Activities on the chronobiological organisation of the immune response in aged rodents were performed mainly at Buenos Aires. They included the definition of age-related circadian changes in cell proliferation in lymphoid organs in Sprague-Dawley rats treated with Freund's adjuvant and developing an autoimmune disease of the joints. This paradigm was used to analyze the changes of circadian rhythms during autoimmune diseases at different ages and the potential capacity of melatonin treatment to restore amplitude of lymph cell proliferation rhythms in aged rats. Strasbourg was especially involved in formation of scientific personnel, including postgraduate students from Buenos Aires and Santander, who completed short as well as long (1-2 years) periods of training.

Results

In the course of the project the following original descriptions were made:

- ⇒ decreased amplitude of circadian changes in anxiolysis-related behaviour in aged hamsters that correlated with decreased brain GABA release;
- ⇒ decreased reentrainment rate of locomotor activity in aged hamsters, recovered by melatonin treatment;
- ⇒ modified reentrainment rate of locomotor activity in senescent-accelerated mice;
- ⇒ long-lasting changes in circadian rhythms of locomotion and anxiety-related behaviour in a model of endogenous depression in hamsters;
- ⇒ significant effects of immune-mediated inflammatory response after Freund's adjuvant injection on ACTH, GH, prolactin and TSH release, partially sensitive to immunosuppression;
- ⇒ (6) 24-hour rhythm in immunosuppression in rats that was in part dependent on local autonomic innervation of lymphoid tissue;

- ⇒ decreased amplitude of 24-hour variations in lymph node and splenic cell proliferation and local autonomic nervous system activity in old rats during Freund's adjuvant arthritis and after pinealectomy;
- ⇒ melatonin efficacy to restore amplitude of circadian rhythm in immune responsiveness of lymphoid tissue.

Selected publications

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Period: February 1995 to May 1997

**GROWTH HORMONE SECRETAGOGUE PEPTIDES : LONG TERM
TREATMENT ON DIFFERENT CELL FUNCTIONS AND BIOLOGICAL
PARAMETERS DURING AGEING.**

Co-ordinator: Università di Bari, Bari, Italia (Diana Conte Camerino)

Objectives

The objectives were to evaluate the effects of a long-term treatment with hexarelin, a GH secretagogue, on the most important indices of bone remodeling and muscle performance in aged animals.

Activities

It is known that muscle performance and bone composition are target of GH action and that during ageing GH release decreases. The project aimed to determine the capacity of hexarelin to stimulate GH release and to restore the different cellular function and biological parameters of aged animals vs. those of adults. Macroscopic electrophysiological parameters and activity of single ion channels of skeletal muscle fibres of aged rats treated with hexarelin or GH were evaluated in Bari. The long term effects of hexarelin treatment on GH control by hypothalamus and neurotransmitters was studied in Argentina by immunocytochemistry. In Argentina the effect of GH releasing peptides on sleep organization and on cardiac and respiratory control during sleep was also examined. IGF-I muscle levels and markers of bone resorption and bone formation of aged dogs were evaluated in Milan.

Results

Chronic GH administration to aged rats restored the membrane electrical parameters of skeletal muscle fibers vs. adult values. In contrast, in aged rats hexarelin treatment produced only a slight increase of GH plasma levels and IGF-1 muscle content. Consequently the passive cable properties and the component ionic conductances of EDL muscle fibres, modified by ageing, were not improved after hexarelin treatment. On the other hand, the GH-releasing peptide restored the kinetic parameters of the sodium channel, also modified by ageing, slightly improving the excitability of muscle fibres of aged rats. In regard to bone metabolism in aged dogs, hexarelin treatment reduced bone resorption while bone formation was apparently not affected. Hexarelin did not have effects on sleep organization or on respiratory characteristics during sleep.

Follow up

Further investigation are required to define better the hexarelin effects. Besides, it would be of interest to evaluate the effects of a chronic treatment with a non peptidic GH-secretagogue. Collaboration is currently being undertaken with a pharmaceutical company to evaluate the effectiveness of treatment with L163,255 on aged rats.

Selected publications

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PROTEIN TYROSINE PHOSPHORYLATION IN BRAIN NEURONES

Co-ordinator: INSERM U114, Collège de France, Paris, France (Jean-Antoine Girault)

Objectives

There is strong evidence to indicate that tyrosine phosphorylation is an important mechanism of regulation in adult brain, involved in the control of synaptic plasticity and neuronal survival. The long-term aim of this project was to elucidate the signal transduction pathways that involve non-receptor tyrosine kinases in brain neurones.

Activities

The present work was based on the observation that "classical" neurotransmitters, including acetylcholine and glutamate, as well as depolarization are capable of activating protein tyrosine phosphorylation pathways in hippocampal slices. The aims of this project were to identify the protein kinase(s) involved in these effects, to determine their mechanism of activation, and to investigate their role in physiological and pathological circumstances.

Results

The intercellular messengers responsible for the regulation of protein tyrosine phosphorylation have been better characterised. It has been demonstrated that acetylcholine and glutamate increase tyrosine phosphorylation via several types of receptors, including both ionotropic and metabotropic receptors. In addition, several lipidic messengers have been shown to exert potent effects on tyrosine phosphorylation in hippocampal slices. These messengers are lysophosphatidic acid (LPA), arachidonic acid, and anandamide, an endogenous ligand of CB1 cannabinoid receptors. The action of depolarization requires Ca^{2+} . Two of the protein kinases involved in these effects have been identified. Focal adhesion kinase (FAK) is activated in response to neurotransmitters, LPA and anandamide. The action of anandamide appears to be mediated by decreasing cAMP levels, whereas the other stimuli are dependent on the activation of protein kinase C (PKC). Several novel splice isoforms of FAK have been discovered in brain and their increased autophosphorylation demonstrated. Another tyrosine kinase which is closely related to FAK and named PYK2 or Cak β is also regulated in brain. However, tyrosine phosphorylation of PYK2 shows little change in response to neurotransmitters, but is markedly stimulated in response to depolarization by a pathway requiring PKC. In non-neuronal cells, FAK and PYK2 are known to transduce signals in response to integrin engagement, and FAK appears to regulate focal adhesions turn over and to prevent anoikis, a form of apoptosis induced by cell detachment from the extracellular matrix.

Follow-up

Current work in Paris aims at identifying the molecular mechanism by which cAMP or PKC regulate FAK and PYK2, what are pathways activated downstream of these kinases, and what is their function in mature neurones. The group in Montevideo is developing animal models to test the role of these tyrosine kinases in pathological circumstances such as convulsions, and hypoxia. Work is also in progress to examine by immunocytochemistry with phosphorylation-state specific antibodies, the cellular and subcellular localization of the regulatory cascades, which involve these tyrosine kinases.

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NEUTRALIZATION OF SNAKE-VENOM TOXIC EFFECTS BY IgG SUBCLASSES ISOLATED FROM IMMUNE HORSE SERUM USING MOAB; OBTENTION OF HUMAN MOAB ANTI-CROTOXIN BY MOLECULAR ENGINEERING

Co-ordinator: Institut Pasteur, Ingénierie des Anticorps, Paris, France
(Jean-Claude Mazié)

Objectives

The programme had two general goals: To obtain recombinant human antibodies directed against crotoxin (the toxin from the venom of *Crotalus* snakes) and to isolate subclasses of horse immunoglobulins, using monoclonal antibodies to neutralize snake venom *in vitro* and *in vivo*.

Activities

This project involved the preparation of antibody tools for the neutralization of snake venom. It was carried out by direct collaboration between three groups in different countries. The work of the Belgian laboratory, carried out in parallel with work in Brazil, involved preparing specific monoclonal antibodies against crotoxin and various subclasses of specific horse immunoglobulin IgG with neutralizing activity. The Brazilian team also worked directly with the Pasteur Institute in Paris, using molecular biology and genetic-engineering techniques to prepare antibody molecules capable of neutralizing snake venom activity both *in vivo* and *in vitro*. Fab fragments and single-chain Fv molecules (scFv) were isolated and their neutralizing activity demonstrated *in vitro*.

Results

- ⇒ An scFv which was effective *in vitro* was isolated. Its heavy and light chains were sequenced and the sequences compared with the CDR2 and CDR3 sequences. This work was carried out using a mouse monoclonal antibody prepared by the Venoms Unit at the Pasteur Institute. The Butantan Institute carried out the entire project under the direction of the Pasteur Institute. Another part of the work involved the isolation of antibody fragments from recombination libraries.
- ⇒ The other axis of scientific activity in the group was directed by the Catholic University of Louvain in Brussels. A Brazilian researcher was trained in the preparation of mouse monoclonal antibodies directed against crotoxin, and subclasses of specific immunoglobulins from horse. A subclass of immunoglobulins that neutralize crotoxin was isolated as part of this work which was carried out in Brazil over the last two years of the contract. Studies are continuing at the Butantan Institute, to produce other subclasses of immunoglobulin with similar neutralizing activity.

Follow-up

The goal of the program was to isolate recombinant human antibodies and monoclonal antibodies from subclasses of horse immunoglobulins capable of neutralizing crotoxin, the toxin from the venom of *Crotalus durissus terrificus*. These two objectives were attained by working in direct collaboration with a Brazilian group. Horse IgG isotypes with neutralizing activity were thus isolated by a South-American researcher and the group from Brussels. Another Brazilian researcher, who spent several long work visits at the Pasteur Institute in Paris, isolated scFv fragments from recombinant antibody libraries and a mouse monoclonal antibody. Neutralization of the toxin was demonstrated *in vitro* and further work is required to investigate whether these antibodies also have neutralizing activity *in vivo*. These studies have already begun and will continue beyond the contract in Brazil.

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EFFECT OF MEXICAN SCORPION TOXINS ON THE POTASSIUM CHANNELS OF NERVOUS MEMBRANES

Co-ordinator: Consiglio Nazionale delle Ricerche, Genova, Italy (Gianfranco Prestipino)

Objectives

The general goal of the project was to characterize, at the molecular level, the function of the voltage-gated potassium channels by the use of novel toxins purified from scorpion venom, particularly from the Mexican area.

Activities

The project focused on the identification of new neuropeptides to be utilized as tools for the purification of channel proteins, as probes for studying channel functions and for mapping the different types of potassium channels present on the neuronal cell membranes. The capture of scorpions and milking for venom, the isolation and sequence determination of toxins were carried out in Cuernavaca (Mexico), along with the chemical synthesis of peptides corresponding to the amino-acid sequences of these toxins. The electrophysiological measurements were carried out in Genova, following three experimental techniques:

- a) Functional reconstitution into planar bilayers of potassium channels isolated from squid axon membranes,
- b) Patch-clamp measurements of potassium channels in cell cultures of cerebellar granule neurons and
- c) Functional expression of cRNA encoding for ShakerB K⁺channels in *Xenopus laevis* oocytes. Furthermore, electrophysiological measurements were also carried out in Cuernavaca on insect cell line Sf9 expressing ShakerB K⁺channels.

Results

In the course of this project about eighty peptides have been purified from the following scorpion venoms: *Centruroides noxius* H. (CnH), *Centruroides limpidus* L., *Pandinus imperator* (Pi) and *Androctonus australis* G.(AaG). A novel class of toxins from Pi affect, with high affinities, the ShakerB K⁺channels. The new Aa1 toxin from AaG blocks selectively, with high affinity, the I_A type component K⁺current of cerebellum granular cells, leaving the other potassium component, identified like delayed rectifier, unaltered. An affinity column prepared with Noxiustoxin, a K⁺channel blocker from CnH, has been used to purify, and subsequently to reconstitute in a planar lipid bilayer, a functional channel from squid Schwann cells.

Follow up

During the last year of the project, seven synthetic fragments of Noxiustoxin have been produced to characterize the putative sites of interaction toxin-channel in delayed rectifier K⁺channels.

Selected publications

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**FUNCTIONAL AND IMMUNOPROTECTIVE PROPERTIES OF ORGANELLE
PROTEINS (ROP2 AND GRA4) FROM *TOXOPLASMA GONDII***

Coordinator: Université François Rabelais, Tours, France (Daniel Bout)

Objectives

Define the function of ROP2 and GRA4 proteins of *T. gondii* in the host cell invasion process and evaluate their immunoprotective properties.

Activities

- * The maturation of proteins of the ROP family during their biosynthesis was studied in Lille.
- * To analyse the topology of GRA4 which is associated with the vacuolar network, the reactivity of antibodies generated in Tours against the regions of GRA4 located on both sides of the putative transmembrane sequence was analyzed on ultrathin cryosections in Lille.
- * The knockout of the GRA4 gene was performed in Tours to analyse the possible alteration of parasite development in those deletion mutants.
- * The immunoprotective properties of recombinant GRA4 and ROP2 proteins expressed in *E. coli* following systemic and mucosal immunizations in mice were evaluated in Tours for GRA4 and in Mexico for ROP2.
- * The role of the dendritic cells in the differential activation of Th1 or Th2 subsets by the recombinant GRA4 and ROP2 proteins, and in the establishment of a protective immune response to *T. gondii* was determined in Brussels.

Results so far

- ⇒ Several constructs of ROP2 (Mexico) and GRA4 (Tours) have been made and expressed in *E. coli*. A method was developed to purify the recombinant form of ROP2, giving a high yield free of detectable bacterial products. Storage conditions of the purified recombinant proteins also were determined. A mouse model of oral infection with the Me49 *T. gondii* strain and a mouse model of lethal infection with the RH strain were established. Vaccination trials were performed in the murine models, using different adjuvants and immunization ways.
- ⇒ The maturation of proteins of ROP family during their biosynthesis has been studied. Results indicate that the proteolytic maturation of ROP4 protein is exclusively in the N-terminal region. The precise definition of the sequence of processed ROP proteins allowed to study their functions, topology and finally allowed the determination of domain exposed to the immune response.
- ⇒ The next study aim was the expression of proteins of the ROP family in their processed form. Once expressed, these proteins will be used to study their ability to bind to cellular membrane in order to confirm their role during host invasion by *T. gondii*.
- ⇒ Naked DNA immunization was developed to study the immunogenicity of GRA4 protein. Intramuscular injection of plasmid pcDNA3-GRA4 induced a good humoral response and a strong resistance to *T. gondii* cyst formation after oral challenge. Vaccination trials will be continued using the mucosal route and co-administration of plasmids encoding cytokines.

Follow up

- During the last year of the project, Mexico developed a method to purify the recombinant form of ROP2.
- Lille studied the maturation of proteins of ROP family during their biosynthesis.
- Tours evaluated the antigenicity of GRA4 and its immunizing property following intramuscular injection of plasmid pcDNA3-GRA4.

Selected publications

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DEVELOPMENT OF TRANSGENIC TECHNIQUES BASED ON EMBRYONIC STEM CELLS FOR THE ANALYSIS OF THE MOUSE *MSX1* HOMEBOX GENE PROMOTER

Co-ordinator: Institut Pasteur, Paris, France (Benoît Robert)

Objectives

The aim of this project was to investigate the potential of transgenesis via ES cells in the mouse, and use this technique to identify sequence elements in the *Msx1* homeobox gene promoter responsible for directing its expression

Activities

The strategy made use of embryonic stem (ES) cells, which can colonise mouse embryos extensively when injected into morulas. Five transgenes, containing sequences from the *Msx1* gene ranging from 4.9 to 0.33 kb 5' of the translation initiation site were constructed in Rio de Janeiro and Paris. A PhD student from Rio has come to work for two years in Paris, and analysed the properties of these transgenes in ES cell cultures and in chimeric embryos. Sequence analysis of the *Msx1* promoter was conducted in Rio. The London laboratory performed a dedicated analysis of *Msx1* expression in the heart.

Results so far

During development, *Msx1* expression is observed in sites of induction between ectoderm and mesoderm and in the heart. In chimeric mouse embryos, the longest transgene recreates most of the *Msx1* endogenous pattern of expression, between E9.5 and 11.5, except for the fronto-nasal mesenchyme. At E12.5, expression is essentially lost from the mesoderm at all sites of expression and remains only in the ectoderm. Most ES cell clones do not express the longest transgene in cell culture, but express it in a number of histological types after differentiation. Shorter transgenes are more sensitive to the genomic context and are often expressed in undifferentiated ES cells. One transgene was expressed ectopically in the foetal muscles of a chimera. Concomitantly, expression was observed in the myotubes that differentiated from the ES cell in culture, suggesting that the properties of a transgene in a chimeric embryo can be used to identify cells of undefined phenotypes in culture. The promoter sequence analysis, combined with previous results from the Paris laboratory, has permitted the definition of a minimal promoter in the *Msx1* gene, active in many cultured cell types, which contains binding sites for transcription factors. Analysis of *Msx1* expression in the heart demonstrates that, starting from a large domain in the cardiac endothelium, it is progressively restricted to the endocardial cushion.

Follow-up

Msx1 promoter analysis is continued in a collaboration between the Paris, London and Rio laboratories. In the latter, ES cell and transgenesis techniques have been implemented and people have acquired the appropriate competence to run the transgenesis unit.

Publications

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Period: February 1995 to January 1998

DESIGN OF INHIBITORS FOR THE BLOOD COAGULATION AGENT β -FACTOR XIIA

Coordinator: CNRS, Université Henri Poincaré, Nancy-1, France (Bernard Maignet)

Objectives

This project focuses on one of the starting events of the intrinsic pathway of the coagulation cascade, and concerns the design of specific inhibitors of β -Factor XIIa in order to contribute to the discovery of new efficient anti-thrombosis therapy.

Activities

Since designing inhibitors of an enzyme for which no structural information was available, was a complex problem, we decided to start with basic investigations concerning:

- ★ the study of basic molecular interactions involved in the catalytic process, in order to get more highlights about the intimate details of the hydrolysis chemical reaction. Understanding the fine mechanism involving the catalytic triad should help propose solutions to block it;
- ★ the study of the forces involved in ligand/protein complexes similar to the one we will consider later for β -factor XIIa. This will give us indications about the way the ligand will be positioned inside the protein, and information about the secondary interactions occurring between the ligands and su β -sites on the enzyme. The model system chosen was the complex between Trypsin and BPTI. Two kinds of researches were performed on this system: $\Delta\Delta G$ calculations to investigate the change in binding energies upon mutations in the enzyme, and hybrid Quantum Mechanics: Molecular Mechanics (QM/MM) calculations to understand the inhibition phenomenon at the electronic level.
- ★ Next, we concentrated our efforts on the building of a reasonable three-dimensional model of β -Factor XIIa using homology modelling. Three models were independently constructed and their relative qualities compared, using various procedures.
- ★ Finally, using what we learned from the above steps, we docked several known ligands to the 3D model of β -Factor XIIa and started the design of putative new compounds.

Results

A set of seven molecules was proposed for a synthesis based on our theoretical models. We are now able to propose tests to validate the models, as well as the synthesis of putative active compounds.

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Period: March 1995 to February 1998

**VASCULAR CELLS AS A TARGET FOR NEOPLASTIC TRANSFORMATION AND
AS COMPONENT OF NEOPLASTIC TISSUES: STUDIES USING POLYOMA
MIDDLE T-TRANSFORMED ENDOTHELIAL CELLS AND REAGENTS
GENERATED THEREOF**

Co-ordinator: Istituto Ricerche Farmacologiche Mario Negri, Milan, Italy
(Alberto Mantovani)

Objectives

The general goal is to investigate the biological characteristics of endothelial cells (EC) after neoplastic transformation by the middle T oncogene of polyoma virus and the relevance of neoangiogenesis for metastasis dissemination, using lines of a mammary carcinoma and/or melanomas with different capacity to give metastasis.

Activities

- * The *in vivo* growth of middle-T transformed EC (H5V line) was evaluated in the syngeneic mouse strain C57Bl/6 and the resistance mechanisms investigated using immunodeficient (nude) mice and monoclonal antibodies (mAb) to specific leukocyte subpopulations and cytokines.
- * Therapy with cytokines was also tested. Identification of EC in mammary carcinomas has been performed by immunohistochemistry, using an anti-mouse CD31 mAb, generated and developed by the investigator's group. The same mAb has been used to isolate EC from different organs in mice, in order to set up methodology and strategy to isolate EC from tumors in the future.
- * Melanoma lines with different metastatic behaviour have been identified to study their production of MCP1, a chemokine with a proposed role in neoangiogenesis.

Results so far

⇒ H5V cells cause vascular tumors in syngeneic mice, with characteristic similar to those of opportunistic vascular tumors, as Kaposi's sarcoma, that is high leukocyte infiltration, important recruitment of host EC, more aggressive behaviour and "KS-like spindle cells" in immunocompromised hosts. Resistance to the tumor is T cell-mediated (CD8); IFN γ and IL-12 are therapeutically active. IL-12 effects on tumor growth are not sustained by an increase in CTL activity. Isolation of EC from lung and from FGF-neoinduced blood vessels was performed by the immunomagnetic selection of CD31+ cells, using the anti-mouse CD31 mAb and dynabeads. Cells were stabilised in culture and phenotyped with specific EC markers, as VE-cadherin constitutive expression and E-selection induction by inflammatory cytokines. A positive correlation was found between the vascularization and metastatic behaviour of different mammary carcinoma lines and work is in progress to evaluate the MCP1 production by different lines established from human melanomas.

⇒ A new mAb to mouse CD34 has been generated and characterized. The characterization of the vascular tumor model, the generation of new mAbs and the methodology for EC isolation have been performed in Milano, while the tumor vascularization analysis and establishment of melanomas lines have been conducted in Buenos Aires.

Selected publications

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Period: February 1995 to February 1998

SNAKE VENOM COMPONENTS ACTING ON HEMOSTASIS: MECHANISM OF ACTION AND DRUG-DESIGN PERSPECTIVES

**Co-ordinator: Institut Pasteur, Paris, France
(Cassian Bon)**

Selected publications

V. Arocas, R.B. Zingali, M.C. Guillin, C. Bon, M. Jandrot-Perrus, 1996. Bothrojaracin: A potent two-site-directed thrombin inhibitor, *Biochemistry*, **9083-9089**.

M. Leduc, C. Bon, 1998. Cloning of subunit of convulxin, a collagen-like platelet-aggregating protein from *Crotalus durissus terrificus* venom *Biochem. J.* **333**, 389-393.

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**IMMUNOLOGICAL AND MOLECULAR DIAGNOSIS OF TAENIA SOLIUM
CYSTICERCOSIS AND TAENIASIS**

Co-ordinator: University of Salford, Salford, United Kingdom, (P.S. Craig)

Objectives

- ◆ Develop, evaluate and apply immunological and molecular methodologies for diagnosis of *Taenia solium* infection and intermediate hosts.
- ◆ Maintain/investigate *Taenia solium* taeniasis model in hamsters and other mammals.
- ◆ Characterize copro-antigens and produce monoclonal antibodies to *Taenia solium* and standardize a copro-antigen test in rodent model and human infection.
- ◆ Assess DNA dot-blot egg hybridization for *Taenia* in faecal samples.
- ◆ Sub-clone *Taenia solium* recombinants GP24 and TS13 for production, analysis, and diagnostic assessment in cysticercosis.

Activities

- ★ *Taenia solium* is transmitted between pigs and humans, and is the causative organism of human neurocysticercosis (and porcine cysticercosis) and human taeniasis. The former results from cystic lesions in the CNS (and in muscle in pigs) and taeniasis after development of the adult tapeworm in the GI tract. The best diagnostic approach is to detect antibodies specific to the tissue-dwelling stage, and copro-antigen and/or faecal DNA detection for the gut stage.
- ★ The Mexican (UNAM and field collaborators) partner was mainly responsible for using and improving animal models for taeniasis diagnosis research, development of DNA egg detection methods and characterization of *T. solium* antigens, using a panel of *T. solium* monoclonal antibodies. The UK partner (University of Salford) was primarily responsible for the characterization of taeniid copro-antigens and the development of specific immunoblot tests of cysticercosis, production of monoclonal antibodies, and development of putative recombinant antigens for immunodiagnosis. In addition, field collaboration in southern Mexico between Mexican and UK institutes enabled the provision of panels of reference sera and faecal samples, and the assessment of prototype diagnostic tests within an endemic community situation.
- ★ The main outcome has been the development of a novel animal model for *Taenia solium* taeniasis, with the first ever production of gravid worms, identification of a myosin-like protein in *T. solium* adult and larval stages with diagnostic potential, the development of a practical species-specific immunoblot test for antibody recognition of the 26Kda *T. solium* antigen, and its confirmation of application in human and porcine hosts, amino-acid sequencing of the 26KDa molecule, partial immunobiochemical characterization of *Taenia solium* copro-antigens, and isolation and characterization of species-specific DNA probes from *T. solium* for egg detection.

