



INTERNATIONAL SCIENTIFIC COOPERATION

Consolidated report of activities
1987-90

EC - ANDEAN PACT COUNTRIES

International scientific cooperation aims to develop strong and durable links between the scientific communities of certain Asian, Latin American and Mediterranean countries and their counterparts in the European Community. For those countries lacking a substantial body of active scientists, such links allow work to be carried out at an international level but with the advantage of the scientists remaining in their home institutions. For European scientists, such links allow access to a new intellectual environment and the opportunity to apply their skills to a different range of conditions and problems.



Report

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INTERNATIONAL
SCIENTIFIC
COOPERATION

Consolidated report of activities,
1987-90

EC - ANDEAN PACT COUNTRIES

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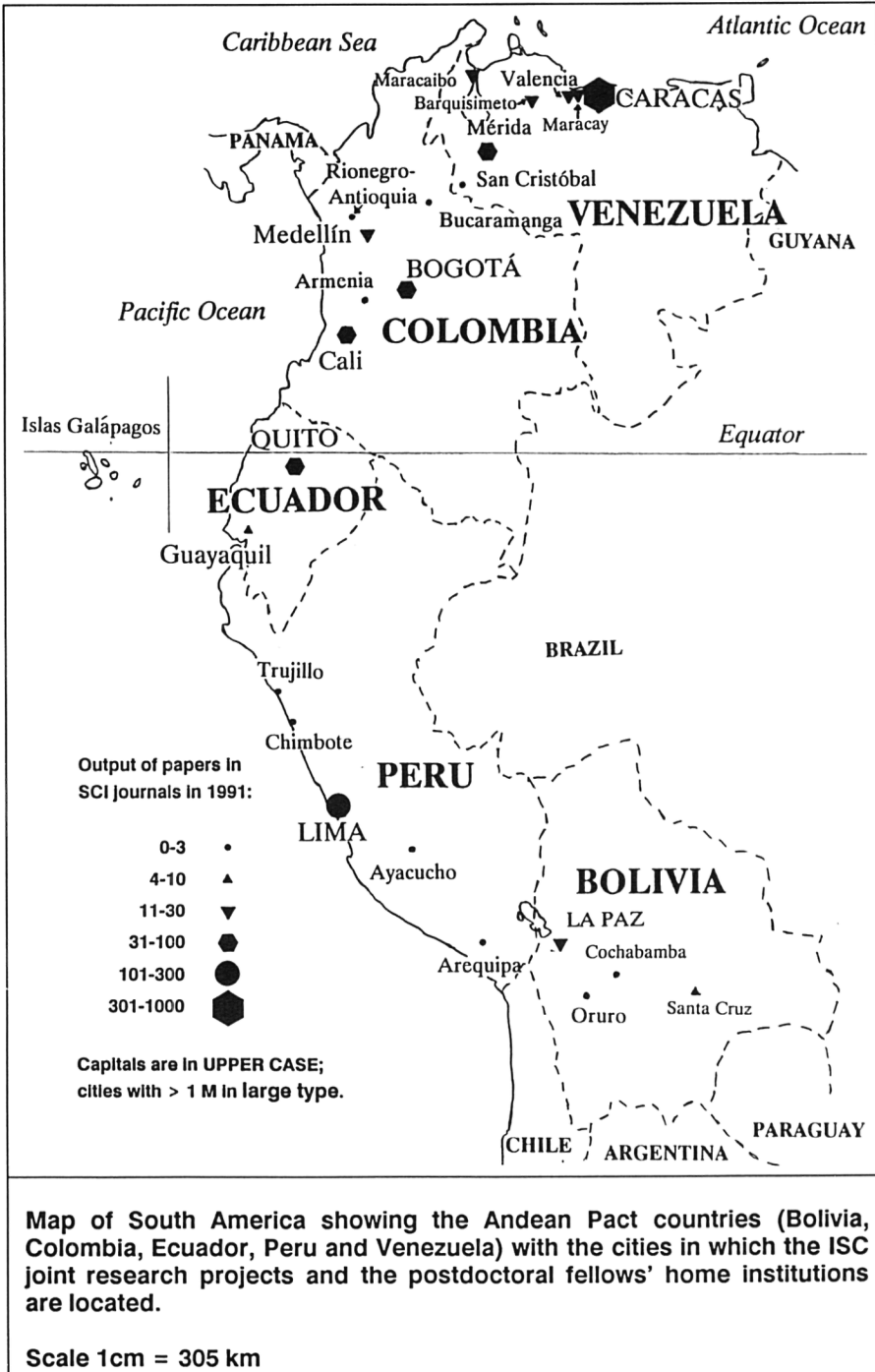
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The boundaries shown on the map are purely illustrative and have no political significance.

FOREWORD

International Scientific Cooperation aims to develop strong and durable links between the scientific communities of certain Asian, Latin American and Mediterranean countries and their counterparts in the European Community. For those countries lacking a substantial body of active scientists, such links allow work to be carried out at an international level but with the advantage of the scientists remaining in their home institutions. For European scientists, such links allow access to a new intellectual environment and the opportunity to apply their skills to a different range of conditions and problems.

Joint Research Projects

These are the central International Scientific Cooperation activity and allow research teams to work together on a problem of mutual interest, each team contributing complementary skills, expertise or resources. A minimum of one European Community research centre and one Third Country research centre must be included, and participating centres should have an established record in the research field and an adequate level of basic equipment and infrastructure. Thus, permanent staff salaries and infrastructure costs must be supported by the laboratories themselves. The Community normally supports the extra (additional or marginal) costs required to carry out the project on a joint basis; specifically, these may include:

Labour costs - salaries of additional staff such as research assistants or technicians employed to work on the project.

Travel and subsistence - to allow reciprocal working visits between participating centres.

Durable equipment - additional pieces of equipment to complement existing equipment.

Consumables - additional supplies used during the course of the work.

Other expenditure - specific additional costs to be justified and negotiated on a case-by-case basis.

In certain cases, the Community contribution to a Joint Research Project may represent a percentage of the full costs of the research.

Proposals for joint research projects are evaluated on the basis of criteria such as scientific interest, innovative value and the extent to which the joint approach might give added value. For those proposals that are financially supported by the EC, research contracts are established between the European Community and the participating centres, one of which is nominated by mutual agreement to manage and coordinate the project and to handle all communications with, and payments by, the Commission. Projects may last from 2 to 4 years. Graduate students may be incorporated into joint research projects and in this way the projects can have a

training function and can enable students to undertake all or part of their work at a second centre.

Postdoctoral Fellowships

These enable qualified Third Country scientists to work in European laboratories and make contact with European scientists; the fellowship may represent a preparatory phase for a joint research project.

Fellowships are normally granted for a period of 6 to 12 months. The Community awards fellows a monthly maintenance grant to cover all costs including food, accommodation and health insurance for the fellow and his/her dependants. Fellows also receive a lump sum to cover travel home at the end of the fellowship period. Cost of travel to Europe is normally borne by the fellow's home institutional or national authority. The European laboratory receiving a fellow is awarded a bench fee to cover research and related costs.

Candidates for fellowships are appraised on the basis of their research and academic records, and on the possibilities for continuing joint scientific research after the fellowship period. For successful candidates, the European Community establishes contracts with the fellows for the maintenance grant and with the host laboratory for the bench fee.

Workshops

These deal with topics selected together with the third country national authorities; they bring together up to 10 or 12 European scientists and a similar number of third country scientists to review progress, present results, discuss ideas and develop contacts that might lead to the preparation of joint research proposals. Workshops may also have a regional orientation and include scientists from neighbouring countries participating in International Scientific Cooperation. The Community can cover the travel and subsistence costs of visiting participants and make a contribution towards the publication of the proceedings. The host country covers all local requirements, such as infrastructure, venue, local transport and secretariat.

International Scientific Cooperation activities are always coordinated in conjunction with the third country national science and technology cooperation authorities and in annual meetings, priorities and procedures are established and reviewed as necessary.

International Scientific Cooperation is open to scientists both from the private and the public sectors, from industry, industrial research centres, universities and government research institutes. Research may be carried out on topics from the natural and exact sciences but must be of a precompetitive nature, i.e. further development should be necessary before a product or process is marketed.

INTRODUCTION

The first International Scientific Cooperation activity with a country in the Andean Pact group (Bolivia, Colombia, Ecuador, Peru and Venezuela) was initiated in 1987, and this volume presents a summary of all activities for which a financial commitment had been made before the end of 1990. It thus covers joint research projects, fellowships and workshops, in various stages of completion: some being totally finished, some in progress and for those projects just starting, a work programme only is presented. These activities conform largely to the patterns described in the Foreword.


The objective of compiling this volume is to show what has been achieved in the framework of International Scientific Cooperation with the Andean Pact countries. The strength of these achievements lies with their firm scientific foundation and this is reflected in the style of presentation of this volume; however, the contents are orientated towards a wide readership, to allow not only the scientists, both from the Andean Pact region and from Europe, to place their work in a wider perspective, but also to provide a concise account of the activities to other scientists, government officials, diplomatic representatives and all those interested in science in the Andean Pact countries and the European Community.

This report covers 26 research projects, 28 fellowships and two workshops, and the summaries included here demonstrate the results of these actions in terms of research findings, productive contacts developed and scientific publications. These results are impressive especially considering that many of the activities are still at an early stage of development and further output will be generated before they are completed.

The subject matter of the report has been divided by chapters into eight areas, Agricultural Sciences, Biological Sciences, Chemical Sciences, Earth Sciences, Environmental Sciences, Health and Biomedical Sciences, Materials Sciences and Physical, Mathematical and Engineering Sciences, which reflect the subjects covered by International Scientific Cooperation proposal evaluation panels and the need to indicate the spread of activities. Though necessary, this division is somewhat artificial and the main features of each area are described in the introduction to each chapter. Within each chapter, research projects have been arranged in ascending order of project number, an approximately chronological order of starting date, with fellowships in alphabetical order.

The successful outcome of the projects is largely the result of the efforts of the scientists involved. In the reports of the joint research projects the names mentioned are those of the principal scientists leading the research groups in the different institutions. It would have been impractical to list all individuals associated with each particular research project; nevertheless, full references have been given to publications resulting from the projects and these indicate some of the other scientists involved. In the reports of fellowships, the fellow's name and institution are given, along with the names of the host scientist and institution.

Another factor in the success of International Scientific Cooperation with the Andean Pact countries has been the contribution of the national governments and regional organisations to promoting the programme and for presenting high-quality proposals to it, and of the Commission staff responsible for the management of the programme.



Giuseppe Valentini
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 Commission of the European Communities
 (1987-91)

Further information on International Scientific Cooperation with the Andean Pact countries is available from:

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Bibliometric study of Andean Pact and EC research output

In order to see the work supported by the International Scientific Co-operation (ISC) programme in context, a study was carried out by General Technology Systems Limited in Uxbridge, England, of the extent of co-operation in scientific production between scientists of the five Andean Pact (AP) countries - Bolivia, Colombia, Ecuador, Peru and Venezuela - and from the European Community (EC) during the years 1986 to 1991.

The source of data was the Science Citation Index (SCI), published by the Institute for Scientific Information in Philadelphia, USA. It covers some 3200 leading scientific journals, the majority of which are international in both authorship and readership and are published in English. There are seven journals included that are published in Spanish-speaking countries of South America. Four of these are from Argentina, two from Chile, and one - *Interciencia* - from Venezuela. Despite these restrictions, it is recognised as providing a good coverage of "mainstream" science and it has the singular advantage of recording all the corporate addresses for the authors of each paper. This enables papers to be identified when the authors are, say, from Venezuela and an EC Member State.

In order to obtain a reasonable sample of the period under review without excessive cost, an analysis was made of the scientific output of 1986, 1988, 1990 and the first half of 1991 (January to June), designated hereafter as 1991i. This sample was designed to allow the effects of the output of the ISC programme, most of which have only recently begun to appear in the international serial literature, to be seen.

First, the total output of the five AP countries was analysed. Papers were attributed to a country if at least one of the corporate addresses was in that country. (Care was taken to exclude the small number of Spanish papers with the words "Pabellon de Peru" in the address which would have been identified as being Peruvian as well as Spanish.) The results are shown in Table 1.

Country	1986	Papers in SCI journals				Mean, N	Pop'n M	N / M
		1988	1990	1991i				
Bolivia	17	17	28	16	22	6.4	3.4	
Colombia	147	128	188	87	157	28.6	5.5	
Ecuador	23	25	40	25	32	9.4	3.4	
Peru	107	123	128	85	127	19.7	6.4	
Venezuela	<u>457</u>	<u>426</u>	<u>424</u>	<u>239</u>	<u>442</u>	<u>17.3</u>	<u>25.5</u>	
Total	751	719	808	452	780	81.4	9.6	
Any AP	<u>747</u>	<u>714</u>	<u>797</u>	<u>451</u>	<u>774</u>			
Multi AP	4	5	11	1	6			

Table 1. Numbers of papers in SCI journals by Andean Pact scientists and relation to population (in millions), 1986-91.

The overall scientific output of the region has thus averaged 780 papers per year but it has grown quite rapidly, particularly in the last two years. Venezuela continues to dominate the region's output, with over half of the total (reflecting its much greater relative wealth than that of the other countries, and corresponding ability to spend on scientific research) but the other four, particularly Bolivia and Ecuador, have increased their output much more in percentage terms.

The bottom row of figures in Table 1 gives the difference between the numbers of papers from each of the countries added together and the numbers from any of them. For example, a paper with authors from Peru and Venezuela would appear in each country's total, but would be counted only once in the "Any AP" row, which is thus less than the sum of the individual country totals. A paper with authors from three AP countries would give a difference of two. Thus the bottom row gives a measure of the multi-nationality of the region's scientific production within the region. It appears that there is no obvious tendency for this to rise or fall; the mean value is 0.8% which is rather low. By way of comparison, the corresponding figure for the five Nordic countries is about 3%, and they all speak different languages.

An attempt was made to see if this low degree of trans-national co-operation within the region could be attributed to the bias of the SCI choice of journals. As mentioned above, there are seven Hispanic South American journals in the SCI, including one from Venezuela. It turned out, however, that very little of the AP scientific output was in these journals, only 27 papers out of 747 (3.6%) in 1986 and 15 out of 451 (3.3%) in 1991i. None of these papers was trans-national within the AP region. It therefore seems unlikely that the apparent lack of intra-regional scientific co-operation seen in Table 1 is solely attributable to the selection of journals.

The second task undertaken was to examine whether AP scientists wrote papers with ones from the EC, and if so, which countries and which Member States were involved. The results are given in Tables 2 (for the AP) and 3 (for the EC) for the mean of the three years and the six-month period used for the previous analysis.

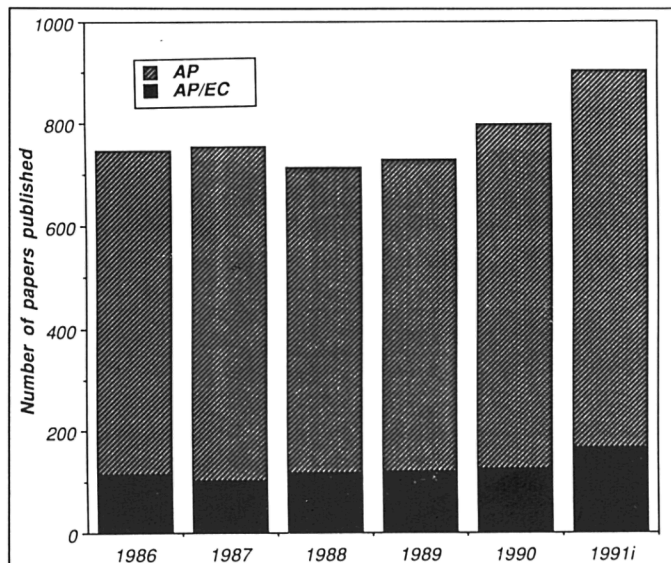


Figure 1. Andean Pact scientific production in SCI journals and co-publication with EC, 1986-91.