Follow-up

Currently, the diagnostic development and animal model are being applied/used in Mexico to undertake further applied and experimental research on *T. solium*. Continuation of molecular laboratory studies on *T. solium* myosin and the 26 KDa molecule are planned in order to clone their respective genes. Furthermore, it is intended to apply the development and knowledge from this Joint Research project to facilitate studies on *T. solium* transmission with Mexico and also in China and south-east Asia

Selected publications

- Chapman A., Vallejo V., Mossie K.G., Ortiz D., Agabian N. and Flisser A.S. 1996. Isolation and characterisation of species-specific DNA probes from *Taenia solium* and *T. saginata*, and their use in an egg detection assay. *Journal of Clinical Microbiology*. **33**: 1283-1288.
- Ambrosio J., Cruz M., Allan J.C., Moran E., Ersfeld K., and Flisser A. 1997. Identification and partial characterisation of a myosin-like protein from cysticerci and adults of *Taenia solium*, using a monoclonal antibody. *Parasitology*. **114**: 545-553.
- Rodríguez-Canul R., Allan J.C., Fletes C., Sutisna P., Kapti N., and Craig P.S. 1998. Comparative evaluation of purified *Taenia solium* glycoproteins and crude metacystode extracts by immunoblot for the serodiagnosis of human *T. solium* cysticercosis. *Clinical and Diagnostic Laboratory Immunology*. **4**: 579-582.
- Craig P.S., Rogan M.T., and Allan J.C. 1996. Detection, screening, and community epidemiology of taeniid cestode zoonoses; cystic echinococcosis, alveolar echinococcosis, and neurocysticercosis. *Advances in Parasitology*. **Vol. 38**: 169-250.
- Fraser A. and Craig P.S. 1997. Detection of gastrointestinal helminth infections, using copro-antigen and molecular diagnostic approaches – minireview. *Journal of Helminthology*. **71**: 103-107.
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**PUBLIC HEALTH STUDY OF PSYCHIATRIC DISORDERS, DRUG ABUSE AND
HEALTH BEHAVIOUR IN SANTIAGO, CHILE**

Co-ordinator: University of Wales, Cardiff, United Kingdom (Glyn Lewis)

Objectives

- ◆ Measure inequalities in the prevalence of psychiatric morbidity, functional somatic symptoms and drug abuse between different socio-economic groups in Santiago, Chile.
- ◆ Determine the strength of the relationship between socio-economic group and use of health care services.
- ◆ Explain the differences in health service use between socio-economic groups by adjusting for psychiatric morbidity, functional somatic symptoms, drug abuse and social support.
- ◆ Elucidate the reasons for using and not using health services and the perception of health services according to gender and socio-economic group.
- ◆ Compare the results with those from the British National Survey of Psychiatric Morbidity, which uses similar design and methods.

Activities

- ★ Out of the total of 4000 interviews, 3473 were carried out.
- ★ Exchange visits occurred in both directions.
- ★ By the beginning of 1998 all the interviews were completed and the data were entered in order to allow analysis.
- ★ An international workshop took place in 1998 in order to discuss the results, compare with results from elsewhere in the, UK and formulate recommendations for the Ministry of Health in Chile.

Results

- ⇒ Psychiatric morbidity has been measured with the Clinical Interview Schedule - Revised, (CIS-R) an interview developed by some of the researchers in this study (G.L. & R.A.). These preliminary results are on data from 1886 adults interviewed up until May 1997. This sub-sample represents approximately half of the total sample size estimated for this study. It is possible that the complete sample will have different results but these are presented for interest.
- ⇒ Rates of psychiatric disorder were almost twice as high in Santiago (31 % prevalence) than in the UK (14 % prevalence). The shape of the distribution on both sites was similar. The rates of psychiatric disorder were higher in those with less education and women had a higher prevalence than men. The quality of housing was also significantly related to psychiatric morbidity though the ownership of housing was not. This is not found in countries like the UK where tenants have higher rates of psychiatric disorder than those who own their own home.

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ISC

Materials Sciences

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**COMPUTER SIMULATION STUDIES OF THE STRUCTURE AND DYNAMICS OF
GLASSY SYSTEMS**

Co-ordinator : University of Cambridge, Cambridge, United Kingdom (Ian R. MacDonald)

Selected publications

Ferrario M., Klein M.L., McDonald I.R. 1995. Cation transport in lithium sulphate-based crystals. *Molecular Physics*. Vol. **86**, no. 4, 923-938.

Sergi A., Ferrario M., Elliott S.R., McDonald I.R. 1995. Molecular dynamics study of the plastic-crystalline phase transition of tetraphosphorus triselenide. *Molecular Physics*. Vol. **84**, no. 4, 727-742.

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**MAGNETISM AND SUPERCONDUCTIVITY IN ELECTRON
SUPERCONDUCTORS: INFLUENCE OF OXYGEN STOICHIOMETRY AND
MICROSTRUCTURE**

Co-ordinator: Institute of Materials Science, CSIC, Barcelona, Spain (Xavier Obradors)

Objectives

The overall objective of the project was to analyse in detail the outstanding role of oxygen stoichiometry in the microstructure, normal state and superconducting properties of the electron doped superconductors $\text{Re}_{2-x}\text{Ce}_x\text{CuO}_4$ (Re = Nd, Pr, Sm, Gd).

Activities

- * The choice of these materials was motivated by the extreme sensitivity of their properties to the oxygen stoichiometry, which could be related in some sense to the fact that they are the unique series of electron-doped oxide superconductors.
- * The groups from Bariloche and Madrid developed a precise methodology of controlling oxygen stoichiometry, by means of thermogravimetric analyses under controlled oxygen pressure. The microstructure and the crystal stability were analysed, mainly in Madrid, through Transmission Electronic Microscopy
- * The magnetic and superconducting properties were investigated in close collaboration between Barcelona and Bariloche. Materials preparation was carried out in all the laboratories (single crystals and ceramics) and an exchange of samples was scheduled.

Results

- ⇒ In the course of the project, the microstructural mechanisms controlling the formation of a superconducting behaviour in the ceramic $\text{Nd}_{2-x}\text{Ce}_x\text{Cu}_{1+\delta}\text{O}_{4+y}$ samples were clarified. It was proposed that a very small concentration of cationic vacancies ($\delta \approx 0.01$) may destroy the formation of a superconducting condensate and only when these vacancies are filled by Cu ions, during high temperature annealing under reducing conditions, the superconductor behaviour appears. These findings demonstrated the extreme sensibility of the superconducting behaviour to the existence of defects.
- ⇒ On the other hand it was demonstrated that small Re ions (smaller than Gd) generate a distorted crystal structure responsible of the formation of weak ferromagnetism and the non-appearance of any superconducting behaviour. A close connection between the magnetic properties and crystal structure was evidenced in these studies, because a reduction of the crystal symmetry allows the formation of non-collinear antiferromagnetic structures. Finally, the irreversible superconducting properties were analysed by means of high frequency electromagnetic waves. It was a particularly useful the analysis, in the microwave range, through a new technique called "Magnetically Modulated Microwave Absorption" (MAMMA).
- ⇒ Overall, the collaboration between partners allowed us to show that the electron-doped superconductors adopt a very complex behaviour. It was also very appealing to observe

the fact that the physico-chemical challenges involved were so demanding that new advances in experimental techniques were achieved in the course of the project.

Follow Up

- At the end of the project, it was widely agreed that the electron-doped superconductors were too complex to become useful electronic materials. There was then a general agreement to shift the research activities towards materials, with a higher practical potential applicability, where the oxygen stoichiometry plays also a central role.
- The collaboration between the different partners then was extended to study manganese perovskite oxides displaying colossal magnetoresistance and Hg-based superconductors, which display the highest transition temperatures. In both systems the knowledge generated during the present project has become a very useful background.

Selected publications

Prado F., Briático J., Caneiro A., Tovar M. and Causa M.T. 1995. Copper content and superconductivity of $\text{Nd}_{1.85}\text{Ce}_{0.15}\text{Cu}_{1+\delta}\text{O}_y$. *Solid St. Commun.* **90**, 695

Seffar A., Fontcuberta J., Piñol S., Obradors X., Peraudau G., Berjoan R. 1996. Copper deficiency and superconductivity in $\text{Nd}_{2-x}\text{Ce}_x\text{CuO}_4$ oxides. *Physica C* 259, 75

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Magnetic field dependent microwave absorption in a $\text{Sm}_{2-x}\text{Ce}_x\text{CuO}_{4\pm\delta}$ single crystal
A.Rouco, J.Fontcuberta, X.Obradors, S.Piñol, L.Fábrega, M.Tovar, J.Briático and M.T.Causa
Physica B 194-196, 1585 (1994).

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**GROWTH AND CHARACTERIZATION OF PURE AND DOPED COPPER
TERNARY IN BOTH BULK AND THIN FILM FORMS FOR PHOTOVOLTAIC
APPLICATIONS AND A DETAILED STUDY OF THEIR MAGNETO-TRANSPORT
AND MAGNETO-OPTICAL-PROPERTIES**

Co-ordinator: Service National des Champs Magnétiques Pulsés, INSA, Toulouse, France
(Jean Leotin)

Objectives

- ◆ Grow large single crystals of pure and doped Cu-III-VII₂ ternaries and their alloys by different techniques;
- ◆ Establish their homogeneity, single phase nature and structure;
- ◆ Study the temperature and magnetic field dependence of their electrical resistivity and Hall effect to determine activation energies and the conduction mechanisms;
- ◆ Study the optical absorption and photoluminescence as a function of temperature to determine the variation of the band gap and identify the origin of the different defect levels.

Activities

To this end, in addition to the use of existing infrastructures, new facilities were installed. These consisted of fourteen zones fully automated horizontal Bridgman furnace, a He⁴ cryostat having a 2 Tesla superconducting coil and a He³ cryostat in Toulouse and in Mérida installation of 22 Tesla pulsed magnetic field, eight zone automatic vertical Bridgman furnace, and an apparatus for the measurement of the thermal conductivity. Large single crystals of CuInSe₂ were grown in Toulouse at different selenization temperatures by a new technique called "Horizontal Varying Gradient Zone Freeze Technique". Single crystals of CuInSe₂, CuInSe₂:O₂, CuInSe₂:Fe, CuInTe₂, CuInTe₂:Zn,Cd, CuIn_{1-x}Fe_xTe₂ were grown by this technique in Mérida and Fe-doped Cu(In_{1-x}Ge_x)Se₂ were also synthesized. The structural characterization, optical absorption, electrical resistivity and Hall effect of all samples were made in Mérida. High mobility and X-ray diffraction showing the absence of secondary phases confirmed the good quality of the ingots. Eg vs T and ρ, R_H vs T up to 4K were also made in Mérida. The photoluminescence and the magnetoresistance (MR) in both low and high magnetic fields between 1.6 a 300K in these materials were studied in Toulouse.

Results

- ⇒ Conduction by activation above 100K, in general, and mainly by variable range hopping (VRH) of Mott type between 1.5 at 100K have been observed in n-CuInSe₂, p-CuInSe₂ and p-CuInTe₂. VRH conduction at such high temperatures, not reported in traditional semiconductors, has allowed us to study in detail the temperature dependence of the MR in both low and high magnetic fields. Results confirm :
- ⇒ the quantum interference model for the negative MR in low field;
- ⇒ Efros Shklovskii model for positive MR above and below the critical field B_c and
- ⇒ the effectiveness of the Coulomb gap in high magnetic fields in CuInSe₂ and CuInTe₂. Study of the photoluminescence combined with the stoichiometric composition of the

samples and the observed activation energies in CuInSe_2 and CuInTe_2 permits the identification of different defect levels. From the variation of E_g with T some useful band parameters are estimated. Three Ph.D., four MS and one licenciatura thesis have come out of these works and more than 30 papers have been published in reputed journals.

Follow-up

It is found that CuIn_3Se_5 precipitates at the surface of In-rich CuInSe_2 thin films. Presence of this phase supposedly increases the efficiency of solar cells based on this material. Hence, we are also studying the physical properties of $\text{Cu}(\text{In}_{1-x}\text{Ga}_x)_3\text{Se}_5$ system. Several papers have already been published.

Selected publications

Arsene M. A; Albacete A; Voillot F; Peyrade J.P; Barra A; Galibert J; Wasim S M; and Hernández E. 1996. Synthesis and growth of large stoichiometric single crystals of copper indium diselenide by horizontal varying gradient zone freeze technique. *Journal of Crystal Growth*, **158**, 97-102.

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DYNAMIC SIMULATION OF CRYSTALLINE WATER MOLECULES USING X-RAY DIFFRACTION CONSTRAINTS

Co-ordinator: CNRS, Illkirch, France (Alberto Podjarny)

Objective

The main objective of the project is to produce an algorithm that will describe the structure and dynamics of a macromolecule and its surrounding water molecules, using both the information of the interactions potential and the experimental X-ray diffraction data. This algorithm will be applied to different experimental cases. The molecules to be studied by the Strasbourg laboratory are aldose reductase, the tRNA helix [U(UA)₆A]₂ and an heterologous tRNA-synthetase complex.

Activities

- * The project focuses on the extension of existing molecular dynamics simulation packages such as GROMOS and XPLOR, to improve the treatment of disordered water molecules in the crystals of macromolecules studied by X-Ray crystallography methods using the diffraction amplitude data.
- * A first attempt, based on the GROMOS package, led to a solute-grid-restrained molecular dynamic program package (SGRMD). This package, starting from known macromolecular models, calculates solvent occupancy maps. It is based in the calculation of the mean occupancy of the solvent region, and was tested in BPTI, Rubredoxin and in the RNA fragment U(UA)₆A.
- * To introduce the X-ray diffraction constraints, a novel water simulation approach, based on the program XPLOR (MC-XPLOR), was developed. This protocol uses a multicopy approach to water modelling, with several copies, simulated by molecular dynamics, and contributes at the same time to the X-ray potential. It was applied to BPTI, Rubredoxin, Aldose Reductase (both in the native form and complexed with inhibitors), the RNA fragment U(UA)₆A and an heterologous tRNA-synthetase complex. To check the validity of the protocol, it was applied to BPTI, using neutron diffraction data (which has a strong water component) and the results were compared with the residence times measured by NMR.

Results

The results of the MC-XPLOR protocol enable the classification of water molecules in terms of their relative order. The water molecules could be separated in different classes, depending on their exposure. Buried waters are the most rigid ones, while surface waters are more mobile and they form channels that follow the crevices in the surface of the protein. The dispersion of the several copies give a clear idea of the mobility of the water molecules, and in the BPTI case it correlates well with the residence times measured by NMR. In the case of Aldose Reductase, the results obtained with the native protein agree well with those obtained for the complexes with the inhibitors tolrestat and sorbinil. Aldose Reductase has two water channels, going from the interior of the molecule (ordered waters) to the surface (disordered

waters) and the multicopy procedure gave a clear picture of the water mobility inside these channels.

Follow up

The existing protocols are being updated to include the mobility of the protein residues near the surface and of the inhibitors in the case of the Aldose Reductase complexes. The mobility of these residues and inhibitors will also be simulated by the multicopy protocol. The result should give a clear idea of the conformational space available to these molecules, which is necessary to properly simulate the docking of protein and inhibitors. This has important pharmaceutical consequences in the case of Aldose Reductase, an enzyme involved in diabetes complications.

Selected publications:

- Grigera J.R., Grigera T.S., Howard E.I. and Podjarny A. 1994. Molecular dynamics simulation of crystal water with X-ray constraints. *International Journal of Quantum Chemistry Biol. Symp.*, **21**: 109-116,.
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- Howard Eduardo I., Grigera J. Raul, Podjarny Alberto D. and Urzhumtsev Alexander 1997. Multicopy modeling of the solvent distribution in macromolecular crystals,. *Acta Cryst.*, **A52S**, C86,.
- Podjarny Alberto D., Howard Eduardo I., Urzhumtsev Alexander and Grigera J. Raul. 1997. A multicopy modeling of the water distribution in macromolecular crystals". *Proteins: Structure and function*, **28** : 303-312,

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**THIN FILMS OF NEW MATERIALS DEPOSITED BY PLASMA ASSISTED
TECHNIQUES**

Co-ordinator: Universidad Autónoma de Madrid, Madrid, Spain (J. M. Martínez Duart)

Objectives

The general goal of this project has been the study and development of thin film materials deposited by plasma assisted techniques, such as PECVD (plasma enhanced chemical vapour deposition) and PECVD (remote plasma enhanced chemical vapour deposition). The envisaged applications are in the fields of micro- and opto- electronics, hard coatings and barrier coatings for packaging.

Activities

The thin film materials grown in this project have been silicon oxide, silicon nitride, silicon oxynitrides and diamond films. Silicon oxide and silicon nitride films were deposited by PECVD activated by a RF (13.56 MHz) discharge. The diamond deposition was carried out in a microwave PECVD system (MWCVD). In these systems, all the gases are exposed to the plasma and the substrates are immersed in it. However, the system used for depositing silicon oxynitrides was of the "remote" type of PECVD. In this system only a subset of the reactant gases is directly plasma excited and the substrate is outside of the plasma region. In this manner, excellent quality films are produced. The main characterisation techniques used were infrared spectroscopy, ellipsometry, electrical measurements (C-V), Rutherford Backscattering Spectroscopy (RBS), microhardness measurements and Atomic Force Microscopy (AFM).

Results

- ⇒ Silicon oxide and silicon nitride films deposited by PECVD showed very good properties for being used in microelectronics as interlevel dielectrics and as gate oxides in MOS devices. The silicon nitride obtained corresponds to stoichiometric Si_3N_4 with a refraction index of 2.0. The index of refraction of the silicon oxynitride films deposited by PECVD could be continuously varied between the values of the oxide (1.5) and the nitride (2.0).
- ⇒ On the other hand, silicon oxide thin films deposited on polymeric film substrates have been studied as transparent gas barrier coatings for the packaging industry. A very interesting relationship between the pore surface fractality and the water vapor and oxygen permeabilities has been developed (see references below).
- ⇒ Finally, diamond thin films with applications as hard coatings have been obtained with low graphite content (approx. 5 %). These diamond films were grown for different concentrations of methane and oxygen in hydrogen.

Selected publications

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Contract number: CII*CT930039

Period: December 1993

**SILICON CARBIDE FILMS PREPARED BY SPUTTERING, PECVD AND
MRPECVD**

Co-ordinator: University of Wales, Swansea, United Kingdom (J.M. Marshall)

Selected publications

S.P. Lau, J.M. Marshall, T.E. Dyer, A.R. Hepburn, and J.F. Davies. 1994. Blue-light electroluminescence from doped $\mu\text{-SiC}$ prepared by excimer (ArF) laser crystallisation. *Materials Research Society Symposium Proceedings*. **339**: 647.

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S.P. Lau, J.M. Marshall, and L.R. Tessler. 1995. Optoelectronic properties of highly conductive microcrystalline SiC produced by laser crystallisation of amorphous SiC. *Proceedings 16th International Conf. On Amorphous Semiconductors*. Science & Technology, Kobe, Japan.

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Contract number: CII*CT930041

Period: February 1994 to June 1996

HYDROPHILIC COMPOSITE ULTRAFILTRATION MEMBRANES

Co-ordinator: GKSS-Forschungszentrum, Geesthacht, Germany (Klaus-Viktor Peinemann)

Objectives

Development of hydrophilic membranes for nano- and ultrafiltration with high water permeability and low susceptibility to fouling.

Activities

Membranes for this project were composite, made of several layers: a non-woven layer of polyether or polypropylene, an asymmetric porous poly(vinylidene fluoride) support and a dense top layer. The top layer consisted mainly of a hydrophilic blockcopolymer of poly(ethylene oxide) and polyamide, which were modified by *in situ* reacting different amines with trimesoyl chloride. The two lower layers, including the asymmetric porous support, are standard products of GKSS. The research project was concentrated on the development of the top layer. The University of Campinas was in charge of the initial developments of the material itself for the selective layer, chemical characterisation and development of membranes in the laboratory scale. Optimisation of the membranes for preparation in a continuous machine, as well more specific characterisation and evaluation of the performance in nano- and ultrafiltration was the role of GKSS.

Results

In the first stage of this work composite membranes were developed with a selective layer of polyether-block-polyamide. Membranes with molecular weight cut-offs between 800 and 4500 g/mol and water permeabilities between 2.3 and 9.4 l/h m² bar were obtained with quite low susceptibility to fouling. In the second stage the selective layer was modified by a *in situ* polycondensation reaction using different amines as starting materials. The objective was to decrease the membrane cut-off to the range of nanofiltration, keeping the water flow as high as possible. With the use of diamines with long polyether segments in the main chain, membranes with cut-off of 600 g/mol and permeability of around 3 l/h m² bar.

Follow-up

The membranes were tested for industrial applications. Wastewater from the metallurgical industry containing mainly water and oil (about 6 %) was concentrated up to about 60 % oil. Membranes were also delivered to a petrochemical industry for tests, having the objective of decreasing the oil content of waterstreams to be discharged as waste from the oil recovery.

Selected publications

Nunes S.P., Sforça M.L. and Peinemann K.V., 1995. Dense hydrophilic composite membranes for ultrafiltration. *Journal of Membrane Science* **106**: 49-56.

Sforça M.L., Nunes S.P. and Peinemann K.V., 1997. Composite nanofiltration membranes prepared by *in situ* polycondensation of amines in a poly(ethylene oxide-b-amide) layer. *Journal of Membrane Science* **135**: 179-186.

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Contract number: CII*CT930044

Period: February 1994 to February 1998

STRUCTURAL RELAXATION AND LONG-TERM BEHAVIOUR IN AMORPHOUS POLYMERS

Co-ordinator: Université Paul Sabatier de Toulouse III, Toulouse, France
(C. Lacabanne)

Objectives

- ◆ Understand the mechanisms of the physical ageing phenomenon.
- ◆ Propose a physico-mechanical model which characterize physical ageing.
- ◆ Develop a technique to characterize the low-temperature ($T < T\beta$) physical ageing.
- ◆ Understand the effect of physical ageing in the secondary relaxations (mainly β -relaxation).
- ◆ Propose a procedure to quantify the long-term behaviour of amorphous polymers from short-term tests.

Results

- ⇒ The responses to a thermal stimulation were followed. By Differential Scanning Calorimetry, the structural relaxation of heat capacity was analysed. The enthalpic relaxation time was extracted from this study. By ThermoStimulated Currents, the structural relaxation of the electrical polarization was measured: it gives the dielectric relaxation time of structural polarization. Both enthalpic and dielectric relaxation times are analogous. In the thermodynamic analysis, the relaxation time has two components: an Arrhenian one and a Vogel-Tamann-Fulcher one. In other words, the "apparent" activation enthalpy increases strongly while approaching T_g . In the dielectric analysis, a discrete distribution of relaxation times (τ_i) is introduced. Since τ_i obeys a compensation law, it is only dependent upon one parameter: the activation enthalpy ΔH_i . The increase of ΔH_i around T_g is coherent with the evolution of the "apparent" activation enthalpy.
- ⇒ The isothermal transient response was analyzed. The non-linearity versus time is well described by a stretched exponential. The evolution versus ageing was characterized.

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Contract number: CII*CT930053

Period: January 1997 to December 1997

**PREPARATION AND CHARACTERIZATION OF A NEW GENERATION OF
IMPROVED TRACK ETCHED MEMBRANES FOR MICROFILTRATION AND
ULTRAFILTRATION**

Co-ordinator: University of Glasgow, Department of Chemistry (Russel Paterson)

Objectives

The main goal of this project was to produce track etched membranes at the IPEN-IEA-R1 nuclear reactor and to conduct a full range of characterization tests for these membranes, by using the gas permeation technique.

Activities

- * The project has been proposed to develop track etched membranes (TEMs) in IPEN-São Paulo, for micro and ultrafiltration applications. The technique consists basically in irradiating thin sheets of polycarbonate plastic in a well collimated beam of fission fragments. The thermal neutron flux close to the centre of the IEA-R1 reactor core is used to produce the fission fragment beam from an uranium sample. After the irradiation, the tracks produced by the fission fragments across the entire thickness of the plastic foil, are selectively etched by an appropriate chemical solution. The track (pore) diameter is controlled by the etching time while the pore density is determined by the irradiation time.
- * The full range of TEMs produced in Sao Paulo are characterized, by using the gas permeation apparatus of the University of Glasgow, mainly to establish the pore size and the pore density for these membranes.

Results so far

A new experimental device for track etched membranes (TEMs) production has been designed, tested and installed near the core of the IEA-R1 (2MW) nuclear reactor at IPEN-Sao Paulo. Membranes with pore diameters ranging from 15 to 100 nm have been prepared using Makrofol KG plastic foils, 10 µm thickness. The pore diameter and the pore density for these TEMs were determined by the nitrogen permeation technique in the University of Glasgow. The gas permeation apparatus has been transferred to IPEN-Sao Paulo and at present moment is being employed as a routine technique for characterization of these porous membranes.

Selected Publications

Yamazaki I.M., Paterson R. and Geraldo L.P. 1996. A new generation of track etched membranes for microfiltration and ultrafiltration. Part I. Preparation and characterization. *J. Membranes Science* **118**: 239.

Contract number: CI1*CT930053

Yamazaki I.M., Geraldo L.P. and Paterson R. 1998. Characterization of polycarbonate nuclear track-etched membranes by means of the gas permeation method. Submitted to Nuclear Inst. Methods. in Physics Research (Section A).

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SURFACE MODIFICATION DUE TO ALKALI ATOM ADSORPTION

Co-ordinator: Forschungszentrum Jülich, Jülich, Germany (Ansgar Liebsch)

Objectives

This project aims at elucidating the electronic structure and the response to electromagnetic fields of clean and alkali-metal-covered metal surfaces, as well as surface alloys between free electron-like metals and alkali metals.

Activities

The scientific project consisted in experimental investigations carried out in Berlin (Germany) as well as in Valparaiso (Chile), and theoretical calculations performed in Jülich. It combined the particular strengths of the groups involved in that a leading researcher in the field of theoretical surface response theory provided important input for the type of experiments to be performed. The specific strength of the experimental groups consisted in the experience concerning alkali-metal adsorption that the groups had achieved, the technical background in aluminium surface preparation, and most importantly, the facilities at the BESSY storage ring that enabled us to perform photoelectron-yield experiments which define the present state-of-the-art and are of an unparalleled quality. Several novel results were obtained, which are of great interest for an understanding not only of the surface response in alkali metals, but also for the characterization of the electronic structure of the surface alloy phase. The experiments and their interpretation in terms of current theoretical models open the path to further studies in which the partners will continue to collaborate.

Results

The experimental investigations were carried out, on the one hand, in Valparaiso, where a fully functional scanning tunnelling microscopy laboratory was installed through funding by the European Community in the course of this project. Scanning tunnelling microscopy is one of the key methods of modern surface science, which can be used in investigations at a fundamental as well as an applied level, encompassing the determination of surface structure at the microscopic (atomic) level as well as the so-called mesoscopic level (surface steps, adsorbate islands, etc.). This instrument is currently set up to study growth modes of metal films, in particular alkali metals, on noble metal substrates such as gold. The collaboration between the partners in the experiments carried out in Berlin led to the successful set-up of a facility for photoyield measurements in the energy region from the visible part of the electromagnetic spectrum to the far ultraviolet (about 2 to 20 eV). This has enabled us to study the region of plasmon excitation on clean and alkali-metal-covered surfaces of aluminium, as well as on thick layers of alkali metals. Moreover, we were able to prepare the surface alloy phases which were recently observed in the Na/Al system, and to study their surface response. For the latter project, the wide range of photon energies was particularly important, since the high-energy response of Al (nearly 13 eV) and the low-energy features of

the alkali metals (2.5 to 7 eV) could be measured in the same experiment. Among the features that were studied is the influence of the atomic lattice on the shape and intensity of the bulk-like and multipole plasmon. A surprising finding was that the lattice affects the multipole plasmon to a much lesser extent than the bulk-like mode. This result could be interpreted in terms of the calculations for realistic alkali-metal layers, which were carried out within the frame of this project.

Follow-up

The project has opened up the path to a better understanding of the electronic structure of alkali-metal surfaces and alloy surfaces. In view of the fact that the experimental set-up seems to be quite unique in its capability to study the optical response in such systems and the recent progress in the theoretical description of the relevant physical processes, the partners have agreed to continue their projects through mutual visits and collaborative experiments. In Valparaiso, the focus will be on studies of the growth modes of metal films, using the scanning tunnelling microscope now installed there. In Berlin, the surface optical response will be at the centre of interest, making use of the BESSY storage ring.

Selected publications

Barman S.R., Häberle P., Horn K., Ishida H., and Liebsh A. 1998. Photo-induced plasmon excitations in alkali metal overlayers. *Physical Review*. **B57**: 6662.

Barman S.R., Häberle P. and Horn K. in press. Collective and single particle excitations in the photoyield spectrum of Al. *Phys. Rev. B. (Rapid Comm.)*. In press.

Barman S.R., Stampff C., Häberle P. and Horn K.. Dependence of collective excitations on surface electron density. *Phys. Rev. Lett.* Submitted

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Contract number: CI1*CT930062

Period: March 1994 to March 1996

**STUDY OF THE STRUCTURAL, OPTICAL, AND TRANSPORT PROPERTIES OF
AMORPHOUS AND MICRO-CRYSTALLINE SILICON CARBON ALLOYS
FABRICATED BY PLASMA-ENHANCED CHEMICAL VAPOUR DEPOSITION**

**Co-ordinator: Università di Roma "La Sapienza", Roma, Italy,
(F. Evangelisti)**

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Period: January 1994 to June 1996

CHEMICAL CROSSLINKING OF POLY(VINYL CHLORIDE)

Co-ordinator: Institute of Polymer Technology and Materials Engineering, Loughborough, United Kingdom (Marianne Gilbert)

Objectives

The aim of the programme was to develop chemical methods for the crosslinking of poly(vinyl chloride) compounds. This requires control of the crosslinking process. For melt processes, it is essential that little or no crosslinking occurs during processing, but that an adequate level of crosslinking is produced subsequently. Thermal stability must also be addressed. Reactions which create crosslinks also tend to accelerate PVC degradation, so the stabilization of crosslinkable compounds was also considered.

Activities

- * Work at CIQA (Mexico) focused on the synthesis of:
 - thiotriazine salts as crosslinking agents for plasticized PVC and
 - novel tin compounds for use as both heat stabilizers and crosslinking agents.
- * Work at IPTME (UK) concentrated on the production of peroxide and silane crosslinked compounds. Characterization methods for crosslinked compounds were developed in collaboration for use at both institutions. Advice was provided from Akros, who also supplied materials for the project.

Results

A magnesium derivative of thiotriazine has been synthesized, and shown to be effective as a thermal crosslinking agent for plasticized PVC. A compound synthesized as a tin stabilizer is also effective in crosslinking. Peroxides have been used successfully as thermal crosslinking agents in compounds incorporating an unsaturated monomer. All the above compounds have been produced by milling at a low temperature with crosslinking occurring during processing at higher temperatures. Peroxide crosslinked foams have also been produced. Silane containing plasticized PVC compounds which can be crosslinked by immersion in water have been developed. These can be processed by milling/moulding, or extrusion. Various techniques have been used to follow grafting and crosslinking reactions.

Follow-up

Research is continuing at both CIQA and IPTME. Silane crosslinking looks of interest for extruded applications, and cable companies in Mexico have expressed an interest in developing such systems. IPTME has established a link with OSi Specialities (UK) Ltd., who, together with Akros, are supplying materials for future work. Rigid PVC is a strong, tough and chemically resistant polymer, with its relatively low glass transition temperature (80°C) being one of its main limitations in use. A current project is concerned with crosslinking rigid

PVC in order to increase its softening temperature, hence applicability. Thermal crosslinking agents capable of being processed via milling/moulding could potentially be used in calendered applications.

Selected publications

Yanez-Flores I.G., Gilbert M. 1994. Development of crosslinked flexible PVC foam formulations. *Cellular Polymers*, **13**: 371-388.

Saethre B., Gilbert M. 1996. Peroxide crosslinking of plasticized poly(vinyl chloride). *Polymer*, **37** : 3379-3386.

Rodríguez-Fernández O.S., Gilbert M. 1997. Aminosilane Grafting of Plasticized Poly(vinyl Chloride). I Extent and rate of crosslinking reactions. *Journal Applied Polymer Science*, **66** : 2120-2129.

Rodríguez Fernández O.S., Gilbert M. 1997. Aminosilane Grafting of Plasticized Poly(Vinyl Chloride). II Grafting and crosslinking reactions. *Journal*

Fiaz M., Gilbert M. 1997. Silane Crosslinking of Plasticized Poly(Vinyl Chloride). *Advances in Polymer technology*. In the press.

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**STUDIES ON LIPID-PROTEIN MOLECULAR INTERACTIONS IN FOOD
PROTEIN USING BIOPHYSICAL MEMBRANE TECHNIQUES**

Co-ordinator: University of Oxford, Oxford, UK (Anthony Watts)

Objectives

The main objective of this project was to study the molecular interactions between food proteins (myosin and β -casein) and lipids in model systems which can be used for novel food emulsions. In particular, the project studied:

- ◆ the interactions between the proteins and several membrane-forming lipids using biophysical methods; and,
- ◆ the orientational characteristics of the proteins at the lipid interface.

Activities

Solid-state ^2H NMR was used to gain further information at the molecular level about the interactions of myosin and β -casein, two widely used proteins for the production of food emulsions, with lipids at the emulsion interfaces. In the first year, myosin was purified (Brasil) for NMR studies (UK) and further modified (Brasil) for FPLC work (UK). Data from the NMR studies was transferred via the internet from Oxford to São Paulo for processing by an innovative programme being developed in Brasil. Attempts were made to model the small protein, β -casein, at an interface similar to those found in emulsions, using a Cray computer (Brasil). This protein would provide a good model for the larger myosin system. A variety of labelled and unlabelled phospholipids were synthesised and confirmed using NMR (UK), in preparation for studying their interaction with the myosin. The purchase of a 300MHz NMR machine by Brasil enabled parallel work in both laboratories to be undertaken and also allowed the transfer of recently developed pulse sequence techniques from UK to Brasil. Two senior people from Oxford spent one month in São Paulo soon after the delivery of the new NMR spectrometer. They carried out many experiments and, where necessary, helped the laboratory with some experimental set-ups and conditions. Temperature-dependent sensitive static and fast spinning lineshape and relaxation experiments on selectively deuterated material were carried out. Deuterium and phosphorus NMR experiments on phospholipid membranes, both before and after adding various food proteins, were undertaken.

Results

⇒ Out of this work, a novel approach to study the interaction of food-related protein and lipid components has been developed. The sensitivity of the methods, previously used to study how ions, peptides, proteins and water interact with biological membranes, has given new information in this area. In particular, the interaction of type-II myosins with membranes has been shown for the first time using these approaches. Myosins are muscle-related proteins and thus found widely in most life-forms. Their interaction with

membranes, in particular the single-headed type I myosin, sheds new insights about how cell membranes could be invoked in cell movement.

⇒ For the food technology aspect of the project, a scientific way of detecting and understanding how proteins and lipids can and do interact, is always useful in this area of science, and new methods can help in the future design of such combinations for consumption, especially when deciding what factors are required to promote the necessary stable interactions needed for their use.

Follow-up

Other food proteins are also available for study (caseins) and the interaction of these with lipids could also be studied in due course. In addition, the effects of external factors such as ions and pH on the stability of protein-lipid complexes involving food-related products, should be studied.

Selected publications

Areas J.A.G., Gröbner G.J., Pellacani L.B., Glaubitz C. & Watts A. 1997. Use of solid-state ^2H NMR for studying protein-lipid interactions at emulsion interfaces. *Mag. Res. Chem.* **35**: 119-124.

Areas J.A.G., Gröbner G., Glaubitz C. & Watts A. 1998. Interaction of a Type II Myosin with biological membranes studied by ^2H solid state NMR. *Biochemistry* (in press).

The results of ^31P -MAS NMR experiments of various lipid mixtures with myosin are in the process of being prepared for publication.

Partners

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Contract number: CII*CT930318

Period: April 1994 to March 1997

MECHANIC AND MAGNETIC PROPERTIES OF THE Fe-Mn-Al ALLOYS SYSTEM

Co-ordinator: Consejo Superior de Investigaciones Científicas, Madrid, Spain
(José Ramón Gancedo Ruiz)

Objectives

- ◆ Study the mechanical properties and the corrosion behaviour of Fe-Mn-Al alloys in the FCC phase in order to select those which present properties similar to those of austenitic stainless steels.
- ◆ Study, experimentally and theoretically, the magnetic properties of Fe-Mn-Al alloys in the BCC phase, in order to obtain the present magnetic phases and correlate these studies.

Activities

For mechanical properties and corrosion studies, FCC alloys were melted using high-purity powder metals, in an induction furnace. After heat treatment they were prepared for the different tests and measurements, i.e. impact, hardness, optical microscopy and x-ray diffraction. Some samples were submitted to wet-dry cycles in two different SO₂-polluted atmospheres and then, studied by x-ray diffraction, XPS, ICEMS and Auger methods. For magnetic properties studies, FCC and BCC alloys were melted, using high-purity powder metals, in an arc furnace and after heat treatment they were prepared for measurement by x-ray diffraction, ac magnetic susceptibility, magnetization and Mössbauer spectroscopy at different temperatures. From previous results, experimental magnetic phase diagrams showing the different phases were obtained and these experimental-phase diagrams were compared with those theoretically obtained by using the Mean Field Renormalization Group method (MFRG) applied to a diluted and random-bond Ising model. Also, the experimental thermodynamic properties obtained were compared with those calculated using Bogoliubov's inequality method applied to a diluted and random-bond Ising model.

Results so far

By carrying out optical microscopy and microhardness tests, it was shown that alloys in the FCC phase with 7.5 at. % Al and 1 at. % C, present the best austenitic microstructure and the largest microhardness. The majority of alloys with these Al and C contents are austenitic but when the Mn content decreases, a certain amount of martensite/ferrite is obtained and causes the hardness value. Austenitic alloys present a high plasticity. The results obtained for the corroded samples suggest a corrosion mechanism similar to that studied for pure iron and weathering steels. Also, the weight gain in the Fe-Mn-Al alloys is from 2 to 5 times lower than in pure Fe and weathering steels. This reduction of the corrosion velocity was attributed to the formation of Mn²⁺ sulphate, which can block the cracks and pores and prevent access of oxygen and SO₂ to the surface.

Experimental magnetic studies of Fe-Mn-Al alloys enabled us to determine some phase diagrams which showed, depending on the content and temperature, the paramagnetic, ferromagnetic, antiferromagnetic, spin glass, re-entrant spin glass phases and, in some cases, a superparamagnetic-like phase. Except for the superparamagnetic-like phase, the theoretical-phase diagrams obtained by using the MFRG applied to a diluted and random-bond Ising model and adjusting the competitive parameters to reported values, our results are in good accord with other experimental ones.

Follow-up

We are now working with FCC alloys with a low Cu in order to improve corrosion behaviour. New samples and experiments, such as neutron diffraction and Mössbauer spectroscopy with external field, are under preparation and execution respectively, in order to prove more conclusively the existence of the superparamagnetic-like phase. New theoretical models, such as Heisenberg, are also being applied.

Selected publications

- Ligia E. Zamora, Pérez Alcázar G. A., Bohórquez A, Plascak J. A. and Rosales Rivera A. 1995. "Phase diagrams for a diluted and random-bond Ising model". *Phys. Rev. B.* **51**, 9329.
- Pérez Alcázar G. A., Ligia E. Zamora, Bohórquez A., González E. and González J. M. 1996. "Magnetic phase diagram of the $\text{Fe}_x\text{Mn}_{0.6-x}\text{Al}_{0.4}$ alloys series". *J. Appl. Phys.* **79**, 6155
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Period: January 1994 to December 1997

LIQUID CRYSTALLINE COPOLYMERS HAVING RIGID AND FLEXIBLE BLOCKS

Co-ordinator: Università di Genova, Genova, Italia (Alberto Ciferri)

Objectives

The overall aim of the project was the preparation of new high-technology materials based on copolymers of a rigid aromatic polyamide and a flexible polyamide or polyether. The resulting material was expected to show superior performance and easiness of fabrication with respect to the conventional high-performance composites based on a mechanical mixture of a rigid and a flexible homopolymer.

Activities

- * The main focus of the project was the synthesis of the copolymers, carried out in Santiago, in Concepcion and in Genova.
- * The next step of the project involved the physico-chemical characterization of the copolymers, carried out in Genova and Madrid.
- * The final step was the determination of the solid state properties, to be carried out in Freiburg.

Results

A series of diblock copolymers based on rigid poly(*p*-benzamide) and several different flexible segments [poly(*m*-phenylene isophthalamide), poly(*m*-benzamide), poly-(propylene glycol), poly(ethylene glycol)] were prepared. The series was based on a given rigid segment length which controls the formation of the ordered liquid crystalline phase and on variable lengths of the flexible segments. The prepared series included pure diblock copolymers "tailored" to an extent which was probably never before reported. These copolymers allowed the unambiguous discovery that attachment of a flexible chain to a rigid one results in an increased stability of the ordered liquid crystalline phase. This discovery allowed us to predict that the copolymers should be processed into films and fibres using a much simpler technology than disclosed in the patent literature for materials such as Kevlar® and high-performance composites.

Follow-up

During the last phase of the project, attempts were made to prepare films and fibres to characterize the surface and mechanical properties; the suitability of a patent application will be considered.

Selected publications

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Period: April 1994 to April 1997

**CHARACTERIZATION, OPTICAL, AND MAGNETIC PROPERTIES, AND
RELATIVISTIC CLUSTER CALCULATIONS**

Co-ordinator : University of Dublin, Dublin, Ireland (Werner Blau)

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METASTABLE ALLOY FORMATION ASSISTED BY EXTERNAL WORK

Co-ordinator: Universitat Aut3noma de Barcelona, Barcelona, Spain (María Teresa Mora)

Objectives

The general goal is the study of the order-disorder mechanisms induced by mechano-thermal treatments in several intermetallic alloys.

Activities

- ★ Sample preparation by mechanical alloying and rapid solidification;
- ★ Determination of Short Range Order (SRO), Long Range Order (LRO) and microstructure by X-Ray Diffraction (XRD), Mössbauer Spectroscopy (MS) and Perturbed Angular Correlation (PAC) spectroscopy;
- ★ Determination of the advance of nanocrystallization from the amorphous phase on Fe-Si-B-based amorphous samples performed by neutron diffraction (ND) at ISIS, Rutherford Appleton Laboratory (UK) facility;
- ★ Specification of thermodynamic and kinetic quantities & energetics of the processes by Differential Scanning Calorimetry (DSC).

Results

The results of the study of metastable alloy formation by solid state reaction in Fe-B, Fe-Al, Fe-Sn, Fe-Si and Fe-Ge alloys have concerned the evolution of short range order, crystallite size and product phases. In most alloys, the first milling stage was characterized by fragmentation and accumulation of strain. The transformation products are very sensitive to the overall alloy composition. The return to equilibrium phases for milled alloys has been extensively studied for Al₅₀Fe₅₀ samples. The extensive analysis of the correlation between MS and DSC results on heat treated powders of Fe-Ni-P-Si alloys obtained by mechanical synthesis and the comparison of the reaction products with those obtained from alloys prepared by rapid solidification has been very fruitful. The data collected from the nanocrystallization process of rapidly quenched Fe-Si-B-based (FINEMET) amorphous alloys allowed us to get a unified view of the thermodynamic and kinetic aspects that drive primary precipitation of DO₃ Si deficient (Fe,Si) nanocrystals from the amorphous phase.

Follow-up

The present effort is made to develop analysis of RE₂Fe₁₇G_x (RE= Y, Sm; G= N, H) and hydridation-deproportionation-desorption-recombination (HDDR) processing of RE₂Fe(Hf)₁₄B hard magnets through informal collaborations with other groups. These studies are addressed to a number of interesting problems such as the identification of substituents (Hf, Zr) site occupancy in the crystal structure and mechanism of gas diffusion. A research on the mechanically induced transformations in binary metallic oxides with the spinel structure has been recently initiated with the same purpose.

Selected publications

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**LATTICE VIBRATIONAL, OPTICAL AND STRUCTURAL PROPERTIES OF
CuInSe₂, AgGaSe₂, AgGaS₂ UNDER HIGH PRESSURE AND TEMPERATURE**

Co-ordinator: Université de Lille I, Lille, France (Jacques Lefebvre)

Objectives

The general purpose of this project is to get a better knowledge of the solid state properties of ternary semiconductors of the I-III-VI₂ group with a chalcopyrite structure. These compounds have potential applications in solar cell devices and non-linear optics.

Activities

During the last year, different points of the joint research project have been developed by the two partners. A large single crystal of CuFeSe₂ has been grown, allowing neutron experiments. Raman measurements at low temperature and X-ray diffraction at high pressure in AgGaSe₂ have been done. Neutron experiments on a single crystal of AgGaSe₂ and a powder sample of CuFeSe₂ were performed.

Results

Most dispersion curves of AgGaSe₂ along the directions [1, 0, 0] and [0, 0, 1] have been measured by inelastic neutron scattering. The results were analysed with different rigid-ion models: Born-von Karman and valence force field models, which give a good agreement with experiments. The nuclear and magnetic structures of CuFeSe₂ were solved: an antiferromagnetic order is observed below the Neel temperature ($T_N = 65$ K). The anharmonic properties of the soft modes (Γ_5 mode or TA[100] mode) were analysed from temperature-dependent Raman and neutron scattering experiments in CuGaS₂, AgGaS₂ and AgGaSe₂. All these phonons have very small temperature coefficients as compared to other ternary semiconductors.

Follow-up

Continuations of this work are planned. We have to explain high-pressure X-ray experiments and low-temperature Raman scattering measurements in AgGaSe₂ and, particularly, the photoluminescence. High-pressure Raman experiments in this compound will be performed in order to determine Gruneisen constants. With a single crystal of CuFeSe₂, it is now possible to study the magnetic short range order near the transition temperature.

Selected publications

Tovar R., Quitero M., Fouret R., Derollez P., Bouree F., Hennion B., Lamarche G. and Woolley J.C., 1996. Crystalline Structure and Spin Correlations in (AgIn)_{0.5}MnTe₂ and (CuIn)_{0.5}MnTe₂ alloys. Cryst. Res. Technol. **31**: 635-638.

González J., Guinet Y. and Lefebvre J., 1996. Temperature dependence of the Raman active phonons in AgGaS₂. Cryst. Res. Technol. **31**: 453-457.

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PHOTOREFRACTIVE NON-LINEARITIES IN SEMICONDUCTING, INORGANIC AND ORGANIC MATERIALS AND STRUCTURES

Co-ordinator: Universidad Autónoma de Madrid, Madrid, Spain (Fernando Agulló-López)

Objectives

- ◆ Develop appropriate theoretical models to describe the photorefractive non-linearities in thin film structures, both inorganic and organic,
- ◆ Evaluate the role of the relevant geometrical and physical parameters
- ◆ Examine possible applications to spatial modulation devices.