Country	Papers per year		
	With EC	Total	%
VEN	58.6	442	13
PER	24.8	127	20
COL	23.1	157	15
BOL	11.4	22	52
ECU	8.3	32	26
AP	125.4	780	16

Table 2. Extent of scientific co-authorship with EC for AP countries, 1986-91i

Table 2 shows that almost one in six AP papers has an EC co-author. This is much higher than the result for Mexico (8%, rising to 10%, over the same period). The percentage for the AP region has also grown, from 15% in 1986 to nearly 19% in 1991i.

Member State	Papers per year		
	With AP	Total	%
		thousand	
UK	36.6	55.0	0.07
F	34.3	30.6	0.11
D	20.3	36.1	0.06
E	19.1	7.4	0.26
I	10.9	16.2	0.07
B	5.1	5.7	0.09
NL	3.1	12.5	0.02
DK	2.0	5.3	0.04
P	1.4	0.9	0.16
GR	0.6	1.7	0.03
EC	126.0	164.5	0.08

Table 3. Extent of scientific co-authorship with AP for EC Member States, 1986-91i

However the actual annual number of such trans-Atlantically co-authored papers is almost identical (126 for AP, 125 for Mexico).

Among the EC Member States, the pattern of co-operation with the AP is quite similar to that with Mexico. The UK heads the list of EC countries, see Table 3; with Mexico it is only third with 28.6 papers per year after France (33.4) and Spain (28.8). Germany also co-operates less with AP countries than with Mexico (25.8 papers per year). Nevertheless, Spanish scientists are still much the most likely of those in the EC to seek an AP partner in scientific co-operation for obvious linguistic and cultural reasons. A possible explanation for the changes in UK and German co-operation seems to lie in the choice of subjects for co-operation, which are examined next.

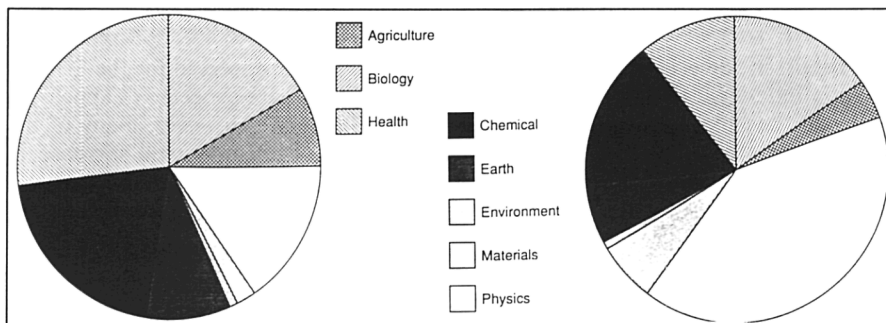


Figure 2. Subjects for Andean Pact and EC scientific co-operation (left) and for Mexican-EC co-operation (right)

Details of the papers on which AP and EC scientists co-operated during the period considered were printed out and allocated to one of the eight scientific fields used by the ISC programme for project classification and which form the chapter headings of this report, see Figure 2.

The allocation to field was made on the basis of both title and journal name; sometimes the address was also taken into consideration (e.g., papers emanating from a hospital or medical centre were presumed to be in the "health" field). The result was somewhat of a surprise. Whereas the pattern of co-operation with the EC was very similar for both Mexico and India, for the AP region it was quite different. Table 4 gives the results in terms of papers per year and also percentages for the AP region as a whole and, by way of comparison, for Mexico.

Field	Andean Pact		Mexico	
	Papers	%	Papers	%
Health and biomedical sc.	33.1	26	12.8	10
Chemical sciences	26.0	21	19.8	16
Biological sciences	21.1	17	19.4	16
Physics & mathematics	19.4	16	50.4	41
Earth sciences	11.4	9	8.0	6
Agricultural sciences	10.6	9	5.2	4
Materials sciences	2.6	2	7.8	6
Environmental sciences	1.1	1	1.0	1
Total Life sciences	64.8	52	37.4	30

Table 4. Scientific fields in which AP and Mexican scientists co-publish with EC authors, 1986-91

The big difference is that AP scientists co-operate much more with the EC on health and to some extent on agriculture, subjects of obvious relevance to Third World countries, very much less on physics and mathematics and somewhat less on materials. Thus it is perhaps to be expected to find more co-operation with the UK (considered to be relatively stronger in the life sciences) and less with Germany (whose strengths are greater in physics) for the AP region as compared with Mexico.

This report includes details of some 33 papers in journals processed for the Science Citation Index. More than half (18) of these appeared, or are expected to appear, in 1991 and they represented 2% of AP output in that year. However, many of the projects are still at an early stage and so this proportion may well increase in subsequent years.

1 AGRICULTURAL SCIENCES

Summary

For the purpose of this report, agricultural sciences are interpreted in a broad sense and include crop production and protection, seed chemistry, treatment of agroindustrial waste, animal health and freshwater aquaculture.

The crop production activities reported here focus on plantain (page 11), pineapple (page 15) and amaranthus (page 23). Plantain is an important component of the diet in Colombia and is frequently grown in association with coffee, the major export crop; however, since plantain is often regarded as a secondary crop, little improvement in its cultivation has been made and the particular problem of its diseases has not been studied much. Pineapple is of great interest in the region because it is native there, and the possibility still exists of finding new genetic material; however, in addition, it has potential as a cash crop for canning and the local yields are low in comparison with those obtained in other parts of the world, suggesting that there is potential for improvement of agronomic practices. Amaranthus is a native Andean crop which has not been subjected to intensive scientific study but which possibly has potential for improvement as well as for cultivation in other regions. Lupin is another Andean crop, but one whose potential is limited by chemical antinutritional factors in the seed (page 26).

The workshop on the utilisation of coffee crop by-products (page 22) was particularly relevant for the region because existing coffee pulp waste disposal procedures lead to environmental contamination, especially of water courses, and because, on the positive side, the pulp offers potential uses such as for preparing compost fertilisers as a growing medium, as a source of biogas and as an animal feed. It could thus contribute economically rather than be an economic and environmental burden.

Two reports deal with molecular-level studies, one (page 18) on plant pathogen interactions with a view to developing rapid screening techniques for identifying disease-resistant material, and the other (page 24) on the study of a model system which might eventually be of use for extension to agronomically important species.

Finally, the fellowship studies deal with fresh-water aquaculture (pages 25, 27, 28), a subject which is not well-developed in the region but which has great potential given the wide range of lakes and rivers and different environmental conditions to be found in the Andean Pact countries.



Figure 1: A patch of plantains affected by the Sigatoka disease at altitude (in the region of Arménia - Quindío).



Figure 2: Symptoms of Sigatoka at an advanced stage of the disease.

Joint research projects**1 Improvement of plantain cultivation in the coffee zone of Colombia****J. Ganry**

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Contract number and duration: CI1*/0378, April 1989 to March 1992.

Background and objectives

With 2 400 000 tonnes/year, Colombia is one of the biggest producers of plantains, accounting for 10% of world production. 97% of its production is destined for the internal market. About half of this comes from the coffee zone, which is dominated by the association of coffee with plantain. The coffee zone lies between longitudes 74°5' to 76°2' West and latitudes 4° to 5°3' North, encompassing the two western Andean cordilleras of Colombia at altitudes between 900 and 2 000 metres.

Despite recent improvements, plantain is still often considered as a secondary crop to coffee. Accordingly, very little effort has gone into acquiring knowledge of it.

The current trend is to maintain the level of production of coffee whilst increasing the production of plantain. In this context, it is important to have a good understanding of the ecophysiological, agronomic and parasitic components of the association of coffee and plantain. Only then can moves be made towards optimisation of the systems of cultivation compatible with socio-economic and land-ownership constraints.

In the light of such problems, the research undertaken within the present project has been partitioned into three main areas:

1. A multi-factorial diagnostic investigation aimed at evaluation of the agronomic and plant health problems in different situations and targeting the most appropriate directions of research.

2. The *Cercosporioses* (Sigatoka and Black Stripe diseases) which constitute a constraint on production or are an important threat to crops. The objective is to devise strategies for effective and economic control of Sigatoka disease whilst preventing the arrival of Black Stripe disease.
3. Enlarging the varietal range; current production, when subject to parasite attack, is weakened by the narrowness of the present range of varieties. The desired objective is the introduction of varieties originating from other areas of cultivation, in particular the African continent, and their evaluation in different ecological and phytosanitary situations in Colombia.