Activities

The project has required the evaluation of available experimental results, proposal of adequate mathematical models and rate equations, analytical and numerical solutions and modelling of experimental and device configurations. In particular, the simulations has been applied to semiconductor multiple quantum well (MQW) structures and polymer films where new interesting physics and perspectives for application are being advanced. The Mexican team has focused on the development of advanced numerical methods to simulate complex time-dependent processes and beam coupling effects in the high modulation regime. The Spanish team has been mainly responsible for working out suitable physical models and obtaining analytical results. The two teams have jointly discussed the results and have evaluated the application capabilities.

Results

The main significant conclusions are as follows : A two-dimensional model describing the performance of thin film structures has been developed and applied to semiconductor MQW structures, operating in both longitudinal and transversal geometries. The relevance for the operation of PROM spatial type modulators has been evaluated. A purely electrostatic analysis has provided an useful qualitative picture of the thin-film space-charge field behavior. Relevant edge effects have been found when the period of the recorded gratings is comparable to the thickness of the structure. These deleterious effects can be minimised by playing with the dielectric constants of the buffer layers. Charge accumulation at the boundaries and fringe curvature has been quantitatively determined. The coupling and energy-exchange processes between beams during device operation have been numerically simulated and simple convenient rules to assess the contribution of the non-linear terms have been suggested. Moreover, the fringe tilting and bending effects have been rigorously described. A new photorefractive polymer structure, that independently optimises the electrooptic and photoconductive response has been proposed.

Selected publications

- Agulló-López F., Aguilar M. and Carrascosa M., 1996. Photorefractive thin films. *Pure and Appl.Opt.* **5**: 495.
- Aguilar M., Carrascosa M., Agulló-López F., Magaña F. and Solymar L., 1996. Short-time photorefractive recording in multiple quantum wells : longitudinal geometry. *J.Opt.Soc.Am.* **B13**: 2630.
- Aguilar M., Carrascosa M., Agulló-López F., Solymar L. and Magaña F., 1997. Quasi-steady space-charge fields in photorefractive multiple quantum wells : edge effects. *Phys.Rev.* **B55**: 5526.
- Aguilar M., Carrascosa M. and Agulló-López F., 1997. An alternative design strategy for thin photorefractive polymer structures. *Adv.Mater.* **9**: 423.
- Murillo J.G., Magaña F., Carrascosa M. and Agulló-López F. Effects of strong modulation on beam coupling gain in photorefractive materials : application to BSO. *J.Opt.Soc.Am.B.* (in press).

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**STUDIES OF ELECTRONIC PROPERTIES OF SUPERLATTICES GROWN
ALONG HIGH INDEX DIRECTIONS AND SEMIINFINITE SUPERLATTICES**

Co-ordinator: Consejo Superior de Investigaciones Científicas, Madrid, Spain
(Victor R. Velasco)

Objectives

- ◆ The theoretical study of the electronic properties of semiconductor superlattices grown along high index directions, in particular the [211] and [311] directions.
- ◆ The theoretical study of the electronic properties of these semiinfinite superlattices and of the embedded quantum wells formed by a substrate, some atomic layers forming the well and a semiinfinite superlattice.
- ◆ The theoretical study of the electronic properties of quasi-periodic heterostructures, such as Fibonacci superlattices.

All these studies have been performed by means of Green function methods and semi-empirical tight-binding models, which are realistic enough to obtain accurate information on these systems.

Activities

- * The study of the electronic properties of the superlattices grown along high index directions was performed jointly by the Madrid, Puebla and Zacatecas groups, while the study of the electronic properties of embedded quantum wells and Fibonacci superlattices, was carried out by the Puebla and Madrid groups.
- * The study of the effects of the strain on the electronic properties of superlattices grown along low symmetry directions was carried out by the Zacatecas group with some cooperation from Madrid.

Results

In the course of this project we have developed a robust theoretical model and a very powerful method to study in a unified way complicated semiconductor heterostructures having many non-equivalent interfaces. The interfaces considered can have a high symmetry or a low symmetry. It has been found that only a small number of the many (211) and (311) superlattices analyzed have direct gaps. For embedded quantum wells it was found, in agreement with the existing experimental information, that the Tamm-states, i.e. the states localized in the embedded well, can be clearly identified only when the thickness of the embedded well is at least two times bigger than the corresponding well in the superlattice. In the case of the quasi-periodic superlattices, such as those following the Fibonacci sequence, it was found that the results coming from the realistic description provided by our model are somewhat different from those predicted by simpler models, and that some interpretation is necessary to compare the predictions of the different models.

Follow up

Although the project is over, collaboration continues with the study of the electronic properties of other semiconductor heterostructures, and some of this work is the subject of the Master and Ph.D. theses of Mexican students.

Selected publications

Arriaga J. and Velasco V. R. 1995. "Electronic states of a semi-infinite superlattice with an embedded quantum well", *J. Phys.: Condens. Matter*, **7**, 3493.

Arriaga J. and Contreras-Solorio D. A. 1995. "Electronic structure of (211) AlAs/GaAs superlattices", Proceedings of the "Segunda Reunión Iberoamericana de Optica", Guanajuato, México (September 1995): SPIE-International Society for Optical Engineering, Vol. **2730**, p. 583.

Arriaga J., Contreras-Solorio D. A. and Velasco V. R. 1996. "Electronic states of (211) AlAs/GaAs quantum wells", *Surf. Sci.*, **367**, 203.

Arriaga J. and Velasco V. R. 1997. "Electronic properties of semiconductor Fibonacci quasi-periodic superlattices", *Physica A*, **241**, 377.

Arriaga J. and Velasco V. R. 1997. "Electronic properties of GaAs-AlAs Fibonacci superlattices", *J. Phys.: Condens. Matter*, **9**, 8031.

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ANALYSIS OF LARGE DATA SAMPLES FROM PARTICLES PHYSICS

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STRUCTURAL STUDIES OF SURFACE ADSORPTION STRUCTURES USING LOW ENERGY ELECTRON SCATTERING

Co-ordinator: University of Warwick, Coventry, United Kingdom (Phillip Woodruff)

Objectives

Develop and apply methods based on electron elastic scattering, notably low energy electron diffraction (LEED) and X-ray photoelectron diffraction (XPD) to a range of structural problems associated with adsorption at well characterised single crystal surfaces.

Activities

The project was based on establishing and using similar facilities at both Warwick and Belo Horizonte, at least in the area of LEED. In particular, the two sites have established essentially identical LEED diffracted beam intensity collection capabilities based on computer-interfaced Peltier-cooled CCD TV cameras and computer workstations for running the multiple scattering simulations codes needed for the data analysis. The XPD facilities were only available at Warwick but with a new X-ray photoelectron spectrometer installed in Brazil, the long-term objective was to also explore XPD in both laboratories.

Results

The initial stages of the project have involved the purchase and setting up of the necessary instrumentation. The work concentrated on LEED studies of the semiconductor surfaces InSb(110), Bi₂Te₃(0001) and Bi₂Se₃(0001) and of Sb adsorption phases on Ag(III). The InSb work was based on data collected prior to the project but was then analysed at UFMG with the new resources. Data from the remaining semiconductor surfaces were collected in Belo Horizonte, while the metal overlayers were studied in Warwick. Analysis of early Warwick-based XPD results has also been completed for methyl thiolate overlayers.

Selected Publications

Fernández A., Espinos J.P., González-Elipse A.R., Kerkar M., Thompson P.B.J., Ludecke J., Scragg G., de Carvalho A.V., Woodruff D.P., Fernández-García M. and Conesa J.C. 1995. Structural aspects of the interaction of methyl thiol and dimethyldisulphide with Ni (111). *J. Phys. : Condens . Matter* **7** : 7781-7796.
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Soares E.A., de Carvalho V.E. and de Castilho C.M.C. 1996. Study of the InSb(110) surface Debye temperature by tensor LEED. *Surface Sci.* **367** : 67.

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SYNTHESIS, CHARACTERIZATION AND OPTIMIZATION OF THIN FILM OLIGOMERS FOR ELECTRICAL APPLICATION PURPOSE

Co-ordinator: Institut des Matériaux, Nantes, France (Serge Lefrant)

Objectives

Develop both basic scientific research and derived applications of conjugated polymers and oligomers: with the improved processability of such compounds, their electronic and optical properties can be utilized in several technical devices such as integrated circuits, sensors, rectifiers, electroluminescent diodes, etc...

Activities

Within the frame of this project, studies have been carried out in different steps.

- * First of all, the synthesis of the conjugated polymers or oligomers has been performed by the two chemistry groups in Chile (Universidad de Chile and Pontificia Universidad Católica de Chile, Santiago). The real challenge was to find new synthesis routes to design new polymers or oligomers to make devices. Those compounds contain basically aromatic or heteroaromatic units.
- * In a second step, the two groups in Nantes (Faculty of Sciences and Institute of Materials) have performed the characterization studies by using several spectroscopic techniques including NMR, FTIR, UV-visible absorption, Raman scattering, XPS etc...
- * Finally, the fabrication of thin films devices has been carried out in Nantes (Faculty of Sciences) with a feedback to the other groups for optimization.

Results

Towards the end of this project, we obtained competitive compounds to be used in thin films devices. Oligothiophenes and soluble oligo-alkylthiophenes were synthesized, and also polyselenophenes. However, the preparation of oligoselenophenes was unsuccessful. Other families, like polyaniline derivatives with halogen substituents, or polycarbazole or polyvinylcarbazole led to very promising results in terms of electronic properties. Thin-film preparation focused on terthiophene compounds evaporated at high temperature, leading to well-crystallized deposition. An important result was obtained by performing evaporation, using a hot filament which led to a significant improvement of the electrical properties of the diodes. Rectifying effects were put in evidence on this preliminary device. Other diodes, based on longer thiophene oligomers or on the carbazole family, were analysed in detail by means of numerous spectroscopic techniques and electrical and/or optical performance measurements.

Follow up

The last six months of the project were mainly devoted to the fabrication of devices like diodes and light-emitting diodes for the Nantes (Faculty of Sciences) group. At the same

time, a great effort was made on the modeling of spectroscopic results (Raman scattering, IR, XPS) at the Institute of Materials for the purpose of improving and controlling the conjugated compounds prepared by the two groups in Chile. This was continued until the end of the project.

Selected publications

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EFFECT OF MONOMER SOLUBILITY IN WATER ON MICROEMULSION POLYMERIZATION

Co-ordinator : Universidad del País Vasco, Bilbao, Spain (Issa A. Katime)

Objectives

The main drawback of microemulsion polymerization for its scaling up to industrial level is the large quantity of surfactant required. Our objectives were to:

- ◆ understand the dynamical behaviour of microemulsion polymerization,
- ◆ expand the kinetic model developed by us for predicting particle size and molar mass distribution,
- ◆ optimise the method discovered by us for making microemulsion-made lattices with high solid content,
- ◆ produce structured polymers with superior properties: core-shell materials with improved mechanical properties, and superabsorbent hydrogels with better controlled release properties.

Activities

Microemulsion polymerization is a new process for producing latex with very small particles, polymers with high molar mass at high rates of reaction, and polymers with unique structures. This has a great potential for industrial, medical, and biological applications. We are working together on

- * the determination of phase diagrams and microemulsion characterization by laser light scattering (QLS),
- * the determination of the monomer partition between the aqueous phase polymerization,
- * the determination of reaction kinetic, and
- * on a mathematical model for the prediction of the reaction kinetics.

Results

- ⇒ We have discovered a new process to produce nanolatex with high solid content by microemulsion polymerization without altering the basic features of the microemulsion-made nanolatex, i.e. small particle size and high molar masses. The process consists of the polymerization to high conversions, of a typical microemulsion to produce a low solid-content latex. Then, more monomer and initiator are added in a semi-continuous mode for several hours to this latex, which results in a final product with more than 45% of solid content.
- ⇒ We developed a model for microemulsion polymerization. Now, the goal is to extend the model to other systems containing other monomers and also cosurfactants.
- ⇒ We are also working to elucidate the main mechanism of particle nucleation in microemulsion polymerization. To do this, fast dynamic kinetic studies are required, using pulsed techniques to turn on and off the free radical reactions.

- ⇒ We are also pursuing the study of the role of monomer solubility in water on the kinetics of microemulsion polymerization. The results with vinyl acetate are quite promising.
- ⇒ With respect to the polymerisation of a u-hexyl methacrylate in three-component microemulsions prepared with DTAB, we have studied the kinetics of the process using two different initiators: potassium persulfate and V-50.

Selected publications

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MOLECULAR THERMODYNAMICS OF PURE FLUIDS AND MIXTURES

Co-ordinator: University of Sheffield, Sheffield, United Kingdom (George Jackson)

Selected publications

A. Gil-Villegas, A. Galindo, P.J. Whitehead, S.J. Mills, G. Jackson, A.N. Burgess. 1997. Statistical associating fluid theory for chain molecules with attractive potentials of variable range. *Journal of Chemical Physics*. **106**, 4168.

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MIXING PROCESSES FOR FLOWING PARTICLES AND POROUS MEDIA

Co-ordinator: Laboratoire Fast, Orsay, France (Jean Pierre Hulin)

Objectives

The general objective of the project was to improve our knowledge of the mixing and chaotic behaviour of particle and fluid interfaces in particle-fluid and liquid-liquid (miscible and non miscible) flows.

Activities

- * Study of Lagrangian chaos, Stokesian turbulence and inertia effects on tracer and particles mixing;
- * Study of gravity induced instabilities for miscible liquid-liquid interfaces;
- * Analysis of dispersive mixing for non-Newtonian fluids flowing in porous media;
- * Study of dynamics of non-miscible liquid-liquid interfaces on heterogeneous surfaces.

Results so far

- ⇒ Trajectories of tracers in organized flows (Taylor-Couette instabilities) were analysed using the same concepts as in the physics of contaminants dispersion in porous media. The trapping of tracers in the rolls and the mean flow contribute to the deviation from Gaussian behaviors. Trajectories of particles in time-dependent flow, vortex and surface waves, were studied as a function of the mass of the particles in order to show the contribution of the drag in the complex chaotic Lagrangian dynamics.
- ⇒ A set of experiments was performed in order to study the gravitational instability between two miscible fluids when the heaviest fluid is placed on the top of the other fluid. The development of the instability was characterized (wavelength, growth rate) as a function of the difference of density, the viscosity, the thickness of the Hele-Shaw cell. The equivalent of the Raleigh instability and of the instability of a falling layer were explored too in miscible fluids.
- ⇒ A procedure for preparing polysaccharide solutions with power law rheological properties and stable and predictable characteristics was developed. The dispersion of a salt tracer in a flow of Newtonian and shear-thinning fluids was then studied in double porosity media made from packing of porous grains. A clear increase of the dispersion with the non-newtonian character was observed and studied as a function of the flow velocity.
- ⇒ The thickness of the residual film left behind by the displacement of a fluid by another in a capillary tube was studied as a function of the displacement velocity and of the ratio between the viscosities of the two fluids : clear variations of the thickness with distance along the flow were demonstrated, particularly when the displacing fluid is not of negligible velocity compared to the other. In a second phase, dewetting experiments were performed - also in a capillary tube geometry - in which the velocity of the contact line of the residual film was studied as a function of the film thickness and of the viscosity ration between the fluids.

Follow-up

- New experiments are running to follow particles motion in central vortex. The trapping and expulsion from the vortex core are studied and will be compared with natural behaviours such as the polar vortex, in order to modelize basic process related to the ozone hole in the south pole.
- We will continue to study the gravitational instability between miscible fluids in a new experimental set-up. Different ranges of parameters will be studied in the linear and non linear regime. A horizontal flow will be created in the layers of fluids in order to add shear strain to the gravitational instability.
- Tracer dispersion measurements will be performed in transparent model arrays of variable width channels in order to study visually and through computerized image analysis the dependence of the microscopical mechanisms of dispersion on the rheological properties of the fluid.
- Fluid film dewetting experiments will be performed in a geometry allowing to reduce the influence of gravity on the displacement process. New theoretical models taking into account the viscosity of both fluids and the shape of the bump building up near the rear contact line will be developed.

Selected publications

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ISC

Physical, Mathematical, and Engineering Sciences

QUANTUM MECHANICS OF FUNDAMENTAL SYSTEMS

Co-ordinator : Université Libre de Bruxelles, Bruxelles, Belgium (Marc Henneaux)

Objectives

The objective of the project was to continue the classical and quantum study of the physical theories describing the fundamental interactions (electromagnetism, nuclear forces, gravity). The characteristic and common feature of these models is that they are invariant under transformations that can be chosen independently at each space-time point (gauge symmetries). Equal attention has been given to the general formalism (geometrical and algebraic structure of gauge systems) and to its applications.

Activities

Collaborative research has been pursued through the following means :

- ★ Exchange of researchers : numerous visits of about 15 days each have been paid on each side. The visits were devoted to joint research, seminars and conferences. They resulted in fruitful exchanges between both senior scientists and younger colleagues.
- ★ Meetings
 - A conference "The Black Hole : 25 Years After" has been organized in Santiago. It gathered a great number of young Latin American scientists around experts in the field. The proceedings of this meeting have been published by Plenum Press.
- ★ A workshop on the same theme has been organized in January 1995.

Results

The contractors and their collaborators:

- ⇒ have discovered black holes in three-dimensional gravity and analyzed their theoretical properties;
- ⇒ have developed the antifield formalism and found the general solution of the Wess-Zumino consistency condition for Yang-Mills gauge models and Einstein gravity;
- ⇒ have clarified the role of time-independent gauge conditions in gravity;
- ⇒ have proved that the BRST path integral is a solution of the constraint equations for both irreducible and reducible gauge systems.
- ⇒ The results of the research have been reported in the form of articles in scientific journals and at international meetings. 38 papers have benefited directly or indirectly from the collaboration.
- ⇒ Training of young researchers: A total of four graduate students (one in the Chilean team and three in the Belgian team) have obtained their doctoral degree in the period of the contract.

Follow-up

The collaboration has been going on. It is hoped that it will continue in the future and that it will be extended to other Latin American countries. In this respect, contacts with research groups in Argentina, Brazil and Uruguay are quite promising.

Selected publications

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NON-LINEAR DYNAMICS AND PATTERN FORMING PHENOMENA

Co-ordinator : Université Libre de Bruxelles, Bruxelles, Belgium (Daniel Walgraef)

Objectives

This research programme was devoted to current problems in non-linear science. Its main objective was to study fundamental questions related to the spontaneous appearance of order and chaos in systems far from thermal equilibrium.

Activities

The project focused on several open problems related to instabilities and transitions leading to spatio-temporal pattern formation in physics, chemistry, biology and materials science.

Results

Among the results obtained by this project are : a dynamical description of microstructure formation in metals and alloys under particle irradiation, the derivation of a dynamical model for the electrohydrodynamic instability of liquid crystals, the study of pattern formation in interacting Turing and Hopf instabilities, the dynamical behaviour of vortices and spirals in the complex Ginzburg-Landau equation, the derivation of effective potentials near Hopf bifurcations, the study of models for noise induced transitions, the formulation of a qualitative theory of topological defects in nonequilibrium systems, the formation of spatio-temporal patterns in non-linear optics and in forced liquid crystals, the study of excitable systems and excitability waves with applications, for example, to fibrillation in cardiac muscles, etc.

Follow-up

The collaborations initiated during the course of this project are continuing to be pursued jointly. Moreover, interactive multimedia presentations on spatio-temporal pattern formation are being made and a book in Spanish entitled "Estructuras Espacio-Temporales lejos del Equilibrio" has been prepared.

Selected publications

- P. Borckmans, G. Dewel, A. De Wit and D. Walgraef, 1994. Turing Bifurcations and Pattern Selection in Chemical Waves and Patterns, R. Kapral and K. Showalter eds, Kluwer, Dordrecht, pp. 323-363.
- T. Frisch, P. Coulet, S. Rica and J.M. Gilli, 1994. Spiral waves in a nematic liquid crystal submitted to a rotating magnetic field. *Physical Review Letters* **72**: 1471-1474.
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MORPHOLOGY, STABILITY AND DISTRIBUTION FUNCTIONS OF GALACTIC STRUCTURES

Co-ordinator: Università di Roma "La Sapienza", Rome, Italy
(Simonetta Filippi, Remo Ruffini)

Objectives

- ◆ Elaborate a theory of the equilibrium and stability of figures arising in the gravitational and morphological study of galaxies.
- ◆ Establish the relevance of Landau Damping Time in the formation of large-scale structures in the Universe.
- ◆ Study the relevance of collective relaxation in determining the structure of elliptical galaxies.
- ◆ Develop a technique for probing the large-scale structure to the case of clusters of galaxies and to carry out a detailed comparison between the results obtained and the current theories of structure formation.
- ◆ Elaborate models of the anisotropy of the cosmic background radiation, generated by a hierarchical matter distribution on cosmological scale.

Activities

Using the computer facilities at I.C.R.A. in Rome:

- * We elaborated a theory consisting of successive generalizations to obtain realistic self-consistent models for gravitating collisionless systems, describing stellar objects and galaxies.
- * We studied the Landau damping time associated with the perturbations in an expanding universe, and derived and solved an integral equation for the spectrum of small-amplitude fluctuations.
- * We developed a "cellular fractal model" of the universe and tested it with data coming from the Hubble Space Telescope.

Our *collaboration with the University of "Tor Vergata"* was focussed on the theoretical comprehension of the mechanism of onset and development of instabilities in simple dynamical systems and on astrophysical applications.

Our *collaboration with C.I.F. in Bogotà*, was focussed on the development of a new statistical technique to test cosmological models using Cosmic Background Radiation data.

Results

- ⇒ A series of very important results were obtained and more than forty articles were published in international magazines, and presented at international meetings.
- ⇒ We formulated a virial theory of n^{th} order containing all the classical results about classical equilibrium configurations and able to pose constraints on the existence of figures of equilibrium for gravitating heterogeneous and non-linear systems.

- ⇒ The Landau damping time proposed new insights on the theories for the formation of structures in the universe and a new interpretation of the degeneracy parameter associated with fermions.
- ⇒ A critical re-analysis of the two-point correlation function of galaxies and clusters of galaxies showed that a double power law shape cut-off can explain observational results such as detection of voids, or the shell and bulk motions of galaxies on large scales.
- ⇒ We established the basis for a stability analysis of spheroidal systems to assess the relevance of rotation and dispersion velocity anisotropy for the onset of the gravothermal catastrophe.
- ⇒ We completed a statistical analysis of the COBE first year sky maps based on the genus and spot number statistic.

Follow up

After the end of the project, we are pursuing work at I.C.R.A. in collaboration with Colombian scientists, and we have presented other results published at the latest Marcel Grossmann Meeting (Jerusalem, June 1997), on the topics described in previous paragraphs.

Selected publications

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THE DEVELOPMENT OF AN INNOVATIVE 3-S PUMP

Co-ordinator : Eindhoven University of Technology, Eindhoven, Netherlands
(P.T. Smulders)

Objectives

- ◆ Strengthen joint research on windpumps,
- ◆ Expand a network of common expertise and facilities,
- ◆ Develop reciprocating, innovative lift pumps that can be coupled to fast-running windmills of modern design.

Activities

The project focused on “3S-issues”:

- 1) easy STARTING in light winds to enhance the amount and regularity of water output ;
- 2) SMOOTHING the loads in the pump rod, especially when the wind-pump operates at speed;
- 3) hydrodynamic SEALING of the piston, i.e. sealing without direct contact between piston and cylinder, thus eliminating leather seals and their inherent wear-and-tear. Solutions would have to meet criteria of reliability, efficiency, low cost and appropriateness for local manufacture and maintenance.

Two starting devices were investigated one of which was abandoned. The other; the “floating valve” (so called because it is lighter than water) was investigated theoretically and experimentally in the laboratory and test field. Research on sealing concentrated on investigating the hydrodynamic flow in the gap between the piston and cylinder, the latter having a smooth surface, while the piston is smooth or has grooves of a particular geometry cut into its surface. Both sub-resonant and over-resonant smoothing were studied, experimentally and theoretically, the first by building elasticity into the pump rod, the second by incorporating a sealed bladder air-chamber at the lower end of the lift rod.