Diagnostic investigation

Materials and methods: The multifactorial diagnostic investigation had five principal stages. The first was the preparation of a questionnaire in line with the objectives. The second was the identification of 165 sample sites, with different types of soil, climates and systems of cultivation. The third stage was data collection. Most of the field work was undertaken by two teams of three persons between 5 March and 20 June 1990. Fourth, an analysis of soil samples, of leaves and of the nematode (threadworm) counts was conducted. Finally, an overall statistical analysis and its interpretation, based on multivariable techniques, was carried out.

Preliminary results: The principal limiting factors revealed by the investigation are, in order of decreasing importance:

Lack of technical aids to cultivation: Despite its quantitative and economic importance, the cultivation of plantains is still carried out by traditional methods, with few technical aids. It suffers from a lack of motivation by the farmers, aggravated by a shortage of man-power which is mostly attracted to the cultivation of coffee.

Ecophysiological and nutritional components: These are of three types. The chemical characteristics of the soil and its surface properties play a key role in plant nutrition. The second is climate, in particular temperature; the climatic gradients which are characteristic of the zone largely explain the diversity of nutritional situations observed. An inventory of these is one of the principal acquisitions of information from the investigation. The third is soil-borne parasites which can have a serious effect on plant nutrition.

Parasitic components: The most important potential threats to future production are the risk of spread of Black Stripe disease and of the nematode *Radolphus similis*. The incidence of the Sigatoka disease (see Figures 1, 2), the banana weevil and other nematodes (*Helicotylenchus*, *Meloidogyne*, *Pratylenchus*) is very variable and should probably be taken into account in certain locations identified during the investigation.

Cercosporioses:

Materials and methods: Work undertaken in the laboratory and in the field included characterisation and behaviour of the two types of disease - Sigatoka and Black Stripe - particularly the effects of temperature; microscopic observation of the forms of reproduction and dissemination; and relationships of hosts/pathogens. Field work included evaluation of the time-scales for incubation and for the evolution of lesions; speed of evolution of the disease; incidence of the disease in crops; and techniques of chemical and cultural control.

Climatological observations: simplified meteorological observation stations were established at six different situations (see Figure 3).

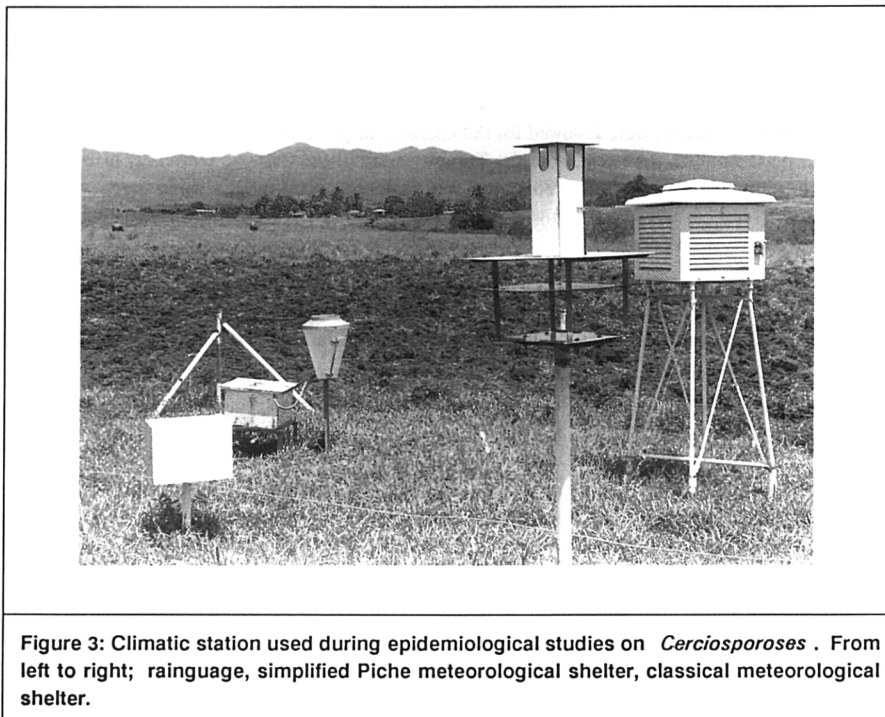


Figure 3: Climatic station used during epidemiological studies on *Cercosporoses* . From left to right; rain gauge, simplified Piche meteorological shelter, classical meteorological shelter.

Preliminary results: Epidemiology and evaluation of the risk of development of Black Stripe disease (*Mycosphaerella fijiensis*) The first observations reveal a very great variability in the behaviour of *M. fijiensis*, linked to climatic factors. There was evidence of a different response of the two types to low temperatures. The temperature factor alone would not explain the lesser development of *M. fijiensis* at altitude. The epidemiological data, in particular for the kind of "host" varieties, must be integrated.

Epidemiology of the Sigatoka disease (*M. musicola*) A very great variability was noted in the behaviour of this type as a function of altitude (between 1 000 and 1 600 m) and of the time of year.

Incidence of disease in crops Experiments are under way at six sites to quantify the incidence of disease (Sigatoka and Black Stripe) in different ecological zones.

Control techniques Different integrated systems of combating diseases were tried, combining, at the most appropriate periods, the application of fungicides with cultural practices such as leaf-thinning.

Varietal resistance Observations were made by ICA on the El Agrado collection, the only collection of a wide scope to be situated at altitude.

Widening the varietal range

Materials and methods. A list of 27 cultivars of interest was established from the Musacée germplasm bank maintained by INIBAP (Catholic University of Louvain). For security reasons, two complementary procedures were followed for the despatch of plant material. Five examples *in vitro* of each of 27 plant varieties were sent from the Transit Centre of the Catholic University of Louvain to the *in vitro* culture laboratory of ICA. 30 examples of each of 21 plant varieties from the preceding list were sent after multiplication by the *in vitro* culture laboratory of IRFA/CIRAD at Montpellier. The behavioural studies were undertaken by both ICA and FNCC.

Preliminary results. 21 varieties have been introduced, of which some are already in the field. In order to accelerate the establishment of experimental plots and to minimise the risk of plant losses, each of the types introduced has been multiplied *in vitro* in sufficient numbers to be tested at 6 to 8 different sites in the coffee zone

Discussion and conclusions

It has been possible to develop a cooperative research programme to improve the production of plantain in the coffee zone of Colombia. The scientific structures for accommodating the project in Colombia are completely satisfactory, both in the FNCC (CENICAFE) and the ICA. Despite an initial delay in setting up the scientific and technical activities, for essentially administrative reasons, the first two years have enabled three principal directions of work to be established, leading already to quite concrete results.

The conduct of a multi-factorial diagnostic investigation has led to identification of the principal limiting factors on the production of plantains. This has already enabled immediate solutions to be proposed which can be adapted to local conditions by the FNCC. It has also enabled targeting of main lines of research which are pertinent to the real problems encountered.

The methodological contribution has been very important, the "investigation" approach being capable of use for the study of more specific problems. The results obtained on *Cercosporioses* are extremely useful in view of the more and more worrying nature of the situation, linked to the spread of Black Stripe disease.

The widening of the varietal range which is under way will help minimise the phytosanitary risks. The diversity and novelty of the problems encountered have helped enrich the scope and applicability of the research and, in consequence, to increase our scientific and technical knowledge of the cultivation of plantains.

2 Improvement of pineapple cultivation in the Peruvian Amazon region

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Contract number and duration: CI1*/0379, April 1989 to March 1992.

Background

The pineapple industry in Peru represents an interesting case within the Andean Pact community, because these fruits are produced on a fairly large scale (60,000 tonnes in 1986) under topographic conditions which are harder than elsewhere. Thus results obtained in this country may pave the way for the development of pineapples in other places where it could help to diversify the sources of income, especially as an alternative to drug plants like coca.

Pineapple cropping techniques however are still at a very inadequate standard, with average productivity between 5 and 12 t/ha as compared to 60-90 t/ha in the most advanced countries. Fruit quality is also poor leading to very tiny profits for the peasants. However, good opportunities for effective research have been provided, on the one hand by the establishment of a programme for tropical crops within the national research organisation, INIAA, and, on the other, through the commercial pull exerted by a pineapple cannery which was ready to cooperate.

Additionally it should be mentioned that Peru contains native genetic resources of pineapple which are not quite the same as those in the other riparian countries in the Amazon Basin. Pooling these resources among the countries of the Andean Pact (and then with all Latin America plus the Caribbean) is of major interest for pineapple breeding in these regions.

Objectives

Three major fields of joint research were defined:

1. Investigation of existing cultivation systems in order to identify the factors limiting yield and quality, and means of alleviating them.
2. Evaluation of the economic impact of pests and diseases and setting up of efficient control methods.
3. Collection of wild and cultivated Peruvian varieties of pineapple, introduction of foreign varieties and participation by Peru in an international breeding programme with other Andean and Latino-American-Caribbean countries.

Only some limited aspects in this scheme could be undertaken within the two year term of the current project, which must be considered as a first step in a longer term cooperation. The objectives of the project itself were focused on the most obvious possible improvements in agricultural practices.

Results

Serious internal and external constraints have slowed down the progress of the project. Detailed plans were only established during the first mission of the project leader in the last two months of 1989. In the course of 1990 the *Sendero Luminoso* problems became worse and the lack of civil security in most of the pineapple cultivation zones prevented the socio-technical survey from being conducted.

However, a general picture of the cropping systems could be formed through information provided during the first mission and the main research lines regarding cropping techniques were subsequently defined.



Figure 1: Variety "Samba de Chanchamayo", selected for its single crown and cylindrical fruit. (Note slope on which it is growing.)

These consisted of soil preparation and erosion control; research of the most appropriate planting dates, materials, and densities; management of soil conditions, fertilizers and flower inducers; and crop rotations.

Similarly a first assessment was made of major pests and diseases. A very severe problem of internal spots in the fruits was identified. This is not quite identical to the fungal black spots known in other countries. A first set of observations on the etiology of this disease could possibly have implicated a fly. No great incidence of other fungus or virus/toxin diseases (rots, pineapple wilt usually associated with a mealybug) was however found. A complex of poorly-known insect fauna was found to be able to cause different types of damage, and there were serious root problems due to nematodes and small myriapods (symphylids).



Figure 2: Variety "Samba", with single crown and lesser slip number obtained by breeding.

While field trials on these topics could not be started for the same reason as for the surveys, efforts were concentrated on genetic improvement, which could be conducted in the vicinity of the pineapple factory. A start was made of selection within the local cultivars 'Samba' and 'Roja Trujillana'; 21 foreign varieties were introduced as a start to the development of a genetic reserve; and the international cultivar 'Smooth Cayenne' was multiplied by field techniques, while its tissue culture has been adapted to local conditions and made ready to start when the laboratory is fully built (during 1991).

Conclusions and perspectives

By the end of 1990 the INIAA acquired from the University a field of 7.5 hectares in a zone without security problems. This will allow an emphasis on the selection/multiplication programmes and a start to some field trials on the themes defined above. We hope that the work will be able to reach its full scale in the course of 1991 as security appears to be improving within the country.

3 Study on the molecular basis of plant-pathogen interactions and the selection of resistant genotypes

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Contract number and duration: CI1*/0549, July 1990 to June 1993.

Background and objectives

The interaction between plants and their pathogens is a "language" of communication, a very complex relationship which determines the fate of the parts involved. Deciphering the "alphabet" of this language may help in developing resistant cultivars.

The work carried out in the laboratory is directed towards understanding the hierarchy of the metabolic changes that take place in host-pathogen interactions *in vitro*, aimed at searching for methods of early screening and direct *in vitro* selection.

Within the framework of this project the main objectives are:

1. The development of methods for early screening of resistant varieties in the model system tomato - *Fusarium oxysporum*.
2. To study the regenerating capacity of plant cell cultures, as a prerequisite for *in vitro* selection.
3. To investigate the molecular aspects of β -glucanase production in host-parasite interactions in both the plant and the fungus.

Results

The work has been divided into three parts in line with the objectives:

Selection: During host-pathogen interactions the plant reacts by switching its metabolism in order to respond to the pathogen. Many biochemical parameters are known to be involved in these circumstances but there is certainly a hierarchy of importance that must be studied to understand which parameters are at the top of this hierarchy and should therefore be selected for.

On the basis of previous experience acquired in the Florence laboratory that points to a critical role for polysaccharides in active defence, a method has been developed and used to select tomato cell cultures that differ from each other in their callose content. Selection was performed on the basis of the fluorescence obtained under UV light of cell colonies painted with aniline blue. The clones thus obtained were further tested for other biochemical parameters known to be included in active defence, such as peroxidase synthesis in dual culture with the pathogen, measured spectrophotometrically and observed by electrophoresis (Figures 1 & 2); ion leakage, a measure of ionic conductance in the presence of fusaric acid (Figure 3); polisaccharide content, observed by fluorescence with calcofluor white; and inhibition of the pathogens in dual culture.

Currently, the induction of phosphorylase proteins (1-3 B-glucanase) in dual culture conditions is under test.

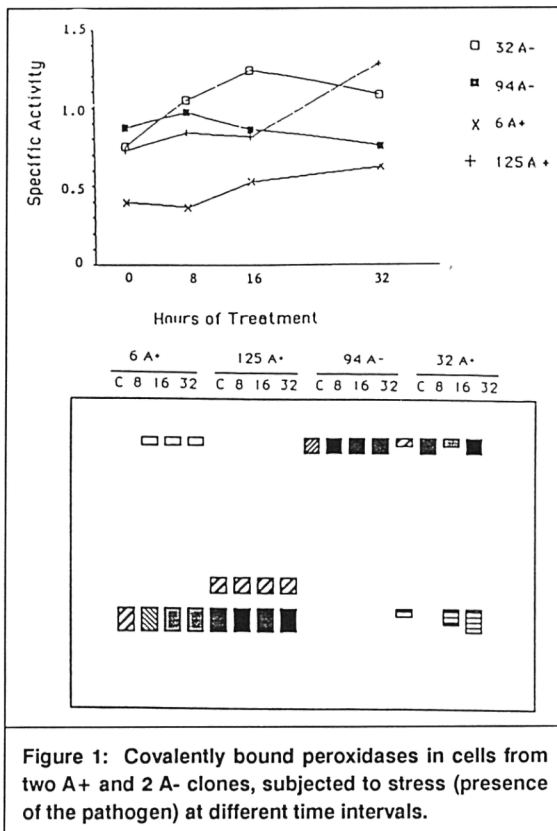


Figure 1: Covalently bound peroxidases in cells from two A+ and 2 A- clones, subjected to stress (presence of the pathogen) at different time intervals.

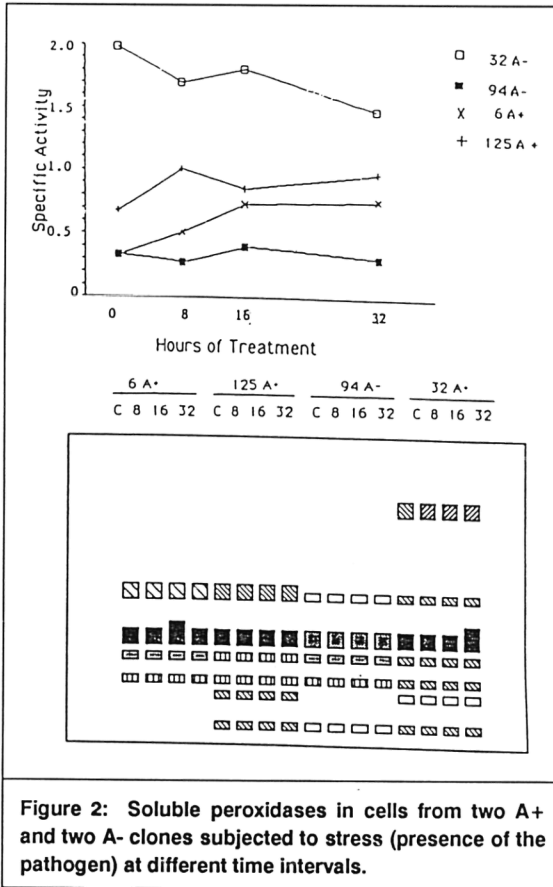


Figure 2: Soluble peroxidases in cells from two A+ and two A- clones subjected to stress (presence of the pathogen) at different time intervals.