Results

All partners agree that the project has been very successful in strengthening ties in their R&D efforts on wind pumps. Also, many students were engaged in the programme at all three universities. This is important for creating future experts in this field. Concerning the 3S-issues: the results of the floating valve are very successful. If properly tuned, this method can increase water output by at least 50% and its availability, i.e. the number of days that water is pumped. Applications of hydraulic sealing were effective for shallow lift pumping (under 20 m). Labyrinth (grooved) seals are preferable to smooth seals. Two geometries for optimal sealing were identified. By building elasticity into the pump rod, peak loads can be more than halved. A sealed air-bladder is a very effective and complete smoothing solution but, nevertheless, less attractive because of the increased complexity of below-ground components. Substantial progress was made in modeling lift-pump flow transients and rod loads.

Follow-up

A regional seminar with EC support was held in November 1994 with participants from Bolivia, Chile, Panama, Peru, Nicaragua, Colombia and Venezuela, as an effective way to disseminate the results of the project and strengthen ties in the region. A number of results were picked up by manufacturers but it is not known exactly which have been incorporated into their standard designs. A follow up to the 3S project has been the 2R project, concerning other aspects of wind-pumping with piston pumps. Unexpected follow up came from India, where the Centre for Scientific Research in Auroville performed some excellent field testing on 5 different systems of load-matching and starting. Their tests have shown unambiguously that the floating valve (or matching valve as it is also called) is a superior and reliable device for increasing output at no extra cost.

Selected publications

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**BIOPROCESS ENGINEERING OF HIGHLY VISCOUS FERMENTATIONS :
XANTHAN AS A MODEL**

Co-ordinator: University of Birmingham, Birmingham, UK (Alvin W. Nienow)

Objectives

- ◆ Undertake a screening programme to obtain the most virulent *Xanthomonas campestris* bacteria capable of giving high yields of good-quality Xanthan gum.
- ◆ Then undertake process engineering studies to activate different agitator types, dissolved oxygen profiles and glucose feeding strategies to be compared.

Activities

- ★ Work was undertaken on a complementary basis in Birmingham (UK) and in Cuernavaca (Mexico).
- ★ Exchange visits took place, and collaboration was very close.
- ★ Eight presentations arising from the work were given at international conferences and some seventeen refereed journal or conference papers were produced including two reviews.

Results

- ⇒ A successful strain-selection programme was undertaken in Mexico, and scale-up studies were undertaken in both countries. The work led to the development of a very reproducible fermentation protocol that resulted in a very precise comparison of different agitator.
- ⇒ It was clearly shown that the traditional small Rushton turbine is inferior to all other agitators tested. An improved fluid dynamic model of cavern formation, i.e. regions of relatively rapid motion surrounded by stagnant regions, was developed.
- ⇒ It was also shown that, by a combination of agitation (to maximise cavern sizes) and gas blending strategies, the specific productivity (quantity of Xanthan produced per cell) is constant at a fixed level of dO₂ if only those cells in the active zone are used for calculation. Finally, levels of gum up to ~65 g/litre were obtained. These quantities were much higher than any value reported previously for agitated fermenters. This figure was achieved by controlling dO₂ and cavern formation to maximise the proportion of well-oxygenated broth in the fermenter, and by using pulsed or continuous glucose feeding, especially in the later stages of fermentation.

Follow-up

Collaboration is continuing actively.

Selected publications

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- Sánchez A., Ramírez M.E., Torres L.G. and Galindo E. 1997. Characterization of Xanthans from Selected *Xanthomonas* Cultivated under Constant Dissolved Oxygen. *World Journal of Microbiology and Biotechnology.* **13**: 443-451.
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**DEVELOPMENT OF MATHEMATICAL MODELS FOR CONTROLLING
MIG/MAG WELDING**

Co-ordinator : Instituto de Soldadura e Qualidade, Lisbon, Portugal (José Oliveira Santos)

Objectives

The general goal of this project is to develop techniques of on line monitoring and control that will enable defect levels to be controlled, repair rates and post weld inspection to be reduced in order to improve the overall cost effectiveness of the MIG/MAG welding process.

Activities

- ★ The project focused on the monitoring and control of welding parameters in order to avoid welding objects normally associated with inadequate control of the welding technique and process. The work was carried out both in ISQ in Lisbon and Universidad Michoacana in Morelia - State of Michoacan - Mexico.
- ★ The monitoring process was first done using MIG welding fluxed cored wires (FCAW) and later with submerged arc welding.
- ★ The parameters considered included: arc voltage got width, preparation angle, arc current, electrical stick-out, travel speed, pulse parameter and pool shape. The responses were measured in terms of lateral and axial penetration fusion area, weld width and the bead concavity. The mathematical modelling was developed in terms of these responses.
- ★ The work was divided between both laboratories under the coordination of ISQ.

Results

- ⇒ The results of the project are used to understand better the physics of the welding processes particularly MIG/MAG welding using fluxed cored wires.
- ⇒ Part of the results are being used to develop a new process control equipment to be integrated in medium term (2 years) in a new welding equipment.
- ⇒ The knowledge acquired during the project also allowed increasing the industrial awareness in Mexico of the FCAW process using pulsed current.
- ⇒ Improved applications of the MIG/MAG welding process leading to a high productivity process were developed in Michoacan companies.

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**NON-LINEAR MATHEMATICAL MODELLING. THEORETICAL AND
NUMERICAL ASPECTS.**

Co-ordinator: Centre National de la Recherche Scientifique, Marseille, France
(Pierre Picco)

Objectives

The long-term goals were to develop new approaches to the study of complex systems. In this project there was a multidisciplinary approach to their analysis. This involved optimization and numerical analysis, discrete mathematics and probability.

Activities

Concerning the discrete mathematics approach, the group co-ordinated by Eric Goles and Carme Torras focused on theoretical models in parallel computers and cellular automata. The group co-ordinated by Carlos Conca and Idelfonso Díaz studied homogenization theory. The optimization group co-ordinated by Rafael Correa, Alfred Auslender and Jochem Zowe studied finite algorithms for global optimization. The activity of the probability group co-ordinated by Servet Martínez and Pierre Picco were devoted to the study of quasi-stationary distributions.

Results

- ⇒ Definition of parallel complexity, which is related to the study of infinite precision in real numbers. Dynamics of sand piles and cellular automata. Plane tiling, which is related to the design of chips.
- ⇒ Study of homogenization of some differential spectral problems which arise in the analysis of fluid-solid structures. Results on the Navier-Stokes equations.
- ⇒ Introduction of directional derivatives for the analysis of multivalued functions and analysis of "Branch and Bound"-type algorithms.
- ⇒ Ratio-limit theorems and domain of attraction for quasi-stationary distributions in diffusions and hyperbolic dynamical systems. Brownian Bridge approach to Polymers.

Follow-up

At this moment, applied research concerning this program is being developed PhD students. We emphasize that a regional program involving Europe, Chile and Brazil is particularly active. This includes research in common, seminars on a regular basis, and international schools.

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Contract number: CII*CT920052

Period: January 1993 to December 1994

**IMPROVED FLOW-INJECTION SYSTEMS FOR MULTI-PARAMETRIC ANALYSIS
OF SAMPLES OF ENVIRONMENTAL, BIOLOGICAL AND AGRICULTURAL
RELEVANCE**

Co-ordinator: Universidade do Porto, Porto, Portugal (José Luis Fontes Costa Lima)

Objectives

The main objectives of the project were the development of novel concepts in flow-injection systems in order to increase the versatility and simplicity of these automation techniques toward chemical multiparametric determination. To achieve these objectives, sandwich techniques and leaping detection were explored and applied to the analytical evaluation of water, soils, vegetables and biological fluids.

Results

Research activities took place in parallel at laboratories of the Portuguese and Brazilian teams involved in the project. The transfer of both groups' experience and knowledge was important not only to achieve and even surpass the proposed goals but also to strengthen the scientific cooperation in subsequent years. Development of flow set-ups with relocatable detectors, low-dead volume potentiometric detectors, proposal of novel approaches for sample and reagents handling, and precipitation reactions in line were allowed by the project. The continuous-flow multi-commutation concept was a new outcome not initially planned but discussed and exploited during the project period. The presence of visiting scientists of both institutions were also important in seminars and workshops for the graduate courses providing new interesting ideas for academic research.

Selected publications

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Contract number: CI1*CT920052

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Period: April 1993 to June 1996

STUDY OF THE EFFECT OF INITIAL CONDITIONS ON THE STABILITY OF A DENSE Z-PINCH

Co-ordinator: Centre National de la Recherche Scientifique, Palaiseau, France and Imperial College of Science, Technology, and Medicine, London, United Kingdom (Peter Choi)

Objectives

The general objective of the collaboration was to study the initial plasma formation processes in a high current linear discharge and the subsequent evolution of the discharge into a high energy density plasma through the development of suitable diagnostics in the visible and X-ray region.

Activities

High energy density plasma created through the application of a large current pulse has potential applications ranging from alternative fusion scheme to ultra-high brightness radiation sources in the X-ray region. The project focused on the development of a range of novel diagnostics in order to characterize the complete evolution of the high current discharge, from the initial breakdown formation and ionization growth, through the period of current rise and plasma compression, to the instability growth and formation of ultra-high energy density structures from the dense plasma column. A range of generators have been used to create the high energy density plasmas, including multi-terawatt MA pulsed power generator to small kJ plasma focus. A range of diagnostics have been developed to study the spatial and temporal development of such transient plasmas, including an 8 frame laser holographic interferometry system with ns exposure and a micro-holographic interferometry technique with resolution down to 20 μm to study the plasma density structure, a shielded magnetic probe array to provide non-invasive characterization of the dynamics of the current carrying sheath, a Slit-Wire X-ray camera to provide quantitative measurement on the micron size hot plasma features and an X-ray filter analysis code to provide accurate plasma temperature diagnostics with non-dispersive filter technique in high Z plasmas.

Results

⇒ The collaboration has been particularly fruitful with a number of new diagnostics developed and a range of new plasma parameters characterized. 54 publications have originated from the collaboration, with 18 in refereed Journals and 24 in published International Conference Proceedings. This success has been helped by an earlier programme of collaboration supported by a British Council Link Scheme, while the funding from EU provided continuity as well as unique opportunities of joint work. Experiments so far have indicated clearly the importance of initial plasma formation on the subsequent plasma development. The development of nanosecond time and micron

space diagnostics in the visible and the X-ray region has allowed essential features to be followed.

⇒ The overall work has led to the development of a novel double shell Gas Embedded Compressional Z-pinch with improved stability and a new Composite Pinch concept for the creation of a high energy density plasma column from solid fibre with enhanced stability. This joint work has also led to the demonstration of a fast capillary discharge soft X-ray source for plasma backlighting studies.

Follow up

The collaboration has provided multiplying effects in the extension of work in a number of new projects supported locally through FONDECYT in Chile. The successful collaboration has been continued through exchanges supported by CNRS-CONICYT. Furthermore, the development in the fast capillary discharge soft X-ray source has directly contributed to the initiation of a large TMR network project on the same topic. The project, involving 8 partners in 6 countries from universities, research institutions and industries, begins in 1998.

Selected publications

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SEISMIC BEARING CAPACITY OF FOUNDATIONS ON SOFT SOILS

Coordinator: Géodynamique et Structure, Bagneux, France (Alain Pecker)

Objectives

The objective of the research is to improve the evaluation of the seismic bearing capacity of shallow foundations on clay soils, with particular emphasis on buildings founded in Mexico City, and to propose recommendations which can be embodied in seismic building codes.

Activities

- ★ Reevaluation of the mechanical characteristics of Mexico City clays on the basis of recent tests to reflect the influence of strain rate and cyclically induced pore pressures;
- ★ Identification of well-documented case histories of foundation failures reported in Mexico City during the 1985 Michoacan Guerrero earthquake;
- ★ Evaluation of the seismic bearing capacity and permanent displacements of the case histories, using dynamic finite element models and analytical formulae developed within the framework of the yield design theory;
- ★ Realization of parametric studies to derive general conclusions.

Results

A new methodology has been developed for the evaluation of the seismic bearing capacity of shallow strip foundations resting on clay-like deposits. This methodology is based on the upper bound approach of the yield design theory for the evaluation of the pseudo-static bearing capacity. Analytical formulae, which can be used in seismic building codes, have been established. In addition, the results have been extended to compute the permanent displacements taking place whenever the pseudo-static bearing capacity is exceeded. This methodology has been successfully tested against case histories.

Follow-up

A good cooperative research has been set up between UNAM and the French partners, and collaboration is continuing on that topic.

Selected publications

- Salençon J., Pecker A., 1995. Ultimate bearing capacity of shallow foundations under inclined and eccentric loads. Part I : purely cohesive soil. *European Journal of Mechanics A/Solids*. **14**, n° 3, 349-375.
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USE-OF-SYSTEM COSTING FOR ELECTRICAL TRANSMISSION AND DISTRIBUTION SYSTEM IN CHILE

Co-ordinator : Imperial College, London, UK (Brian Cory)

Objectives

Development of novel techniques for the Chilean CIS power network to provide real power use-of-system costing and reactive power costing for voltage control. These techniques can be used to charge customers for the transport and delivery of both real and reactive power at bulk supply terminals.

Activities

- * Software was researched, written and delivered to Chile, based on a probabilistic technique applied to real-power flows which accounted for variations in the hourly, daily and seasonal costs of producing electrical energy. An optimal combination of hydro and thermal energy was derived over long, medium and short terms, using generation models the cost of which was related to the level of the main reservoir employed for regulating purposes. The probabilistic method represented equivalent costs with an expected value and standard deviation to give a nodal expected marginal cost of delivered power and an acceptable standard deviation.
- * For reactive power, an optimization technique based on a commercial package was recommended, from which the marginal cost of real-power losses due to reactive power flows required to maintain acceptable voltage profiles could be calculated. Although the losses due to reactive power flows were comparatively small, nevertheless for a long reticulated system as in Chile, these losses can become significant enough to be paid for as part of the power delivery tariff.

Results

Methodologies developed in the UK have been transferred for use of the CIS planners and operators in Chile. These methodologies with associated software have been used for the calculation of system marginal real-power generation costs utilizing the optimal mix of hydro and thermal energy sources taken over a designated time period. The collaborators in Chile have continued with this line of research, including the assessment of the cost of system security and the application of transparent charging methods for use-of-system by both generators and consumers.

Follow-up

Arising from the contacts made during this collaborative research, visits have been made to Chile, further collaboration including consultancy to the Northern Utility in Chile.

Selected publications

Wijayatunga P.D.C., Farmer E.D. & Cory B.J., 3rd-5th July 1991. Probabilistic costing of transmission services, 3rd Int. Conf. on Probabilistic Methods Applied to Power Systems, IEE, London.

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Farmer E.D., Perera B.L.P.P. & Cory B.J., August 1996. Revenue reconciled optimum pricing of transmission services, IEEE Trans. on Power Systems, Vol. 11 (3), 1419-1426.

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VIBRATIONAL ENERGY TRANSFER IN OZONE-OXYGEN/NITROGEN GAS MIXTURES FROM INFRARED DOUBLE-RESONANCE MEASUREMENTS, AND MULTIPHOTON EXCITATION OF OZONE-FREONS GAS MIXTURES BY HIGH-POWER TEA CO₂ LASERS

Co-ordinator : C.N.R.S., Paris, France (Lucien Doyennette).

Objectives

- ◆ Determine the rate coefficients of the vibrational energy transfer processes which occur in the collisional relaxation of ozone mixed with O₂ or N₂. These rate coefficients are necessary for a realistic modeling of the concentration altitude profile of ozone in the upper atmosphere where substantial departures from local equilibrium are observed for this gas;
- ◆ Investigate the dissociation of ozone induced by the infra-red multiphotonic dissociation (IRMPD) of freons in order to contribute to the study of the chemical processes leading to the destruction of atmospheric ozone by freons.

Activities

- ★ In the LPMA (Paris), a double resonance method using two CO₂ lasers was used to investigate the vibrational relaxation of ozone. Ozone molecules were prepared into a defined rotational level of the ν_1 or ν_3 vibrational states by pulsed 9P6 or 9P30 CO₂ laser lines, or of the $\nu_1 + \nu_3$ state by the pulsed frequency-doubled 9P8 TEA CO₂ laser line. State-to-state energy transfer was investigated by probing selected vibrational transitions with cw CO₂ laser lines. Measurements were performed in O₃-O₂ and O₃-N₂ gas mixtures, in the 200-300 K temperature range useful for atmospheric applications.
- ★ In the CEILAP (Buenos Aires), the IR multiphotonic absorption (IRMPA) of ozone was obtained by exciting O₃ in the ν_3 mode by various laser lines of a powerful TEA CO₂ laser. Measurements were performed in neat ozone and in mixtures with O₂, N₂ and air. The dissociation of O₃ due to the IR multiphotonic dissociation (IRMPD) of CF₂Cl₂ (first used as freon), irradiated by the 1 OP42 laser line of the TEA CO₂ laser, was also investigated.

Results

⇒ Using our technique, we have been able to investigate the main energy transfer processes occurring in the relaxation of O₃, i.e.: a very fast intermode transfer between the Coriolis-coupled states of the (ν_1, ν_3) dyad; a slow intermode transfer from the states of the (ν_1, ν_3) dyad to the ν_2 state; fast near-resonant V-V transfers between the states of the (ν_1, ν_3) dyad and those of the ($\nu_1 + \nu_2, \nu_2 + \nu_3$) dyad and of the ($2\nu_1, \nu_1 + \nu_3, 2\nu_3$) triad; and at last a V-T/R deexcitation from the lowest states ν_1, ν_3 and ν_2 . The rate coefficients of all these processes have been determined in the 200-300 K temperature range. They have also been evaluated from a theoretical analysis and compared with their experimental determinations.

- ⇒ The IR multiple-photon absorption of ozone shows a departure from linear absorption. The absorption cross-section and the number of photons absorbed per molecule were measured as a function of the laser fluence for all the available P4 to P58 lines of the 9.5 μm laser band for different O_3 pressures. The increase of the absorption cross-section with pressure and its decrease with fluence indicate the existence of bottlenecks to IR laser absorption due mainly to saturation. This fluence and pressure dependence of the absorption cross-section indicate that the IR multiple-photon absorption of O_3 does not follow the Beer-Lambert law.
- ⇒ The products of the decomposition of $\text{CF}_2\text{Cl}_2/\text{O}_2$ and $\text{CF}_2\text{Cl}_2/\text{O}_3$ mixtures irradiated by the 10P42 laser line were identified by infrared and mass spectrometry. Infrared fluorescence from the photoproduct CF_2O was observed in 5.13 μm and analysed.

Follow up

Study of the relaxation of O_3 is now completed and the corresponding results have been used by physicists for modeling the atmospheric ozone concentration altitude profile. In Buenos Aires, the work on dissociation of O_3 by CF_2Cl_2 is in progress.

Selected publications

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- Codnia J., Azcarate M. L. 1995. Disociación multifotónica IR del CF_2Cl_2 en presencia de O_2 . *Anales 1995 de la Asociación Física Argentina.*
- Codnia J. and Azcarate M. L. 1996. IR absorption of ozone under CO_2 laser excitation. *Optical Eng.* **35(1)**, 86-93.
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FIRST PRINCIPLES CALCULATIONS OF ELECTRONIC AND VIBRONIC PROPERTIES OF COMPLEX MATERIALS

Co-ordinator: Aarhus University, Aarhus, Denmark (N.E. Christensen)

Objectives

This project in theoretical solid state physics aims at quantitative predictions of physical properties (structural, optical, electronic and dynamic) of "complex materials". These include high-temperature superconductors (HTSC), unconventional semiconductors, superlattices, oxides (perovskites) and transition-metal silicides.

Activities

- * First-principles quantum mechanical calculations of electronic states are used to derive total energies and interatomic forces. This allows the prediction of structures as well as vibrational states (phonons).
- * The tasks were shared between the groups in Denmark (Aarhus) and Argentina (La Plata).
- * The very heavy computations for HTSCs were performed in Denmark, but Danish computers were also accessed from La Plata.
- * Full information obtained for phonon modes are combined with optical calculations allowing prediction of Raman intensities and thus comparison to recent experiments (Stuttgart).
- * Additional understanding of the properties of the materials is obtained by comparing hyperfine parameters to Mossbauer experiments (Aarhus).

Results so far

- ⇒ We have studied surface states on the bismuth HTSC, Bi2201, calculated phonon modes in YBa2Cu3O7 as well as some of the Hg-compounds.
- ⇒ Electronic structures, including Fermi surfaces and details such as extended van-Hove singularities and selfdoping are examined. Electric field gradients in HTSCs, also under pressure, have been derived.
- ⇒ Elastic properties of the metals (Mo and W) at extreme pressures (multimegabar) have been predicted.
- ⇒ Various calculated physical properties of oxides and iron-silicides have been used in analyses of experimental data for these technologically important materials.

Follow-up

An important result of the support obtained from the European Commission is that the Argentinian partner has been able to establish an efficient network of research groups specializing in this field in Argentina. These activities are clearly at the cutting edge, and future collaboration would also be extremely useful for the European side. Intensive work in the HTSC field is planned, but the prospects are not encouraging due to the difficulty in obtaining follow-up funding in basic research in physics/chemistry.

Selected Publications

- Peltzer, y Blanca E.L Svane A., Christensen N.E., Moreno M.S., Rodríguez C.O., Cappannini O.M., and Methfessel M. 1993. Calculated static and dynamical properties of beta-Sn and Sn-O compounds. *Phys.Rev.* **B 48**, 15712
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EARTHQUAKE-RESISTANT CONSTRUCTION OF LOW-COST ADOBE HOUSES

Co-ordinator: Technical University Delft, The Netherlands (A.W.M.Kok)

Objectives

The general objective of the research project was to perform jointly an applied scientific investigation to elaborate and to verify alternative technologies that can be used for the construction of new, low-cost, two-level adobe houses and for the repair of existing houses in Peru. These technologies should ensure a much better resistance against seismological events and should be incorporated into the Peruvian building codes.

Activities

- ★ To determine the structural efficiency of alternative technologies, a series of physical experiments were carried out on scale models of the houses.
- ★ To apply computer simulations to the analysis of the structural properties, computer programs considering the non-linear dynamic properties of these structures were developed, tested and applied.
- ★ To transfer numerical technologies, finite element courses were taught in Cusco. The physical experiments were carried out in the University of Cusco laboratories. The management of the project was carried out by IIUR (Cusco) and CICAT (TUDelft). Seminars, conferences and publications were organized to transfer the new technologies to the appropriate target groups.

Results

- ⇒ The practical result of this project is that alternative technologies for the construction of new earthquake-resistant houses and the reinforcement of (damaged) existing houses have been developed, tested and verified. One promising alternative for new houses is the so-called 'tapial-quincha' house, suitable for the Amazon area in the north-east of Peru. A promising alternative for the repair of existing houses in the Cusco area is the application of ferro-cement technology, a concrete sheeting on a wiring in the walls.
- ⇒ The academic result of this project is that both TUDelft and the University of Cusco have gained many new insights into new adobe construction technologies and numerical simulation of structural properties.

Follow-up

In order to transfer these new technologies to the end-users - the people - a socio-economic feasibility study is being carried out and a programme for diffusion is being developed. By means of a graduation project of TU Eindhoven in Cusco a considerable contribution has been made to the feasibility study.

Selected publications

Sovero G., Malpartida C., Kok A. 1993. Modelling of reinforced adobe walls in bending with discrete elements. TUDelft report 25.2.93.2.2
Kok A. 1995. Lumped pulses and discrete displacements: a physical way to understand numerical dynamics. Doctoral thesis, Delft University Press, ISBN 90-407-1118-6, NUGI 841.
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TRAPPING AND COOLING OF RUBIDIUM. APPLICATION TO THE STUDY OF MULTIPHOTON PROCESSES

Co-ordinator : Université Paris-Nord, Villetaneuse, France (Martial Ducloy)

Objectives

The general goal has been an experimental study of multiphoton processes in cold rubidium atoms trapped in a magneto-optic trap.