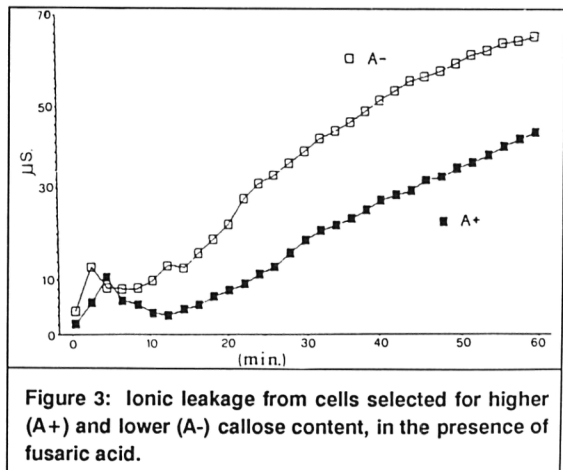


Figure 3: Ionic leakage from cells selected for higher (A+) and lower (A-) callose content, in the presence of fusaric acid.

Regeneration: Three commercial varieties of tomato (UC 105, Marmande and Tondino) were tested under different hormonal balances in order to establish the regenerating capacity and the best hormonal conditions for the production of callus as well as the best medium for regeneration of cell cultures. Tondino has shown surprisingly high regenerative capacity and the hormonal requirements have been established for its regeneration from cell cultures, at least from young cell cultures (four months old). This method and this particular genotype will be used for regenerating plants from cells selected for their high polysaccharide content and will be further tested for *in vivo* resistance to *Fusarium* (Figure 4).

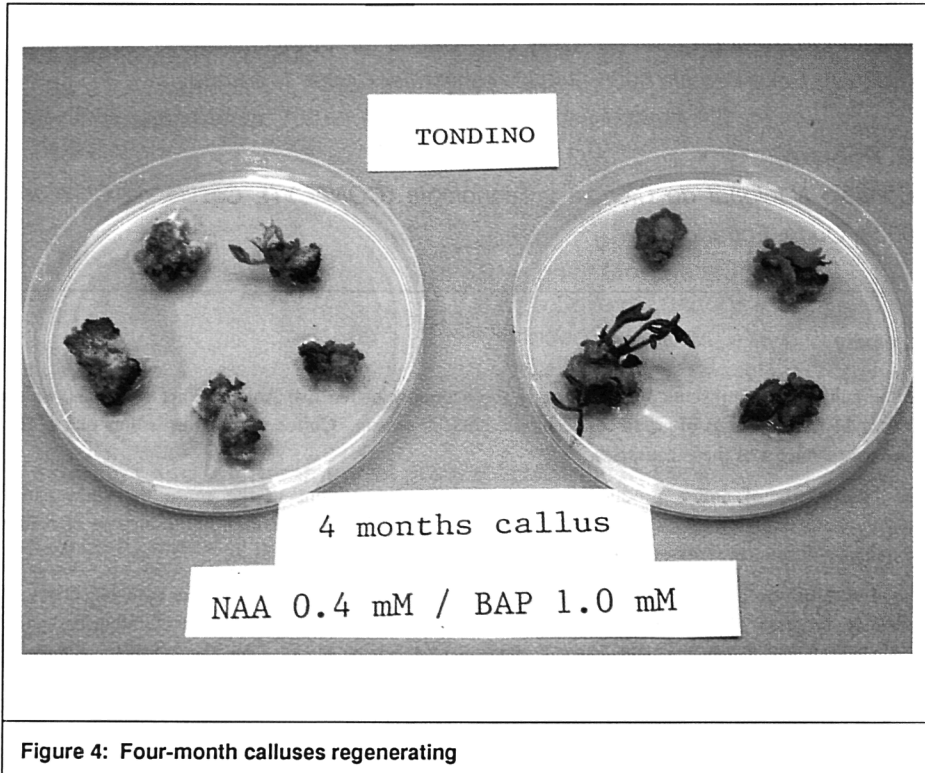


Figure 4: Four-month calluses regenerating

Molecular biology: A full comprehension of host-pathogen interaction implies an understanding of the patterns of gene activations of both partners from recognition onwards. Earlier results obtained in the laboratory revealed that plant tissue may respond to pathogens through amplification of specific sequences, and several authors have shown that specific transcription activation is required for active defence. Work is at present being undertaken on the amplification and transcription of tomato in the laboratory, on the plant side. To start a line of research on gene activation and amplification in the fungus, a genomic library of *Fusarium oxysporum* f. sp. *lycopersici* was constructed in the phagic vector EMBL3. Five clones containing repetitive sequences, two of which were found to contain r-DNA, were isolated to be used for amplification studies. The library is now being used to isolate, with heterologous probes, sequences belonging to the structural genes known to be involved in virulence processes.

In the context of this project, M. Guardiola is preparing a doctorate.

Workshop

Utilisation of coffee crop by-products

Joint Coordinators:

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G. Romero

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Summary

Coffee cultivation represents a key sector in the economy of the five Andean Pact countries, which produced 932,000 tonnes of green coffee in the 1988/89 season. Coffee production is thus one of the principal resources of these countries.

The by-products of coffee production continue to be a problem in the sense that they cause environmental contamination. Their utilisation could permit both an increase in the income of coffee producers and reduce, if not eliminate, these sources of contamination.

The workshop was held in Quito, Ecuador, from 11 to 15 June 1990 and had the twin objectives of reviewing progress of studies on the utilisation of coffee crop by-products and of intensifying the relations between European and Latin American researchers working on the subject. Some 29 scientists from 13 countries participated.

The main by-products considered included moist by-products such as pulp and sediment deriving from soluble coffee production plants, dry by-products such as husks, and substandard black, broken or defective beans eliminated during the classification process.

The techniques considered for the utilisation of these by-products were; for the pulp, anaerobic digestion and biogas production, anaerobic or aerobic fermentation for the preparation of compost or plant growth substrate, and animal feed; for sediment, combustion after pressing and drying, and fertilizer; for dry waste, briquetting and energy or gas production, and animal feed; and for defective beans, extraction of chemical compounds such as oils and caffeine.

In addition, topics such as the contamination of the environment by the waste products and the recycling of effluents were considered in the programme.

Postdoctoral Fellowships

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Selection and improvement of grain amaranthus in Peru.

Fellowship period: July 1990 - June 1991

Summary

Experimental sowings of three accessions of *Amaranthus caudatus* and one accession of *A. cruentus* of Peruvian origin were made at 15 day intervals at finca "El Encin" starting 15 July 1990 and irrigated as necessary.

Flowering and seed ripening occurred, respectively, at 50 and 90 days after sowing for *A. cruentus* and at 60-70 and 124-140 days for *A. caudatus*.

Lipid percentage and seed diameter were 7.02-7.04 and 1.65-1.75 mm in *A. caudatus* and 5.02 and 0.80 mm in *A. cruentus* respectively. In general, the plants appeared well-adapted to the environment.

In addition to the agronomic work, haploid *Amaranthus* material was also generated.

L. Destefano Beltran**M. Van Montagu***Peru**Laboratorium voor Genetika,
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Ledeganckstraat, 35,
9000 Gent, Belgium.***Signal transduction in *Arabidopsis*. Characterisation of transacting factors by transposon-tagging**

Fellowship period: April 1991 - March 1992

Background

Arabidopsis thaliana is becoming the model system for studies in plant molecular biology. Its most important assets include a small genome size and a very high seed production together with self-fertilisation. These factors allow for ready recognition of recessive mutants and a short generation cycle. In sum, these features make this small crucifer the first choice for studying basic mechanisms in plant molecular biology.

Objectives

These are the development of a transposon-tagging strategy to isolate mutants in regulatory genes; characterisation of the mutants; and isolation of the putative regulatory genes from the mutants.

Materials and methods

The study of transacting factors (which influence and determine tissue specific expression) will allow a better understanding and control over the expression of any gene. Also, the work with *A. thaliana* offers many advantages as mentioned above, since it is possible to isolate one or more genes from it and then use it to study a similar gene or genes in other plants of agronomic importance. Finally, this work will allow the fellow to master a series of techniques so he will be able to study tissue-specific expression of different proteins in other plants of agronomic importance for Peru.

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Study of substitution of fish meal in rainbow trout diets

Fellowship period: January 1990 - December 1990

Summary

The objective of this study was to find a diet for rainbow trout in which the fish meal of commercial diets is substituted by other protein sources such as meat meal, blood meal and soyabean meal. Feeding trials were carried out with these substitutes at initiation, growth and fattening stages of the trout development cycle. Although substitute diets led to a longer trout rearing time, economic analysis indicated that they would be more appropriate for Bolivian conditions.

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Lupin seed chemistry and fermentation

Fellowship period: October 1989 - September 1990

Summary

This project consisted firstly of the use of procedures such as PAGE, SDS PAGE, chromatography, FPLC and spectral methods for the recognition, quantification and characterisation of lupin seed protein and their use in the study of protein modification by practical treatment methods. Secondly, solid state fermentation trials were carried out with a view to producing fermented soya-like products; different strains of yeast, bacteria and moulds were used and results evaluated in terms of chemical, nutritional and sensorial characteristics.

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Study of growth and production of black cachama (*Colossoma macropomum*) and white cachama (*C. bidens*) at high densities in tanks and floating cages

Fellowship period: October 1989 - December 1990

Summary

Feeding trials were undertaken at a water temperature of 26°C and oxygen concentration of 5.0 ppm. In floating cages, black cachama grew better than white cachama, with production ranging from 471 to 756 g/m³ cage/day. In fibreglass tanks, white cachama grew faster achieving a weight of 210 g in 190 days as opposed to 170 g in black cachama. Equations were generated for the relation between body weight and oxygen consumption in the two species.

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Sheep pulmonary adenomatosis: analysis of early replication sites of SPA retrovirus

Fellowship period: July 1991 - June 1992

Objectives

The general objective of the proposed research is to use the homologous antisera to the SPA-retrovirus p25 polypeptide as a biological marker to gain new information on the pathobiology of the disease. The specific aims are:

1. To identify SPA-retrovirus (SPARV) - infected cells in naturally occurring and experimentally induced-SPA tumours.
2. To identify initial viral replication sites in experimental SPA tissues.
3. To initiate clones containing SPA viral genes.

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Rearing technologies for warm-water and cold-water fish

Fellowship period: March 1991 - February 1992

2 BIOLOGICAL SCIENCES

Joint research project

4 Structure of use of economically important Venezuelan wild plants

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Contract number and duration: Cl1*/0550, May 1990 to April 1994.

Background and objectives

A large number of scientific papers has been published on the plant anatomy and morphology (structure of tree barks, leaves, fruits and seeds) of Venezuelan forests as have many scientific papers dealing with the structure of Venezuelan plants from different ecological sites. There is already a third generation working in plant anatomy so that one may speak of a complete "school" of plant anatomy in Caracas.

Members of this group have a great knowledge of indigenous plants; they know also that most of them are useful to a certain degree. Useful - and particularly medicinal - plants are very well known by the Indians and by many people living in the country. Medicinal wild plants are sold in the country at "free shops". There one may buy the fresh leaves of an Andean plant which cure asthma or the bark of a *Zygophyllacea* used against rheumatic pains. There are remedies against almost all diseases, even against cancer. The number of plants from which such remedies are obtained is immense. As the flora of the tropics is incomparably larger than that of Europe, the number of useful plants has not been enumerated up to the present.

The objective of this project is to identify, classify and determine the current and potential structure of use of Venezuelan wild plants which are or could be economically important.



Figure 1: The arboreal form of *Espeletia*, Compositae, at a site in the Andes near Mérida, Venezuela, altitude about 4000 m.

Materials and methods

The material collected is taken invariably from wild plants, native of Venezuela. The plants come from forests of different kinds, from savannas, from the mountains (see Figure 1) or from the coastline, in other words from very different habitats. Where possible, entire plants are collected, but for trees and shrubs only the useful parts, such as leaves, barks flowers, fruits, seeds, roots or rhizomes. Wood is only occasionally studied, as a large bibliography already exists on this subject.

The methods used are principally those of anatomical studies. Permanent slides are prepared to study the tissues under the microscope. With different reagents, oily substances, cutine, suberin, cork, starch, lignin, slime etc. are identified. Photographs are taken of the microscope images (see Figure 2). If possible, photographs are also taken of the entire plants in their natural habitat. The outer habit of the plants is described as well as the inner structure, seen under the microscope. It is also intended to obtain collaborators for more detailed chemical studies.

Results

The majority of tropical wild plants are useful to a certain degree, although only wood is currently of economic importance. The economic use of tropical wild plants embraces the following. Foodstuffs include: grain, seeds, nuts, fruits which may be eaten raw or preserved, e.g. dried or processed as juice, marmalade, jelly, jam, candied, canned etc; vegetables obtained from leaves, roots, rhizomes, tubers, stalks, shoots, buds, fruits (legumes) and seeds; and condiments, used in small amounts to improve the taste of foodstuffs, obtained from bark, flowers, rhizomes etc.

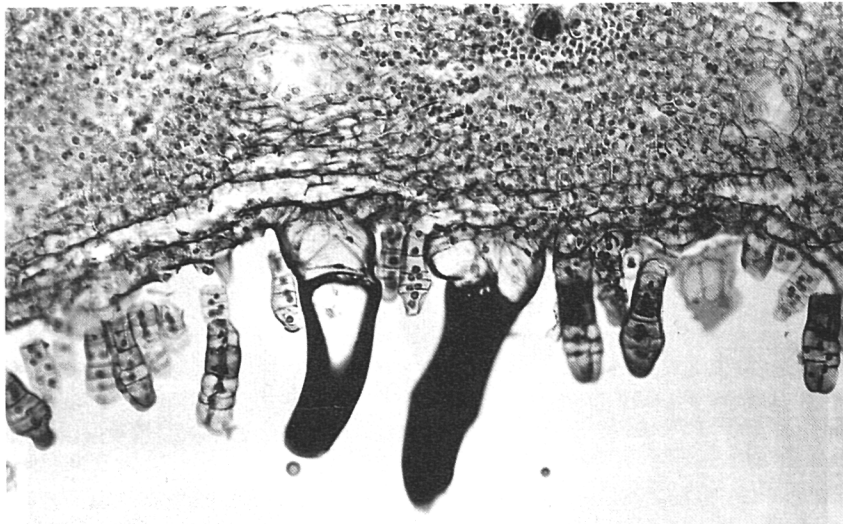


Figure 2: Glandular hairs of the leaf of *Espeletia* which contain etheric oils.

Animal fodder may be grass, fruits, nuts or vegetables. Many plants such as the fruit of oil palms can provide oils and fats. The bark and shoots of species of *Hevea* (other than *brasiliensis*), of *Ficus*, *Sapium* and other *Moraceae*, *Euphorbiaceae* or *Apocynaceae* and *Sapotaceae* can yield gum and resin. Fibres may be obtained from bark, wood, shoots, leaves e.g. of many palm species, seeds and even fruits. Plants with pretty leaves (variegated), or large attractive flowers, have value as ornamentals. (See Figure 3). Perfumes are obtained from flowers and the etheric oils of leaves. Plants are the source of several stimulants other than coffee and chocolate which also come from South America.

Very many tropical wild plants are sources of drugs. The medicinal plants may be subdivided into some forty or so categories, from aphrodisiacs to poisons, according to their application. More than a thousand medicinal plants are already known in Venezuela. The other useful plants in the categories listed above may complete a further thousand.

One example: *Angelonia salicariaefolia* H. & B., *Labiatae*, is a perennial herb, 30-60 cm high. The lanceolate or linear-oblong leaves have no petiole and are 3-8 cm long. The margins are dentate. The blue flowers measure 2 cm in diameter. They are arranged in large racemes. The calyx has five teeth. The corolla is bilabiate. The four stamens have short filaments. In Venezuela the plant occurs in the coastal cordillera, but is also used as an ornamental. The medicinal plant is sold at the market in Mérida (Andes) as a remedy against pectoral diseases and as a sudorific.

Another example is the "Quintas" or "quina-barks", different from the already well-known cinchona bark of Peru. There are several *Cinchona* species of the *Rubiaceae* family in Venezuela, as well as a *Rutaceae* which likewise supplies the alkaloid quinine.