Activities

The central result of the project as a whole has been the consolidation in Uruguay of a specialized team working in close collaboration with the European partners. This led to several international publications involving a Uruguayan contribution, some of which even being derived from the experimental set-up now available at IFFI. Indeed, a fully operational experimental set-up, compatible with the international state of the art, has been designed and installed as a part of the project. This involved in particular the development and control of the laser sources, as well as the build-up and optimization of the atomic trap: in particular, a cold atom cloud containing approximately 10^8 atoms has been obtained within a volume of 1 cubic millimeter, while the trap charging time is 0.3 seconds. Moreover, the trapping is achieved routinely, and the cold atom cloud remains stable during times of the order of the hour.

Results

The spectroscopic aspects of the two photon (stepwise) excitation of the 5D states ($5D_{3/2}$ and $5D_{5/2}$) of ^{85}Rb in a cold atom sample have been studied in Montevideo. The excitation process of the above states was analyzed using stepwise excitation involving photons of the trapping beams (at 780 nm) and a photon from the probe laser of 760 nm. The observed excitation spectra have a complex structure determined by the excited state hyperfine structure and the dynamics of the excitation process mainly dominated by dynamical Stark effect induced by the trapping beams. The excitation of the 5D state was analyzed by the complementary detection techniques of observation of up-converted fluorescence and of monitoring of the resonance fluorescence of the trapped atoms. The influences of laser detuning, laser intensities, laser field inhomogeneities as well as of the magnetic field gradient were investigated. This is of notable interest, as transitions ending on the 5D states of rubidium have been proposed as suitable secondary frequency standards. Also, an original study of non-linear optics taking advantage of the noise fluctuations of a diode laser has been performed, which finally reveals susceptible to improve the understanding of some laser stabilization methods developed by the Université Paris-Nord partner. There, the activity in relation with the project was centered on the development of new methods of laser-frequency stabilization. One of the main idea was to optically lock the emission frequency of a laser diode onto the resonance of a retroreflected nonlinear optical signal originating in polarization spectroscopy. The principle scheme was first implemented on the Rb resonance used for the

cooling beams in magneto-optical trapping, and further extended to the narrow Ba intercombination line.

The project also permitted the continuous leadership of Université Pierre et Marie Curie group in the field of laser-cooled atoms, with special emphasis on the concept of *optical lattices*, and measurement of vibrational lines of atoms inside the light trap. The occurrence of dark states is very promising to achieve higher densities in the traps.

Follow-up

The Montevideo group has developed research on new aspects of dark resonances. In the same spirit of developing relatively simple set-ups with laser diodes, they succeeded in observing inhibition or enhancement of resonances through dark or bright states. These effects are connected with the narrow Raman resonances observed both at P.M. Curie, with cooled atoms, and at Paris-Nord for laser stabilization purposes. The Montevideo group also worked on the atom-surface interaction with cooled atoms, through co-operation with a Brazilian team (Recife), which has been an active partner of Paris-Nord for a long-time.

Selected publications

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Courtois J.-Y., Guibal S., Meacher D. R., Verkerk P. and Grynberg G. 1997. "Propagating elementary excitation in a dilute optical lattice". *Phys. Rev. Lett.* **77**:40-43.

Loe-Mie R., Papoyan A.V., Akulshin A. M., Lezama A., Rios Leite J.R., López O., Bloch D. and Ducloy M.. 1997. "Nearly all-optical frequency-locking of a laser diode on the 120 kHz intercombination line of Ba". *Opt. Commun.*

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MODULI OF BUNDLES IN ALGEBRAIC GEOMETRY

Co-ordinator: University of Liverpool, Liverpool, United Kingdom (Peter Newstead)

Objectives

- ◆ Study moduli spaces of vector bundles in algebraic geometry, especially on algebraic curves;
- ◆ Contribute to the development of algebraic geometry in Mexico.

Activities

The contract funded a number of visits by Brambila-Paz to Liverpool, on several occasions in conjunction with visits by outside experts from India and other countries. During these visits, many aspects of the theory of moduli spaces of vector bundles were studied. The project also helped to fund several workshops in Mexico and supported individual visitors to Mexico and visits by Mexican mathematicians to Liverpool and elsewhere in the EU, thus bringing together mathematicians from Mexico, the countries of the EU and elsewhere for discussions and research. The funding provided for financing visits and was of great benefit, both in the assistance provided to the project and in the development of contacts between Mexican, European and outside mathematicians. We believe that the benefits of our results and of the contacts made will be further demonstrated in future research.

Results

The project has now finished and has achieved both its objectives. Under Objective 1, many results have been obtained on the moduli spaces of vector bundles in algebraic geometry and a number of papers have been published or submitted in various areas of the subject. Particularly notable results where the project made a significant contribution were obtained in the following areas : stability and deformations of the Poincaré bundle, cohomology of moduli spaces, Brill-Noether varieties, restrictions of the Picard bundle and stratifications of the moduli space. Under Objective 2, the project helped to finance major schools, symposia and workshops in Mexico in the years 1994-96. These provided fora for discussions on many aspects of research in algebraic geometry and led directly to work either published or to be published.

Follow-up

Work is continuing in most of the areas studied during the project; in particular, new results on Brill-Noether theory have recently been obtained. It is expected that further grant applications will be made to continue building on what has been achieved.

Selected publications

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**JOINT PROJECT FOR UHV GRAZING INCIDENCE X-RAY DIFFRACTION
EXPERIMENTAL STATIONS USING SYNCHROTRON SOURCE AT LURE-
FRANCE AND LNLS-BRAZIL**

Co-ordinator : Centre National de la Recherche Scientifique, Gif sur Yvette,
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**SINGULARITIES AND GROUPS: GEOMETRY OF SINGULARITIES,
REDUCTIVE GROUPS AND THE MCKAY CORRESPONDENCE**

Co-ordinator: Université Joseph Fourier, St Martin d'Hères, France
(G. Gonzalez-Sprinberg)

Objectives

The scientific aim is the development of the theory of singularities and groups concerning the following subjects : resolution of singularities and exceptional divisors, arc structure singularities, toric varieties and Newton polygons, singular points of differential equations, group actions and quotients, the McKay correspondence, quantum groups, invariant theory.

Activities

- * A large number of seminars and working sessions were held at the Centro de Matemática (Montevideo, Uruguay) as well as at the Institut Fourier (Grenoble, France). The majority of the subjects discussed in these talks appeared in research and expository articles.
- * A Graduate School on singularities and applications was held in Montevideo at the Centro de Matemática in March 1995; a workshop on singularities took place simultaneously with the participation of researchers from the contracting universities (from France and Uruguay) and others (from Argentina, Brazil and Chile).

Results

Examples of results obtained in the course of the project include : a description of the essential divisors of toric resolutions, a description of toric clusters, a criterion of the existence of smooth curves on singularities, a constructive resolution of quotient singularities and applications to a generalization of the McKay correspondence, the description of the quantum double as a twisted product, criteria for the finite generation of invariants of commutative Hopf algebras, a generalization of Chevalley-Shephard-Todd theorem on polynomial invariants, the applications of clusters to study the foliations.

Selected publications

- Gonzalez-Sprinberg G., Lejeune-Jalabert M., Malgrange B., Sebastiani M., 1995. Singularités et applications. Version préliminaire. Institut Fourier et Centro de Matemática, 198 pages.
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SEISMIC BEHAVIOUR OF FRICTION PILES IN SOFT CLAYS

Co-ordinator: Université Joseph Fourier-Grenoble 1, Grenoble, France (Marc Boulon)

Objectives

The specific goal of this scientific cooperation project between the University Joseph Fourier (UJF) and the Universidad Nacional Autónoma de México (UNAM) was to study the seismic behaviour of friction piles in soft clays. The long term goal is to develop simple field criteria for the design of friction pile foundations which can be included in building codes.

Activities

- ★ A review of literature on the evaluation of the shaft friction of piles in clays was first carried out.
- ★ To assess the seismic behaviour of friction piles in soft clays, an experimental study was then conducted by means of: laboratory direct cyclic shear tests directed to undrained conditions (constant volume conditions) at the clay-pile interface, laboratory load tests on model piles in a small calibration chamber allowing consolidation, with measurement of pore pressure developed at the clay-pile interface.
- ★ Finally, a numerical study was performed by means of:
 - an evaluation of existing clay-pile interface constitutive models based on results of the above tests.
 - the proposal for a new clay-pile interface constitutive model taking the main interface properties into consideration.
 - the integration of this model in a numerical code based mainly on the finite element method.

Results

- ⇒ Natural, undisturbed samples under direct shear tests in undrained conditions yield results consistent with the expected behaviour of Mexico City clay. Shear tests on reconstituted material with variable stiffness and normal stress level confirm the importance of the stress path on the yield stress.
- ⇒ Monotonic loading of a friction-pile model in the calibration chamber gives a low effective pressure (low shaft friction), which can be explained by an incomplete consolidation. Cyclic loading indicates a decrease of the mean stress level resulting from an increasing pore pressure.
- ⇒ The proposed interface constitutive model gives qualitative results reproducing the mean features of the clay-pile interface behaviour. Incrementally non-linear interface and soil constitutive equations are well adapted to model clay-pile interaction behaviour.

Follow-up

During the time of this contract, a first attempt was made in order to put together the academic approach and the empirical analysis of soil-structure interaction in case of friction pile in clay subjected to seismic action. In the future, many various real situations should be investigated through this framework for comparing both relevance of numerical models and of building codes; the partners wish to do this as soon as possible.

Selected publications

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RESEARCH ON DYNAMICAL SYSTEMS

Co-ordinators: Université Aix-Marseille II, Marseille, France (Jean-Paul Brasselet)

Objectives

The main goal of the contract was the study of dynamical systems through complex differential equations, vector fields and holomorphic foliations.

Activities

During the period of the contract, major interchange took place between researchers in Brazil and in European countries (mainly France, Italy, and Spain). During their stays, Brazilian and European mathematicians gave lectures and worked together. As a result, a number of joint papers were written on the subject of Complex Dynamics. Moreover, many individual contributions originated from the exchanges induced by the project. There was also a remarkable increase in the number of young mathematicians who were incorporated into our group, both in Brazil and in Europe. This was clearly reflected in the scientific meetings we had in Medina del Campo (Spain) in 1995 and at the CIRM (Marseille, France) in 1996. Another congress will take place in IMPA in September 1998.

Results

- ⇒ The exchange of ideas that was possible throughout the project helped to obtain a better picture of the situation in Complex Dynamics, giving the solution to basic problems in the theory, such as the complete classification of flows with holomorphic transverse structure, the classification of regular foliations with transversal projective structure, the complete classification of complex foliations admitting a Liouvillian first integral.
- ⇒ Other important results were obtained at local (complex analytic differential equations and vector fields) and global levels (complex foliations). At the local level, saddle nodes were studied, topological invariants for general singularities were described (indices, residues). We gave a complete classification of real analytic centres, allowing the study of differential equations with central configuration. Toric singularities of vector fields were described. The notion of holonomy is important in the classification of holomorphic differential equation or complex vector fields, and important classification results (about separation; limit sets) were obtained in this direction.
- ⇒ From the global point of view, from the results previously mentioned, we studied the degree of foliations, giving relation with the genus and the Kodaira dimension. We gave a classification of logarithmic foliations, foliations with algebraic limit sets. We studied the rigidity and stability of projection foliations in relation with holonomy. There were many foliations with singularities with 'good' transverse structure and holomorphic foliations with resolvable projection holonomy.

Follow-up

The project has come to an end from an administrative point of view, but researchers continue to work together, due to important relations of the subject with meteorology, ecology, biology, physics, etc. Also, Complex Dynamic is a subject of confluence of many areas in mathematics and there will be the congress in IMPA in September 1998, where the participants in this project will meet.

Selected publications

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STUDIES IN FIELD THEORY

Co-ordinator: Universidad de Santiago de Compostela, Santiago de Compostela, Spain
(José M.F. Labastida)

Objectives

The main goal was the study of a variety of aspects related to the nature of fundamental interactions in the context of quantum field theory and string theory. Some of these aspects are:

- ◆ non-perturbative aspects of supersymmetric theories,
- ◆ integrable systems,
- ◆ topological quantum field theories,
- ◆ three-dimensional gauge theories at finite temperatures.

Activities

The research groups involved have pursued different paths of research to achieve the variety of results listed below. The work has benefited very much from a meeting held by the three groups at La Plata in 1997. In that meeting, the researchers of the three groups had the occasion to present their results and to exchange ideas on future developments.

Results

Among the most salient results achieved are:

- ⇒ the development of a framework to study non-perturbative features of softly broken supersymmetric gauge theories;
- ⇒ the formulation of new topological quantum field theories which are important in the study of the topology of four manifolds, leading to a generalization of Donaldson invariants; and
- ⇒ the exact computation of the parity-breaking part of the partition function for massive QED₃, showing that gauge invariance is preserved even under large gauge transformations.

Follow-up

Several ongoing projects are at present under development. Among these are:

- the study of T-duality transformations in the context of some specific integrable systems,
- the study of some aspects of duality in supersymmetric gauge theories in four dimensions, and
- the analysis of the implications of quantum field theory in the theory of knots and links.

Selected publications

- Labastida J.M.F. and Mariño M. 1995. Non-Abelian Monopoles on Four-Manifolds. Nucl. Phys., **B448** 373.
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- Fosco G., Rossini G. and Schaposnik F.A. 1997. Induced parity-breaking term at finite temperature. Phys. Rev. Lett. **79** 1980.
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- Falomir H., Gamboa Saravi R.E., Schaposnik F.A. 1997. Editors Trends in Theoretical Physics, CERN-Santiago de Compostela-La Plata Meeting held at La Plata, Argentina, AIP Conference Proceedings 419, American Institute of Physics, Woodbury, New York 1998. ISBN 1-56396-743-X.

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SENSORS FOR ULTRAVIOLET RADIATION

Co-ordinator: Universidad Autónoma de Madrid, Madrid, Spain (Fernando Cussó)

Objectives

The objectives of the project were the development and use of sensors for UV radiation, obtaining systematic ground-based data in different locations of Latin America and correlation with other measuring systems.

Activities

The project involved a broad range of activities, from the growth of single crystals and their testing as wavelength converters and dosimeters, to the development of ground-based UV detectors and calibrations, as well as to the establishment of a network of permanent stations for obtaining systematic measurements, which were analyzed and compared with other measuring systems.

Results

- ⇒ It has been possible to develop UV (ozone) detectors for ground-based operation, based on the differential optical absorption of the incoming solar radiation, using the existing technology of silicon photodetectors. While at the beginning of the project, wavelength conversion (UV to visible) was expected to be necessary, the appearance of UV-extended photodetectors has proved to meet the requirements for solar-UV detection.
- ⇒ The photodetectors which were built proved to have excellent reliability and resistance through continuous operation for a period of time extending over more than three years, including operation in very severe conditions (one of the systems operated in Antarctica, at Esperanza Base, near the South Pole, for a year).
- ⇒ Systematic data were obtained over the entire period of the project in several stations covering latitudes from 19 N to 55 N. Eleven permanent stations: 8 in Argentina and 3 in Chile, operated continuously and the data series extend over the full timespan of the project. An additional station was operating in the Universidad Autónoma de Madrid.
- ⇒ The comparison with other standard UV-meters for ozone determination (satellite and Brewer) shows a satisfactory correlation, normally better than 5% and always better than 10%, which is adequate for many applications. A better correlation would be dependent on the bandwidth of the wavelength-selecting element (interference filters in our case) which has been identified as the limiting factor.
- ⇒ Using similar principles of operation, detectors of atmospheric aerosols were built and successfully proved. Promising applications of thermoluminescent techniques for cumulative (dose) measurements were also tested.

Selected publications

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Period: January 1994 to January 1997

**SOME NON-LINEAR BOUNDARY VALUE PROBLEMS FOR DIFFERENTIAL
EQUATIONS**

Co-ordinator: Università degli Studi di Udine, Udine, Italy (Fabio Zanolin)

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**SELF-CONSISTENT MODELING OF THE SPECTRO-PHOTOMETRIC AND
CHEMICAL EVOLUTION OF INTERACTING GALAXIES**

Co-ordinator: Universitat de Barcelona, Barcelona, Spain (Gustavo Bruzual A.)

Objectives

The objective of this research project is to develop a comprehensive set of models to study in a self-consistent manner the dynamical, spectral, chemical, and morphological evolution of interacting galaxies. These models will be applied to investigate the dynamical processes that occur in clusters of galaxies, and how these processes modify the chemical and spectrophotometric evolution, and the initial morphological distribution of the cluster galaxies, as well as the evolution of the intracluster medium (ICM). The building blocks for the new models will be the computer code developed by Pelló & Sanahuja in order to study the photo-chemical evolution of galaxies in rich clusters, and the Isochrone Synthesis Spectral Evolution code of Bruzual & Charlot (BC93), which follows the spectral evolution of stellar systems under general assumptions about the form of the initial mass function (IMF) and the star formation rate (SFR).

Activities

- ★ Upgrade the BC93 spectral evolution code to allow easy computation of the spectrum of a stellar population subject to an arbitrary star-formation rate, which will be continuously modified by galaxy interactions. Extend the spectral evolution code to follow the chemical enrichment of the stellar population as evolution proceeds. Include in the spectral evolution code a high resolution UV atlas of stellar spectra in order to make realistic predictions of the expected spectra of different types of galaxies which can be readily compared with observed spectra.
- ★ Improve the performance of our dynamical code to study routinely the evolution of 1000 galaxies belonging to a synthetic cluster.
- ★ Build a comprehensive set of models to study the dynamical, spectral, chemical, and morphological evolution of interacting galaxies, which will assemble in a self-consistent manner the individual modeling of each of these aspects developed in the past.
- ★ Apply these models to study the origin and evolution of the different morphological types of cluster galaxies, as well as the evolution of the intracluster medium (ICM).
- ★ Analyze the role played by the environment, since the initial morphology of the galaxies can plausibly be modified by interactions like tidal stripping, ram pressure stripping or merging. Synthetic models will yield values for spectrophotometric and chemical variables that characterize each morphological galaxy type and will allow us to compare them with observations.
- ★ Build synthetic cluster data sets to isolate the volume of parameter space in which good fits to the observational properties of clusters at various redshifts are obtained. The most significant of these data sets include: color distributions, individual galaxy spectra, luminosity function, mean galaxy separation, galaxy morphology segregation, brightness

profile of individual galaxies, fraction of mergers, appearance of cD galaxies, fraction of active galaxies, BO effect, chemical abundance of the cluster galaxies and of the ICM.

Results

- ⇒ Goal (1) of the project has already been achieved. By the end of October 1995 a full set of spectral evolution models for stellar populations of metallicity lower than solar had been developed and debugged. There are models available for $Z = 0.0001, 0.0004, 0.004, 0.008, 0.02, 0.05, \text{ and } 0.10$. These models have been developed in collaboration with S. Charlot (BC96). Chemical evolution is not built in into the models, but a double convolution integral allows to compute the spectral evolution of a composite population with an arbitrary star formation rate and chemical enrichment law. This step is fundamental towards the goal of computing the evolution of galaxies in clusters.
- ⇒ Goal (2) is essentially finished. We are currently working in the coupling of the dynamical code of the Barcelona-Toulouse group and the spectro chemical evolution models of BC96 (goal (3)). During 1998 we will concentrate on the issues listed under goals (4), (5), and (6) in the previous section. In 1998 we expect to fully achieve all the goals of our joint project.
- ⇒ We have as well applied the results of our models to various research problems not foreseen at the time the project was written: (1) Gravitational lenses and faint galaxy samples; (2) Measuring spectral evolution of galaxies; (3) Interacting Galaxies; (4) LBDS 53W091.

Selected publications

- Ebbels T.M.D., Le Borgne J.-F., Pelló R., Ellis R.S., Kneib J.-P., Smail I., and Sanahuja B. 1996. "Identification of a Gravitationally Lensed $Z = 2.515$ Star-Forming Galaxy". *Mon. Not. Royal Astron. Soc.* **281**, L75-L81.
- Bender R., Ziegler B., and Bruzual A. G. 1996. "Redshift Evolution of the Stellar Populations in Elliptical Galaxies", *Ap. J. Letters*, **463**, L51-L54.
- Pelló R., Miralles J.M., Le Borgne J.-F., Picat J.-P., Soucail G., and Bruzual A. 1996. "Identification of a High Redshift Cluster in the Field of Q2345+007 Through Deep BRIJK' Photometry", *Astron. Astrophys.* **314**, 73-86.
- Bruzual A., Charlot G. & S. 1996. "A Library of Galaxy Spectral Evolutionary Models, in A Data Base for Galaxy Evolution Modeling". eds. C. Leitherer et al.", *Pub. Astron. Soc. Pac.*, **108**, 996-1017.
- Pozzetti L., Bruzual A. G., and Zamorani G. 1996. "Pure Luminosity Evolution Models for Faint Field Galaxy Samples". *Mon. Not. Royal Astron. Soc.*, **281**, 953-969.
- Bézecourt J., Pelló R., and Soucail G. 1998. "Number Counts and Redshift Distribution. of Gravitational Arclets as a Probe of Galaxy Evolution", *Astron. Astrophys.* **330**, 399-411.

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SPATIO-TEMPORAL DYNAMICS IN LASER

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Period: From 1993 to 1996

MOLECULAR CLOUDS IN THE GALACTIC CENTRE

Co-ordinator: Max-Planck Institut für Radioastronomie, Bonn, Germany
(T.L. Wilson and C. Henkel)

Objectives

The determination of the distribution, mass, chemistry, composition and dynamics of molecular clouds in the central region of our Galaxy. We also plan to carry out quantitative comparisons with the central regions of other galaxies with that in our Milky Way.

Activities

The project focuses on measurements of the central parts of our Galaxy. Since this region is visible only from southerly latitudes, we have obtained the data in Chile. Crucial to the success of this project is the data-taking instrument. The millimetre wave receiver and the 1.2-meter telescope are the property of the National University of Chile. The upgrades of the computer used to point the 1.2-meter telescope, a new spectrometer, and the data-reduction software were supplied by the Max-Planck-Institut f. Radioastronomie (MPIfR). The measurements were carried out jointly by the personnel from MPIfR and the National University of Chile, over a 24-month period.

Results

We have carried out the first complete sampling of the 18-oxygen isotope of carbon monoxide in the centre of the Milky Way, using the 1.2-meter millimetre wave radio telescope in Chile. The data reductions, and evaluation of the first set of data were presented in a publication in 1997 (see below). Further papers are planned. Using funds from this grant, we have improved the spectrometer, which is now in operation.

Follow-up

Further plans to upgrade the 1.2-meter telescope's receiver system, with funds granted by the Volkswagen Stiftung, will be carried out in 1998/99. Then we will measure the characteristics of other molecules in the galactic centre region.

Selected publications

Dahmen G., Huettemeister S., Wilson T.L., Mauersberger R., Linhart A., Bronfman L., Tieftrunk A.R., Meyer K., Wiedenhoefer W., Dame T.M., Palmer E.S., May J., Aparici J., Mac-Auliffe F. *Astronomy & Astrophysics Suppl. Series 126*, 197-236.

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**THEORETICAL AND COMPUTATIONAL DEVELOPMENTS IN DENSITY
FUNCTIONAL THEORY OF MANY-ELECTRON SYSTEMS**

Co-ordinator : Università di Bologna, Bologna, Italy (Renato Colle)

Objectives

- ◆ Application of Local-Scaling Transformations (LST) for the explicit determination of kinetic- and exchange-energy density functionals:
 - Closed-shell and open-shell atoms
 - Diatomic molecules
 - Poly-atomic molecules as a collection of locally scaled atoms
- ◆ Solving the many-electron correlation problem:
 - determination and analysis of Kohn-Sham exchange and correlation potentials, orbitals and eigenvalues for atoms
 - determination of explicit functionals for the correlation energy $E_c[\rho]$ derived from correlated wave functions
 - analysis of the Colle-Salvetti expression for the correlated 2- matrix
 - use of scaling conditions to calibrate electron correlation functionals
 - determination of locally-scaled operators
 - analysis of the dynamical and non-dynamical components of the correlation energy
 - determination of cluster-expansion correlation-energy functionals
- ◆ Local scaling and the Born-Oppenheimer approximation (diatomics).

Activities

- * Software development for the determination of LST in atoms and diatomic molecules.
- * Paper-and-pencil work for the construction of explicit functionals, locally-scaled operators, and cluster-expansion expressions. Calibration of scaling conditions for $E_c[\rho]$.
- * Calculation of atomic energies using explicit density functionals. Separation of dynamical and non-dynamical components of $E_c[\rho]$.
- * Numerical determination of Kohn-Sham potentials and eigenvalues (atoms).
- * Analysis of the non-Born-Oppenheimer problem for a solvable model (H_2^+).

Results so far

- ⇒ We have constructed explicit kinetic- and exchange-energy (non universal) functionals for atoms which yield the best results available in DFT (HF limits for the test Li and Be atoms). These functionals are variational and contain universal, quasi-universal, and specific modulating factors.
- ⇒ We have shown that LS-DFT leads to highly accurate KS potentials and eigenvalues.
- ⇒ We have developed a promising route for obtaining correlation energy functionals and have been able to analyze the dynamical and non-dynamical components of $E_c[\rho]$.

⇒ We have developed a viable numerical scheme for the evaluation of $E_c[\rho]$ through line integration.

Follow-up

- Approximate representation of the quasi-universal and specific modulating factors in order to obtain easy-to-handle representations of the kinetic-exchange and correlation-energy functionals. This work could contribute toward the development of a systematic procedure for constructing functionals in DFT.
- Extension to polyatomic molecules (viewed as collections of locally-scaled atoms). Development of appropriate software and test of energy functionals, and their approximations.
- Development of explicit kinetic-energy functionals for molecules and solids, and their application in dynamical simulation via the Carr-Parinello approach.

Selected publications

Valderrama E., Ludeña E.V., and Hinze J. 1997. An analysis of correlation energy in the context of local-scaling density functional theory. *Journal of Chemical Physics*. **106**: 9227-9235.

López-Boada R., Ludeña E.V., and Pino R. 1997. Hartree-Fock energy-density functionals generated by local-scaling transformations: applications to first-row atoms. *Journal of Chemical Physics*. **107**: 6722-6731.

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Period: June 1994 to June 1997

**AMPLIFIED SPONTANEOUS EMISSION AND EXCITED STATE
INTRAMOLECULAR PROTON TRANSFER IN HYDROGEN BONDING SYSTEMS**

Co-ordinator: Max-Planck-Gesellschaft, Garching, Germany (G.H.F. Diercksen)

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Contract number: CII*CT930352

Period: August 1994 to July 1998

STATISTICAL METHODS IN MANY-BODY QUANTUM THEORY

Co-ordinator: Technische Universität München, München, Germany (Peter Ring)

Objectives

- ◆ The general goal is the development of new theoretical methods for describing finite many-body quantum systems at finite temperature.
- ◆ The principal aim is the prediction of finite size effects in mesoscopic systems.

Activities

Investigation of new theoretical tools for describing strongly correlated finite fermi systems at finite temperature, by means of mathematical methods based on path integrals.

Investigations including most of the numerical calculations were performed in La Plata and Munich.

Results

- ⇒ The main outcome has been the development of a powerful method for evaluating the partition function and the response function of finite correlated fermi systems, based on a consistent simultaneous treatment of RPA correlations and large-amplitude statistical fluctuations. The method has been able to provide almost exact results for various thermodynamic observables in several testing models, which makes it an attractive economic alternative to more complex numerical Monte-Carlo evaluations, currently in use.
- ⇒ The applications have been aimed at the study of critical phenomena in small systems and their smoothing due to finite size effects, with emphasis placed on the description of superfluidity in hot nuclei and in mesoscopic structures of condensed matter.

Follow-up

Several applications of the methods developed are currently being performed, including in particular the study of ultras-small superconducting metallic grains and hot heavy nuclei formed in heavy ion reactions.

Selected publications

Rossignoli R., Canosa N., Ring P.. Effective finite temperature mean field approximations in finite systems. Nuclear Physics. 1995. **A591** 15.

Rossignoli N. Canosa, P. Ring R 1998. Thermal and quantal fluctuations for fixed particle number in finite superfluid systems. Physical Review Letters **80**. 8153.

Rossignoli R., Ring P. 1998. The RPA strength function in the presence of thermal fluctuations. Nuclear Physics.

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LATTICES AND NUMBER THEORY

Co-ordinator: Université de Bordeaux 1, Bordeaux, France (Jacques Martinet)

Objectives

The general goal of this project was to improve on known results related to various extremality properties of Euclidean lattices, in relation to various questions of number theory (algebraic number theory, analytic number theory, ...).

Results

Among the most striking results are papers [FS1] and [BN]. The first (which shall appear soon in 'Inventiones Mathematicae') gives a positive answer to a ten-year old conjecture about relative regulators of number fields. The second one, which appeared in a special issue dedicated to Martin Kneser of 'Crelle's Journal', is an important contribution to a classical problem in the theory of unimodular lattices. Besides the above two references, it is worth noticing that the ideas described in paper [BM5] have raised questions which proved interesting for specialists of Abelian varieties or of differential geometry. Finally, a great deal of work has been done in Bordeaux and Santiago on various subjects related to the notions of perfect and of extreme lattices (those lattices on which the density of the corresponding sphere packing attains a local maximum). The book [M] is now a reference on this subject.

Follow-up

Although the project has ended, the collaboration between the Number-Theory teams in Bordeaux and in Santiago is still running. Two directions are particularly active:

- Joint work in progress by Friedman (Santiago) and Skoruppa (Bordeaux) should yield a better understanding of some analytical invariants of number fields
- Work done in Santiago by Baeza and Icaza has been recently revisited by Coulageon in Bordeaux, who is considering a new notion of extremality.
- Work in progress by Roblot (Bordeaux) owes much to the collaboration with Friedman.

Selected publications

Bachoc C., Nebe G. 1998. [BN] : Extremal lattices of minimum 8 related to the Mathieu group M_{22} . *J. reine angew. Math.* **494**:155-171.

Bergé A.-M., Martinet J. 1995. [BM5]: Densité dans des familles de réseaux. Application aux réseaux isoduaux.. *L'Enseignement Mathématique.* **41**:335-365.

Friedman E., Skoruppa N.-P. [FS1]: Relative regulators of number fields. *Invent. Math.* (to appear)

Martinet J. [M]. 1996. Les réseaux parfaits des espaces euclidiens. Masson, Paris (110 pages).

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Period: January 1995 to January 1998

GENERAL RELATIVITY AND THE EARLY UNIVERSE

**Co-ordinator : Université Libre de Bruxelles, Bruxelles, Belgium
(Edgard Gunzig)**

Selected publications

- E. Gunzig, R. Maartens, A. Nesteruk. 1998. Inflationary cosmology and thermodynamics, *Class. Quant Grav.*
G.L. Comer, N. Deruelle, D. Langlois, 1997. Long-wavelength iteration scheme and scalar-tensor gravity, *Phys. Rev. D*, Vol **55**, No6, pp. 3497-3504
M. Castagnino, E. Gunzig, A landscape of Time-asymmetry. *Int. Jour of Theo. Phys.* In press.
E. Calzetta, A. Campos, E. Verdaguer. 1997. Stochastic semiclassical cosmological models. *Phys. Rev. D* **56**, 2163

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LIFT PUMP/RISER/ROD INNOVATION, 2R-PUMP

Co-ordinator: The University of Reading, Reading, UK (John D. Burton)

Objectives

The goal is to develop an extractable, wind-driven lift pump capable of being anchored in a tube well without the need for an expensive riser pipe. Thus the tube well itself guides the water to the surface, and the lift rod system used to raise and lower the pump cylinder components.

Activities

- ★ The project has focused mainly upon anchor/release systems for retaining the pump cylinder at the foot of the tube well, and of examining the anchor and lift rod dynamics whilst running. Guidance of the lift rods in the absence of a riser pipe was also explored.
- ★ An important part of the project has involved the exchange of personnel between the cooperating centers in Latin America and Europe.

Results

- ⇒ Reading's free seated, unanchored 2R pump has been laboratory and field-tested. The latter proved to be entirely satisfactory and the arrangement was run for a number of months driven by a CWD2740 mill, on a 43 m deep, 78 mm diameter tube well at Silsoe college, Bedford, (UK).
- ⇒ The Los Andes grip-release anchor system has proved to be a much more complicated arrangement to perfect

Follow-up

After the end of the 2R project contract period two final year student projects (see References 1 and 2) have continued to develop the grip-release anchor system. This work, carried out between March and December 1997, has improved the dynamic understanding by laboratory testing of the systems described in Appendix 1 of Ref. 3. Moreno's work on elasto-hydraulic anchor systems was particularly valuable and seems to indicate that this is a good approach. All rubber anchor sleeves were tested to destruction and it would seem that this type of anchor will be suitable for lifts of up to 60 m. Radial clearance between tube well walls and expandable rubber sleeves should be kept to no more than 5 mm. A local rubber components manufacturer in Bogotá has been involved in these developments.

Selected publications

Kuratomi, Andres, 1997. Bomba para molinos de viento con soportes flexibles. Proyecto de grado, IM-97-II-20. Depto. Ing. Mecanica, Universidad de Los Andes, Bogotá.

Moreno, Dayana, 1997. Bomba 2R. Estudio y Desarrollo de pruebas de un sistema de anclaje elasto-hidraulico. Proyecto de grado, IM-97-II-26. Depto. Ing. Mecanica, Universidad de Los Andes, Bogotá.

Burton J.D., and Davies D.G., 1996. Dynamic Model of a Wind Driven Lift Pump. Appendix 1, Proc. Institution of Mechanical Engineers, London, Part A, Journal of Power and Energy. **Vol. 210** : pp 279-293.

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APPLICATION OF ALGEBRAIC METHODS TO MOLECULAR AND NUCLEAR MANY-BODY SYSTEMS

Co-ordinator : Universidad de Sevilla, Sevilla, Spain (José M. Arias)

Objectives

The main objective of the project is to apply algebraic techniques to the study of molecular and nuclear structure and scattering problems. In the case of molecular spectroscopy the final goal is to design an algebraic model for the study of vibrational excitations based on $U(2)$ algebra and including the relevant discrete symmetry of the molecule. For nuclear structure the main purpose is to analyze collective excitations in nuclei far from the line of stability. Finally, in relation to scattering problems, the aim is to apply algebraic techniques for treating scattering in nuclear and molecular systems.

Activities

The project was designed to exploit the potentialities of the algebraic methods in different many-body systems, particularly, molecular and nuclear spectroscopy. In the context of the project, the development of the molecular and scattering parts was done mainly by the groups in Mexico and Spain, while in the nuclear part all the groups (France, Mexico and Spain) were involved.

Results so far

- ⇒ Concerning molecular physics, during the project we have been able to develop a new algebraic model that uses the continuous group $U(2)$ for the description of the interatomic interactions and includes the appropriate point group to describe the symmetry of the molecule. This model has been called the Anharmonic Oscillator Symmetry Model (AOSM). The AOSM is now well established. Its connection to configuration space treatments and its application to several molecules have been made.
- ⇒ In the field of nuclear physics, we have dealt mainly with medium N approximately equal to Z nuclei. We have examined a semi-empirical mass formula with an $SU(4)$ -type Wigner term and have shown it to yield better results than the conventional Wigner terms. In addition we have been working in the extensions of the Interacting Boson Model (IBM) for medium nuclei in the 28-50 shell. A systematic study of the symmetry limits of IBM-3 has been undertaken and the intrinsic-state formalism of IBM-1 and IBM-3 has been exploited. The problem of $T=0$ versus $T=1$ pairing has been treated in IBM-4.
- ⇒ We have studied algebraic approaches to scattering problems in molecular and nuclear systems. The effect of the nuclear dipole polarizability is important in nuclear scattering involving nuclei close to the drip lines. This problem has been investigated for some halo nuclei. For molecular scattering, we have analyzed the scattering of electrons by polar diatomic molecules. The scattering process atom-molecule has been analyzed from an algebraic point of view. A scattering hamiltonian for one-dimensional atom-diatom

collision has been derived and its generalization to three-dimensional systems has been discussed.

Follow-up

In molecular physics further applications to plane molecules and the ammonia molecule are under study. Both cases present new situations not treated yet with AOSM. In the first case vibrations out of the plane should be included. In the second one Fermi resonances and inversion play an important role. It is worth noting the same algebraic techniques used in AOSM can be applied to solid state physics and statistical mechanics. Applications to vibrational excitations of a linear chain and to the solution of the Heisenberg model are being considered. In nuclear physics, a detailed study of IBM-4 is under way. The scattering of electrons by polar molecules by using coherent states is also being investigated

Selected publications

A. Frank, R. Lemus, R. Bijker, F. Pérez-Bernal and J.M. Arias (1996), A general algebraic model for molecular vibrational spectroscopy, *Annals of Physics* (N.Y.) **252** 211-238.

F. Pérez-Bernal, R. Bijker, A. Frank, R. Lemus and J.M. Arias (1996), On the relation between algebraic and configuration space calculations of molecular vibrations, *Chemical Physics Letters* **258** 301-306.

P. Van Isacker and D.D. Warner (1997), T=0 versus T=1 pairing in the interacting boson model, *Physical Review Letters* **78** 3266-3269.

M. V. Andrés, J. C. Christley, J. Gómez-Camacho and M.A. Nagarajan (1997), Dipole Coulomb excitation in ^{11}Be scattering, *Nuclear Physics* **A612** 82-90.

J.E. García-Ramos, J.M. Arias, J. Dukelsky, E. Moya, and P. Van Isacker (1998), A Hartree-Bose mean-field approximation for the Interacting Boson Model (IBM-3), *Physical Review* **C57** five pages (February).

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**OPTIMIZATION: SENSITIVITY, NONSMOOTH PROGRAMMING AND
MONOTONICITY, AND ALGORITHMS**

Co-ordinator : Institut National de Recherche en Informatique et en Automatique (INRIA),
Rocquencourt, France (J. Frédéric Bonnans)

Objectives

Study of sensitivity analysis of solutions of optimization problems, local monotonicity of generalized gradients, and related algorithmic questions.

Activities

An intensive common work was done, mainly on the occasion of visits, concerning the different subjects of the projects.

Results so far

- ⇒ We have extended most results on sensitivity analysis of nonlinear programs to the Banach space and cone constraint framework, providing general formulas for derivatives of solutions, and giving application to semi infinite problems as well as optimal control of partial differential equations.
- ⇒ Concerning the monotonicity properties, we have introduced a notion of \square -monotonicity which allows us to characterize and extend the relations between subgradient monotonicity and the \square -convexity. This result can be applied to the study of the existence of \square -solutions for the differential inclusions as : $\dot{x} = -A(x)$. On the other hand we have proved an extension of the Hahn-Banach Theorem to nonconvex sets by using the subgradients to the distance function and some variational results. As a consequence we extend the second welfare theorem in economics.

Follow-up

- On sensitivity analysis: recent introduction of a “second order regularity” concept that allows second-order analysis of semi-definite programs under weak hypotheses.
- On monotonicity and nonsmooth analysis: we will apply the characterization of some particular problems concerning differential inclusions and on the other hand we will give new applications of our variational-geometry property proved and mentioned above.

Selected publications

J.F. Bonnans, R. Cominetti 1996. Perturbed optimization in Banach spaces I,II,III. SIAM J. Control Optimization **34**:1151--1171,1172--1189,1555--1567.

J.F. Bonnans, R. Cominetti, A. Shapiro 1998. Second order optimality conditions based on parabolic second order tangent sets. SIAM J. Optimization, to appear.

A. Jofré, D. Luc, M. Théra 1998. ϵ -subgradients and ϵ -monotonicity, To appear in Nonlinear Analysis.

A. Jofré, J. Rivera 1998. A nonconvex separation property and its applications, Submitted to Mathematical Programming.

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**UFRJ - CERN COLLABORATION IN THE DELPHI EXPERIMENT AT LEP AND
IN THE R & D PROGRAMME FOR THE LHC**

Co-ordinator: CERN - European Organization for Nuclear Research, Geneva
(PhilippeGavillet)

Objectives

- ◆ At UFRJ (Rio de Janeiro) : To stimulate the constitution of a coherent High Energy Physics (HEP) Research Group, contributing actively to outstanding physics studies and applied projects, including the DELPHI experiment at LEP, and acquiring the various competences needed to participate in all aspects of modern particle physics experiments.
- ◆ In DELPHI (CERN): To benefit from the collaboration of a new Institute, taking responsibilities in its physics programme in well-defined domains with implications in detector development, data processing and online computing.

Activities

- ★ Physics:
 - a) Study of the interaction process of electron and positron, giving rise to tau+ and tau- particles, to test the Standard Model theory at a centre of mass energy of 91 GeV.
 - b) Search, at high LEP energies (130 to 172 GeV), for new super-symmetric particles, predicted by various extensions of the Standard Model.
- ★ Detector development:

Participate, within an internal UFRJ, CERN, and CEN-Saclay collaboration, in the construction of a Synchrotron Radiation Detector (SRD) to monitor the synchrotron radiation emitted by the electron and positron beams of the LEP collider.
- ★ Data processing:

Build a powerful computing infrastructure at UFRJ, configured to provide the standard computing environment to analyse HEP data.
- ★ Online computing:

Contribute to the system management of the Distributed Computer Control System (DCCS), in charge of the control and data acquisition of the DELPHI detector and take part in the related online software developments.

Results so far

- ⇒ Physics:
 - a) Tau physics: Accurate measurements of characteristic physical constants (polarization, branching ratio, mixing angle) have been performed. They confirm well the validity of the Standard Model.
 - b) Search for new particles: Although no evidence for super-symmetric particle(s) has yet been found, precise limits on the masses of super-symmetric leptons have been determined.
- ⇒ Detector development:

A device, sensitive to low-energy photons, based on a silicon detector with fast-response electronics was built. Commissioned in 1996, the SRD has been fully operational in 1997

and has played a key role in protecting the DELPHI detector against high-level synchrotron radiation backgrounds.

⇒ Data processing:

A new computer architecture, based on two HP712 Workstations funded by the Project, was implemented in 1996. All the DELPHI software was installed and since then the UFRJ Group disposes of a modern and well-supported computing environment for its activities in DELPHI.

⇒ Online computing:

The UFRJ team played an essential role in the operation of the DELPHI DCCS and developed several important online applications.

Follow-up

- The Collaboration between UFRJ and DELPHI, initiated when LEP started in 1989, will go on until the end of the LEP programme, in the early years of 2000.
- The UFRJ Group now has the competence to undertake physics analysis autonomously, to take part in the design and construction of particle detectors, and to collaborate in the offline and online computing of HEP experiments. It will orientate its future activities towards the participation in the new LHC programme at CERN. First contacts have been established with one experiment, LHC-B, and the possibility of collaborating in the physics simulation, the design and construction of the muon detector, part of the detector front-end electronics, the offline computing, and the data acquisition system is envisaged.

Selected publications

S.Amato, I.Boyko, S.de Brabandere, L.de Paula, V.Lefebure, N.McCubbin, J.R.Mahon, F.Matorras, M.E.Pol, D.Reid, B.Stugu, (July 1996). An updated measurement of Tau polarization : Proceedings of the 28th International Conference on High Energy Physics : (ICHEP), Warsaw.

S.Amato, F.Cavallo, S.de Brabandere, L.de Paula, V.Lefebure, J.M.Lopez, J.R.Mahon, F.Matorras, F.Navaria, M.E.Pol, D.Reid, A.Ruiz, (Aug. 1997). Measurements of the Tau Hadronic Branching Fractions : Proceedings of the 29th International Conference on High Energy Physics : (ICHEP), Jerusalem.

P.Anderson, K.Hultqvist, A.Lipiniacka, M.Berggren, M.Gandelman, J.H.Lopes, C.Green and Ph.Gris, (Aug. 1997). Search for Sfermions at $\sqrt{s}=161$ and 172 GeV : Proceedings of the 29th International Conference on High Energy Physics : (ICHEP), Jerusalem.

M.A.Gaspar, M.Barbi, B.M.Marechal, L.de Paula, P.Siegrist, M.Besancon, T.Bolognese, X.Bravo, M.Gros, J.P.Passerieux, F.Pierre, J.Poinsignon, D.Vilanova, E.Merle and J.Renaud, (Sep. 1996). Synchrotron Radiation Monitoring for LEP2 using the DELPHI-TPC Silicon Detector : Proceedings of the XVII Brazilian National Meeting on Particle Physics, Serra Negra.

D.Carvalho, Ph.Gavillet, V.Delgado, J.N.Albert, N.Bellas, J.Javello, Y.Miere, D.Ruffinoni, G.Smith, (Sep. 1995). On the Relevance of Efficient Integrated Computer and Network Monitoring in HEP Distributed Online Environment. Proceedings of the 1995 Computing in High Energy Physics Conference (CHEP), Rio de Janeiro CERN/ECP report 96-01.

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Contract number: CI1*CT940124

Period: March 1995 to April 1996

**COMPUTER SIMULATION OF TRANSPORT ACROSS BIOLOGICAL
MEMBRANES**

Co-ordinator : Rijksuniversiteit Groningen, Groningen, The Netherlands (H.J.C. Berendsen)

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INDEX OF PROJECTS BY SUBJECTS

International Scientific Co-operation (ISC) 1992–1994
Projects by subjects (in numerical order of contracts)

Agricultural sciences

Contract number	Title
CII*CT920018	Production and nutritional value of coffee-pulp silage
CII*CT920029	Environment and pest locusts and grasshoppers of Brazil
CII*CT920061	Grazing-associated factors and characterization of mycological species in the co-ordinated epidemiological comparison of equine dysautonomia in Scotland and Patagonia
CII*CT920072	Algal pathology: infectious diseases and factors determining susceptibility in economically important seaweeds
CII*CT920075	Aromatic potential of muscat-grape varieties from Chile
CII*CT920077	Interaction between soil saprophyte fungi and vesicular-arbuscular mycorrhizae to recover fertility of degraded soils in the province of Buenos Aires (Argentina)
CII*CT920090	Evaluation and selection of coffee germplasm for resistance to prevailing nematodes in Central America
CII*CT920103	Cryopreservation of gametes, embryos, and larvae of marine organisms of commercial importance to mankind
CII*CT930027	Thermal requirements and host range of <i>Nacobbus aberrans</i> and immunoassays for its detection and quantification
CII*CT930028	Ecophysiology and sensitivity to cercosporioses in plantain at high altitude in Colombia
CII*CT930045	A study of the disease in pigs caused by the paramyxovirus LPMV
CII*CT930047	Comparison of genetic variability in European and South American populations of potato-cyst nematodes measured by variation in DNA and virulence towards plant resistance genes
CII*CT930058	Organization and expression of the potato mitochondrial genome
CII*CT930060	Factors affecting sensory quality of lactic-acid preserved meat
CII*CT930300	Determination of some factors affecting the nutritional and biotoxicological value of fishmeal for use in feeds for shrimp culture and the establishment of quality-control norms
CII*CT930307	Analysis of the interactions between the complement system and <i>Echinococcus granulosus</i> , and assessment of immunoprophylactic potential of parasite molecules identified as involved in complement evasion
CII*CT930319	Studies on the use of palm fats and mixtures of fats and oils in poultry nutrition
CII*CT930324	Role of antigen-presenting cells in determining the TH1/TH2 balance <i>in vivo</i>
CII*CT930335	Regulation of triacylglycerol synthesis and accumulation in developing cereal embryos by abscisic acid and water potential

Contract number	Title
CII*CT940006	Phenological forecasting techniques for timing of insect control measures as part of integrated pest management systems
CII*CT940011	Occurrence and significance of virus infections in benthic marine algae
CII*CT940032	Definition of the biological bases for the conservation, restoration and culture of highly valuable characids in the Magdalena River basin, Colombia.
CII*CT940040	Biological and molecular characterization of Chayote Mosaic virus: a new tymovirus inducing an important disease in cultivated Chayotes (<i>Sechium edule Swartz</i>) in Central America
CII*CT940041	Biological control of plant parasitic nematodes of food crops in peru
CII*CT940042	The <i>Rhizobium tropici</i> respiratory chain: isolation of mutants with enhanced respiration and symbiotic nitrogen fixation
CII*CT940060	Structure and function of the transcriptional activator protein NIFA from nitrogen-fixing bacteria
CII*CT940065	The use of plant cystatins as inhibitors of viral infections in transgenic tobacco and potato
CII*CT940067	Degradation of pasture lands and their reclamation using legumes: a major environmental challenge for Brazilian agriculture
CII*CT940074	Comparison of Mexican and European isolates of <i>Colletotrichum lindemethianum</i>
CII*CT940079	DNA replication in higher plant cells: the use of geminiviruses as a model system to study interactions between viral and cellular proteins
CII*CT940082	A functional approach to salt tolerance (FAST)
CII*CT940110	Delivery of transgenes into tissues of adult fish
CII*CT940113	Disposition kinetics, tissue residues and embryotoxic effects of antiparasitic drugs in food-producing animals
CII*CT940133	Modelling the transmission dynamics and determining the stability of <i>Echinococcus granulosus</i> in Uruguay to determine cost-effective methods for control

Biological sciences

Contract number	Title
CII*CT920020	A comparative study of the effect of Sulhydryl inhibitors on the excitation-contraction coupling of single cardiac cells
CII*CT920022	Basis for evaluating the fertilizing ability of mammalian spermatozoa
CII*CT920031	Development of cell polarity in intestinal epithelia and the use of cell lines derived from colon cancer: studies on water and electrolyte permeability
CII*CT920033	Fasciculin as a tool to study the role of Acetylcholinesterase in the brain in both cholinergic and non-cholinergic transmission

Contract number	Title
CII*CT920038	Structural and functional studies of the enzymes from the NAG regulon of <i>Escherichia Coli</i>
CII*CT920040	Molecular studies on desiccation-induced gene products and the regulation of their expression in the desiccation tolerant resurrection plant <i>Craterostigma plantagineum</i>
CII*CT920070	Structure-function relationship in light modulated enzymes of higher plant chloroplasts
CII*CT920084	Multidisciplinary analysis of phenotypic differentiation of neuronal cell types induced by central GLIA
CII*CT920097	Molecular basis of extension rate modulation by phytochromes in light-grown plants
CII*CT930017	Evolutionary study of the molecular basis of neural induction and limb pattern formation
CII*CT930037	Axonal mRNAs: identification, origin and role
CII*CT930042	Form and variability in Chilean <i>Nothofagus</i> Spp
CII*CT930048	The construction of a detailed reaction model of the NaK-pump
CII*CT930050	Environmental influences on the ecology and physiology of sub-Antarctic fish
CII*CT930052	Effects of the physiology of organisms of changes brought about by genetic engineering
CII*CT930054	Biodiversity with applications in biotechnology of yeasts in tropical ecosystems
CII*CT930063	Studies of ADP-Ribosylation in protozoa and plants, and DNA topoisomerase in protozoa
CII*CT940005	Molecular regulation of vertebrate glutamate receptors
CII*CT940013	Membrane skeletal muscle receptors and antagonists of the hormone 1,25-Dihydroxyvitamin D ₃ (Calcitriol)
CII*CT940016	Regulation of the citrate transport system from <i>Lactococcus lactis</i> biovar <i>Diacetylactis</i>
CII*CT940017	Cellular sorting, co-operative membrane topogenesis and ligand specificity of purine and proline permeases in <i>Aspergillus nidulans</i>
CII*CT940026	Exoenzymes of <i>Tetrahymena</i> : production and practical uses
CII*CT940034	Studies of substrate affinity in reactions catalyzed by native and recombinant alpha-amylases
CII*CT940071	Genetical and molecular studies on genomic imprinting
CII*CT940101	Phosphatidylinositol metabolism and control of plasma membrane H ⁺ -ATPase in yeast
CII*CT940116	Ca ²⁺ cellular dynamics, protein phosphorylation (with special reference to the glial fibrillary acidic protein 6FAP) G-protein activation and glutameric neurotransmission in the vertebrate brain
CII*CT940127	Molecular cell biology and modelling studies of the nicotinic acetylcholine receptor and its membrane environment
CII*CT940129	Regulation of intracellular calcium in muscle

Chemical sciences

Contract number	Title
CII*CT920008	Synthesis of chiral biologically active compounds and/or key intermediates for their production, using transition metal asymmetric catalysis
CII*CT920019	Flavour chemistry of some Colombian fruits having economic value
CII*CT920028	Production and advanced structural characterization of coal tar pitches
CII*CT920030	Synthetic, structural, kinetic, and catalytic studies on transition metal clusters containing novel unsaturated phosphorus ligands and their comparative behaviours with organic analogues
CII*CT920041	New catalysts for improving the reductions of sulfur and nitrogen compounds in environment
CII*CT920042	Transition metal-carbene complex-mediated synthesis of organic compounds of pharmacological interest
CII*CT920049	Determination of full structure and partial synthesis of new polyether toxins of marine origin
CII*CT920055	The extraction of gold using a natural resource from Peru
CII*CT920056	Oxide surface science-related laterite catalysts
CII*CT920057	Crystal chemistry and chemical bonding of non stoichiometric 2201 bismuth cuprates and related phases in connection with their superconducting properties
CII*CT920062	Characterization and mode of action of Draculin, an anti-coagulant protein from vampire bat saliva and isolation of the anticoagulant and antiplatelet factors from the same source
CII*CT920089	Preparation study of the properties and analysis of biodegradable surfactants from vegetable origin
CII*CT920097	Molecular bases of extension rate modulation by Phytochromes in light-grown plants
CII*CT920093	Modified noble metal catalysts for the synthesis of higher alcohols
CII*CT930029	Development of NADH electrochemical biosensors for analytical purposes
CII*CT930097	Chemometrics and qualimetrics in pharmacy and fine chemistry
CII*CT930329	Design, synthesis and characterization of homogeneous and heterogenized catalytic precursors, containing sulfur ligands
CII*CT930358	Isolation, production, characterization of glycosyltransferase enzymes for new carbohydrates
CII*CT940025	Biocorrosion, biofouling of stainless steels in industrial environments. Prevention and protection
CII*CT940044	Bifunctional zeolite catalysts for the synthesis of specialty or fine chemicals, synthesis of carbonylated compounds
CII*CT940062	Mechanistic studies of hydrodesulfurization and hydrodenitrification reactions of heterocycles in Mexican crude oil
CII*CT940064	Models for adsorption on metal oxides

Contract number	Title
CII*CT940143	Ensuring international comparability of chemical measurements of Chilean laboratories through the International Measurements Evaluation Programme (IMEP) of the European Communities

Earth sciences

Contract number	Title
CII*CT920036	The influence of shallow stratigraphy on long-duration ground motions in Mexico City basin: experiments and modelling
CII*CT920044	Metamorphism in extensional continental settings: a case study from the basin and Range province
CII*CT920054	Palaeobiogeography and tectonic evolution of the upper Silurian-Devonian sequences in Argentina. Geological correlations with northern and southern Palaeocontinents. Relations with the genesis of mineral resources
CII*CT920088	The Palaeozoic evolution of the Andean lithosphere (30-32°S)
CII*CT920098	Study of volcanic collapse calderas developed in transcurrent fault zones (the Calama-Olacapato-Toro volcano tectonic lineation, Central Andes, Argentina), and their associated mineral deposits (Au, Ag, Sb, Pb, Zn, Cu, and borates)
CII*CT930033	Evolution of meso-cenozoic magmatism and metamorphism in the northern Patagonian Andes (44-47°S Lat.)
CII*CT930061	Modelling waves on the Mexican seas, using ERS-1 wind and wave data to run a third-generation wave model
CII*CT930091	Andean tectonics of Argentina
CII*CT940069	Geological study of Andean saline basins: Chile (17-27°s. Lat.)
CII*CT940075	Geochemistry and alteration patterns of the outflow and peripheral zones of a fossil hydrothermal system
CII*CT940078	COTCOR - Comparative Geophysical transect across Costa Rica
CII*CT940098	Genesis of emerald deposits in the eastern cordillera of Colombia: structural geology, geochemistry and environmental impact of mining
CII*CT940102	Three-dimensional modelling of the northern gulf of California
CII*CT940103	Pilot project for regional earthquake monitoring and seismic hazard assessment in the Andean region
CII*CT940104	Assessment of seismic hazard in El Salvador
CII*CT940109	The seismic cycle in southern Chile: evolution and monitoring
CII*CT940112	Geophysical study of the southernmost Chilean margin
CII*CT940114	Palaeomagnetic constraints and structural block rotations in orogenic zones in Mexico and Spain
CII*CT940139	Ice and magma interaction processes
CII*CT940140	Modelling of water and solute transport (recharge and pollution) in volcanic and sedimentary deposits underlying Guatemala City

Environmental sciences

Contract number	Title
CI1*CT920043	Effects of wastewater re-use on urban groundwater resources
CI1*CT920073	Determination of atmosphere methanol and ethanol derived from the use of alcohol as vehicle fuel
CI1*CT920081	Removal of volatile organic contaminants from ground and waste waters by pervaporation
CI1*CT920082	Study of the composition of background and biomass burning atmospheric aerosols in the Amazon basin
CI1*CT920094	Development and application of cost-effective methods for biological monitoring of rivers in Costa Rica
CI1*CT930015	Marine resources at the Beagle Channel prior to the industrial exploitation: an archaeological evaluation
CI1*CT930030	Elimination of aromatic nitroso and nitro compounds from waste water by electrochemical reduction and subsequent chemical treatment
CI1*CT930055	The fate of organochlorine micropollutants in a tropical river system: Paraiba do Sul (Brazil case study)
CI1*CT930090	Nitrogen oxides and hydrocarbon abatments in exhaustion gasses
CI1*CT930096	Novel technologies for the treatment of industrial effluents, using immobilised microalgae and aquatic plants
CI1*CT930099	Determination and dynamics of heavy metals and organochlorines in the Valdivia River estuary in southern Chile
CI1*CT930306	Use of biomarkers for evaluating the presence and effects of pentachlorophenols in the Biobio River basin
CI1*CT930334	Coastal zone management in Santa Catarina Island (Brazil)
CI1*CT930336	Dendroclimatic reconstruction from southern South American temperate forests
CI1*CT930338	Behavioural ecology of invertebrates on Chilean shores, exhibiting different degrees of human disturbance
CI1*CT930340	Fate, cycling and environmental effects of agrochemical residues from cotton culture in coastal lagoon environments of Nicaragua
CI1*CT930345	The effect of nutrient balance and physical factors on the occurrence, toxicity, and control of cyanobacterial blooms in the Patos Lagoon, Brazil: a laboratory and field study
CI1*CT930346	Anaerobic digestion control strategies for wastes containing xenobiotics and recalcitrants
CI1*CT940018	The use of non-destructive biomarkers to assess the health status of endangered species of marine mammals in the southwest Atlantic
CI1*CT940027	The study of the geomorphology, hydrography and circulation of Argentinian estuaries
CI1*CT940028	Plant functional types and community responses to global

Contract number	Title
	climate change. A comparison between taxonomically distinct floras
CII*CT940030	Project PARAT: a study of the transfer of particulate and dissolved phases southern south America to the S.W. Atlantic ocean
CII*CT940033	Informal settlements in Santiago de Chile: the role of spatial configuration in neighbourhood, housing and community consolitation
CII*CT940035	Studies of photocatalytic reactions and reactors for water pollution abatement
CII*CT940059	Estimation of actual evaporation for irrigated crops and semi-arid rangeland in northwest Mexico from satellite data
CII*CT940066	Experimental and numerical study of precipitation formation in northeast Brazil
CII*CT940076	Study of the fate and impact of pollutants on the Costa Rican coastal zone
CII*CT940077	Numerical simulation of gas flow in porous media with application to landfill
CII*CT940099	Assessment of the impact caused by the introduction of a <i>Pinus caribaea</i> forest on the arthropod community in a Venezuelan natural savanna.
CII*CT940100	Allochthonous organic matter as a resource for stream communities: comparison of key processes among different geographical areas
CII*CT940111	Model validation, atmospheric variability and climate sensitivity over South America
CII*CT940128	Reaction dynamics and photochemistry with applications in atmospheric chemistry and combustion

Health and biomedical sciences

Contract number	Title
CII*CT920003	Prevalence of human papillomavirus infections in women with cervical dysplasia, carcinoma of the cervix and in a normal Honduran population
CII*CT920007	Immunopathology of lesions in parasitic and autoimmune disease - role of extracellular matrix
CII*CT920010	London-São Paulo collaborative project on the molecular genetics of the major psychoses
CII*CT920017	Molecular approaches to the study of the secretory pathway in <i>Entamoeba histolytica</i>
CII*CT920027	Mechanisms of antigen-processing, studies in T-cell tolerance and establishment of a model of B cell-mediated systemic disease induced in transgenic mice by passive transfer of T-cell-specific against a B-cell-specific endogenous antigen

Contract number	Title
CII*CT920045	Analysis of cytokine production in systemic <i>lupus erythematosus</i> and related disorders
CII*CT920060	Characterization of <i>Leishmania</i> parasites and their vectors from Central America, using molecular techniques
CII*CT920068	Rabies and vaccination in vampire bats
CII*CT920071	Analysis of the T-cell receptor specificity in lymphocytes infiltrating the intestinal mucosa in celiac disease children of Hispanic origin
CII*CT920074	Incidence and pathogenesis of HTLV-I induced adult T-cell leukaemia/lymphoma in Brazil
CII*CT920078	Effect on dietary long-chain polyunsaturated fatty acids on intestinal repair in chronic diarrhoea and malnutrition
CII*CT920080	The role of neurotransmitters in the regulation of gonadotrophin secretion during pre-pubertal period and in the onset of puberty in the female rat
CII*CT920083	Biosystematic studies on medically important simuliidae species complexes in centres of endemism in the Brazilian Amazon
CII*CT920085	Active sensory imaging and the central regulation of perception: an experimental and theoretical study of electrolocation in pulse-emitting electric fish
CII*CT920100	Study of volcanic emissions and their effects on health at Poas Volcano, Costa Rica
CII*CT930016	Identification, purification, and characterization of specific proteinases from <i>Trypanosoma cruzi</i> and drug targeting for Chagas' disease
CII*CT930024	Immune response in human cystic hydatid disease
CII*CT930025	Pathogenesis of pituitary tumours: a multidisciplinary approach
CII*CT930026	Construction of potential rotavirus vaccines and evaluation of their capacity for inducing both humoral and cellular immunity
CII*CT930032	Effects of cytochrome P450 inhibition on pulmonary oedema formation induced by ozone and parathion
CII*CT930035	Interaction among cytokines and proteoglycans in schistosomiasis
CII*CT930036	The epidemiology of <i>Leishmania braziliensis</i> in the unforested eastern Andean highlands of Huanuco Department, Peru
CII*CT930049	Identification of the interface of actin and actin-binding proteins, using a set of bi-functional reagents
CII*CT930051	Role of nutritional status in carcinogenesis and mutagenesis by air pollution samples from Santiago (Chile)
CII*CT930056	Ontogeny and genetic control of the natural antibody repertoire
CII*CT930092	Integrative study on the role of interleukins in the regulation of pituitary activity, immune function and cellular growth
CII*CT930098	Molecular and functional aspects of dystrophins in the animal and human nervous systems
CII*CT930301	Differentiation of hypothalamic neurons in presence of their target cells in culture systems
CII*CT930302	The epidemiology of malaria and control strategies in Nicaragua

Contract number	Title
CI1*CT930305	Cytogenetic, toxicological and epidemiological studies in populations chronically exposed to inorganic arsenic in Argentina
CI1*CT930308	Structural elements determining interferon sensitivity of gene expression
CI1*CT930309	The impact of community treatment with Ivermectin on the transmission of <i>Onchocerca volvulus</i> in Guatemala as established by conventional and modern techniques
CI1*CT930310	Cervical cancer in developing countries: a prospective population-based study of the etiologic role of human papilloma viruses, their molecular characteristics and the potential of PCR testing and hydrolysed DNA assay to improve the cost effectiveness of screening programmes
CI1*CT930314	Analysis of human cutaneous leishmaniasis in Mexico: mechanisms for recruitment of host cells and induction of immunoeffector functions
CI1*CT930325	A study of the molecular basis to the chromosomal size variation in <i>Leishmania peruviana</i> with particular reference to GP63-containing chromosomes
CI1*CT930326	Research project: DNA supercoiling, regulation of gene expression and virulence in <i>Salmonella typhimurium</i>
CI1*CT930354	Effects of lipoproteins and cholesterol on "G" proteins
CI1*CT940001	Urinary tract infections by <i>Proteus mirabilis</i> : an investigation into the role of fimbriae and flagellae as virulence factors and immunogens
CI1*CT940003	Heat stress, salt appetite and neurogenic hypertension
CI1*CT940012	Antigenic and genetic analysis of human respiratory syncytial viruses isolated world-wide
CI1*CT940036	Central gabaergic mechanisms, melatonin and ageing. Experimental chronobiological studies
CI1*CT940037	Growth hormone secretagogue peptides: long-term treatment on different cell functions and biological parameters during ageing
CI1*CT940038	Protein tyrosine phosphorylation in brain neurones
CI1*CT940043	Neutralization of snake-venom toxic effects by IgG subclasses isolated from immune horse serum using MOAB; obtention of human MOAB anti-crotoxin by molecular engineering
CI1*CT940045	Effect of Mexican scorpion toxins on the potassium channels of nervous membranes
CI1*CT940057	Functional and immunoprotective properties of organelle proteins (ROP2 and GRA4) from <i>Toxoplasma gondii</i>
CI1*CT940058	Development of transgenic techniques based on embryonic stem cells for the analysis of the mouse MSX1 homeobox gene promoter
CI1*CT940061	Design of inhibitors for the blood coagulation agent β -factor XIIA
CI1*CT940068	Vascular cells as a target for neoplastic transformation and as component of neoplastic tissues: studies using polyoma middle

Contract number	Title
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CI1*CT940073	Snake venom components acting on haemostasis: mechanism of action and drug-design perspectives
CI1*CT940081	Immunological and molecular diagnosis of <i>Taenia solium</i> cysticercosis and taeniasis
CI1*CT940134	Public health study of psychiatric disorders, drug abuse and health behaviour in Santiago, Chile

Materials sciences

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CI1*CT920016	Computer simulation studies of the structure and dynamics of glassy systems
CI1*CT920087	Magnetism and superconductivity in electron superconductors: influence of oxygen stoichiometry and microstructure
CI1*CT920099	Growth and characterization of pure and doped copper ternary in both bulk and thin-film forms for photovoltaic applications and a detailed study of their magneto-transport and magneto-optical properties
CI1*CT930014	Dynamic simulation of crystalline water molecules using X-ray diffraction constraints
CI1*CT930038	Thin films of new materials deposited by plasma-assisted techniques
CI1*CT930039	Silicon-carbide films prepared by sputtering, PECVD and MRPECVD
CI1*CT930041	Hydrophilic composite ultrafiltration membranes
CI1*CT930044	Structural relaxation and long-term behaviour in amorphous polymers
CI1*CT930053	Preparation and characterization of a new generation of improved track-etched membranes for microfiltration and ultrafiltration
CI1*CT930059	Surface modification due to alkali atom adsorption
CI1*CT930062	Study of the structural, optical, and transport properties of amorphous and micro-crystalline silicon carbon alloys fabricated by plasma-enhanced chemical vapour deposition
CI1*CT930303	Chemical crosslinking of poly(vinyl chloride)
CI1*CT930304	Studies on lipid-protein molecular interactions in food protein using biophysical membrane techniques
CI1*CT930318	Mechanic and magnetic properties of the Fe-Mn-Al alloys system
CI1*CT930322	Liquid crystalline copolymers having rigid and flexible blocks
CI1*CT930330	Characterization, optical, and magnetic properties, and relativistic cluster calculations
CI1*CT940029	Metastable alloy formation assisted by external work
CI1*CT940031	Lattice vibrational, optical and structural properties of CuInSe ₂ , AgGaSe ₂ , AgGaS ₂ under high pressure and temperature

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CI1*CT940046	Studies of electronic properties of superlattices grown along high index directions and semi-infinite superlattices
CI1*CT940056	Analysis of large data samples from particles physics
CI1*CT940063	Structural studies of surface adsorption structures using low-energy electron scattering
CI1*CT940070	Synthesis, characterization and optimization of thin-film oligomers for electrical application purpose
CI1*CT940123	Effect of monomer solubility in water on microemulsion polymerization
CI1*CT940132	Molecular thermodynamics of pure fluids and mixtures
CI1*CT940141	Mixing processes for flowing particles and porous media

Physical, mathematical, and engineering sciences

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CI1*CT920006	Non-linear dynamics and pattern-forming phenomena
CI1*CT920013	Morphology, stability and distribution functions of galactic structures
CI1*CT920021	The development of an innovative 3-S pump
CI1*CT920025	Study of earthquake sources and propagation of seismic waves in Mexico
CI1*CT920032	Ultrasonic non-linear experiments in fluids
CI1*CT920037	Bioprocess engineering of highly viscous fermentations: xanthan as a model
CI1*CT920039	Development of mathematical models for controlling MIG/MAG welding
CI1*CT920046	Non-linear mathematical modelling, theoretical and numerical aspects
CI1*CT920052	Improved flow-injection systems for multi-parametric analysis of samples of environmental, biological and agricultural relevance
CI1*CT920053	Study of the effect of initial conditions on the stability of a dense Z-pinch
CI1*CT920069	Seismic bearing capacity of foundations on soft soils
CI1*CT920076	Use-of-system costing for electrical transmission and distribution system in Chile
CI1*CT920079	Vibrational energy transfer in ozone-oxygen/nitrogen gas mixtures from infrared double-resonance measurements, and multiphoton excitation of ozone-freons gas mixtures by high-power TEA CO ₂ lasers
CI1*CT920086	First principles calculations of electronic and vibronic properties of complex materials
CI1*CT920092	Earthquake-resistant construction of low-cost adobe house

Contract number	Title
CI1*CT930001	Trapping and cooling of rubidium. Application to the study of multiphoton processes
CI1*CT930031	Moduli of bundles in algebraic geometry
CI1*CT930034	Joint project for UHV grazing incidence X-ray diffraction experimental stations using synchrotron source at LURE (France) and LNLS (Brazil)
CI1*CT930043	Singularities and groups: geometry of singularities, reductive groups and the McKay correspondence
CI1*CT930046	Seismic behaviours of friction piles in soft clays
CI1*CT930057	Research on dynamical systems
CI1*CT930315	Studies in field theory
CI1*CT930316	Sensors for ultraviolet radiation
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CI1*CT930331	Spatio-temporal dynamics in laser
CI1*CT930332	Molecular clouds in the galactic centre
CI1*CT930333	Theoretical and computational developments in density functional theory of many-electron systems
CI1*CT930339	Amplified spontaneous emission and excited state intramolecular proton transfer in hydrogen bonding systems
CI1*CT930352	Statistical methods in many-body quantum theory
CI1*CT930353	Lattices and number theory
CI1*CT940004	General relativity and the early universe
CI1*CT940047	Lift pump/riser/rod innovation, 2R-pump
CI1*CT940072	Application of algebraic methods to molecular and nuclear many-body systems
CI1*CT940115	Optimization: sensitivity, non-smooth programming and monotonicity, and algorithms
CI1*CT940118	UFRJ-CERN collaboration in the Delphi experiment at Lep and in the R&D programme for the LHC
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	Universidade Federal do Ouro Preto	CI1*CT940101	109
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GERMANY	Albrecht-Ludwigs Universität	CI1*CT930322	391
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	Estación Experimental del Zaidin	CI1*CT920077	13
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