Medicinal plants are usually sold by their common names - botanical identification is therefore sometimes very difficult and mistakes easily arise.



Figure 3: I. Roth studying the giant flower of the "Golden Cup" (*Solandra nitida*) which reaches a length of 25 cm or more.

Conclusions

There is an immense variety of tropical wild plants in Venezuela and elsewhere in South America which could be used for different purposes. Many of them could also be cultivated. However, to get to know all these plants and their uses, a very intensive study is necessary. It is also urgent, as more and more of the indigenous flora is destroyed by projects of urban development, burning and clearance.

3 CHEMICAL SCIENCES

Joint research projects

This is one of the most important subject-areas for International Scientific Cooperation with the Andean Pact Countries, and the topics covered in this chapter include the chemistry of natural products, novel techniques for the extraction of metals from ore, catalysis and basic chemistry.

Studies of the chemistry of natural products (page 34) have focussed principally on compounds extracted from plants and marine sponges, their crystal structure and biological activity, while other such studies have been made of legume seed lectins (page 65) and plant proteases of possible food industry application (page 62).

The mining industry is central to the economy of some Andean Pact countries and one of the studies reported here deals with a new chemical extraction technique for copper presenting a reduced risk of environmental contamination (page 39).

The studies of catalysis (pages 46, 61) are orientated towards the use of laterite, a material occurring naturally in the region in large quantities and in different forms, of zeolites and of possible applications in the petroleum industry (page 59).

Basic chemical studies have been made in the synthesis of tellurium and selenium compounds (page 56), contributing to organometallic chemistry and new materials science, and other studies have been made of the weathering process in steel (page 67).

5 Chemical studies on Colombian natural products

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F. delle Monache

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Contract number and duration: CI1*/0447/0448/0449, November 1989 to December 1992.

Introduction

Complementary studies are being carried out at the three centres involved in this project with the principal objective of searching for substances with biological activity and also in order to elucidate taxonomic and ecological relationships for the taxa studied.

Crystallographic studies on substances isolated from *Iryanthera tricornis* (Myristicaceae)

Following the agreed plan, the first phase consisted of obtaining crystals suitable to carry out the structural studies necessary to elucidate the crystalline and molecular structure of the compounds isolated. Given the difficulties associated with this process, after many attempts it was only possible to obtain suitable crystals of two products extracted from *Iryanthera tricornis*.

1. 2'-4'-dihydroxy-4-6'-dimethoxy- $\alpha_1\beta$ dihydrochalcone.
2. 1-(2'-4'-dihydroxyphenyl)-3-(3",4"-methylenedioxyphenyl) propane hemihydrate.

The effort required for the separation of active natural products from plants is well-known, since one can easily obtain other products that do not correspond to the active natural product being addressed. Moreover, once it has been obtained, the only really sure way of confirming its molecular structure is through the use of the single crystal X-ray diffraction technique. The critical problem is to obtain well-diffracting single crystals of proper size, which are not artefacts produced by the crystallization technique. Given this complex scenario it should be considered a relative success to have been able to crystallize and resolve the structure of the two compounds referred to above.

Compound No. 1 was crystallized from the leaves of *Iryanthera tricornis* and recrystallized with a solution of acetone-hexane (m.p. 444-445^oK.). It is also found in the woody trunk and in the fruit of *Iryanthera laevis*. This compound is of practical interest because, as has recently been demonstrated, it acts as an inhibitor of the Gram-positive bacterium *Bacillus anthracis*.

The crystals obtained were in the form of flat, transparent, prisms; from among them we selected a crystal of suitable quality measuring 0.20 x 0.08 x 0.15 mm. The crystal was mounted on an automatic Enraf Nonius diffractometer equipped with a graphite monochromator working with $K\alpha$ radiation from molybdenum of wavelength = 0.71069 Å. The unit cell parameters were determined by measuring 25 reflections between 3° and 13° and refining the angles by least squares. The following parameters were obtained:

$$a = 4.856 (3), \quad b = 28.896 (7), \quad c = 10.776 (3), \quad \beta = 98.04 (4)^\circ \quad V = 1497 (1) \text{ \AA}^3$$

The symmetry is monoclinic and the spatial group corresponds to $P2_1/c$. The number of molecules in the asymmetric unit is equal to ($Z = 4$). A calculated density of $D_x = 1.34$ was obtained, assuming a composition of $C_{17}H_{18}O_5$ (molecular weight $M_r = 302$).

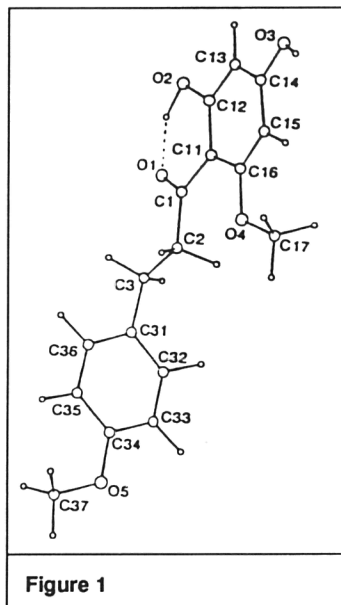
Later, we measured with the $\omega - 2\theta$ technique a total of 5,191 reflections up to a maximum angle of θ of 25°. Of these 2,632 were unique and 792 reflections considered as observed [$I > 2.5 \sigma(I)$]. These reflections correspond to values of (h, k, l) in the ranges $-5 < h < 5$; $-34 < k < 34$; $0 < l < 12$.

In order to check for a possible intensity decay during the measurement process, three standard reflections with known intensity were measured every 50 reflections. No significant reduction of their intensity was observed. The intensities were corrected for Lorentz and polarization effects but not for absorption since the calculated absorption coefficient (μ) was low (0.922 cm^{-1}).

The structure was solved by direct methods and was refined with anisotropic temperature factors using the full matrix least-squares program SHELX 76. The hydrogen atoms and hydroxy groups were found through a differential Fourier synthesis. The positions of the remaining hydrogen atoms were calculated geometrically and included in the last refinement cycle. Different global temperature factors were refined: $U = 0.106(16)$ and $U = 0.121(13) \text{ \AA}^2$. The final residual index obtained was $R = 0.068$. All calculations were carried out on a Microvax II.

The structure obtained is represented in Figure 1. The phenyl ring is flat within the limits of experimental error. The normals to the mean planes of the aromatic rings form an angle of 81°, which determine the overall geometry of the molecule.

The molecule packing is conditioned by the hydrogen bridges. Inter- and intra-molecular hydrogen bonds were found;



$$2.77(1) \text{ \AA} \quad 01 \dots \dots \dots H3' \quad \dots \dots \dots O3'(x-1, 1/2-y, 1/2+z)$$

$$2.50(1) \text{ \AA} \quad 02 \quad \dots \dots \dots H2 \quad \dots \dots \dots O1'(x,y,z)$$

This last intramolecular bond causes the torsion angle values of $O1-C1-C2-C3[5.42(9)^\circ]$ and of $O1-C1-C11-C12[5.63(9)^\circ]$ to be low. These results give an adequate explanation of the spectroscopic results obtained for this compound. Figure 2 shows the unit cell content seen from the a axis. The intermolecular hydrogen bonds which assure the cohesion of the compound in the solid state (de Matheus *et al.*, 1991) are indicated with dotted lines.

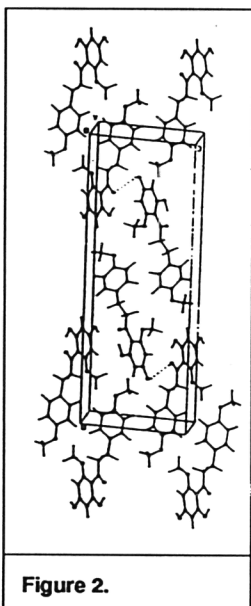


Figure 2.

Compound No. 2 was described for the first time by Alves de Lima *et al.* who described the method of extraction of its derivative diarylpropene from the wood of the trunk of *Iryanthera coriacea* Ducke. In addition they described its IR, PMR and MS spectra. Later the extraction of the compound 1-(2'-4'-dihydroxyphenyl)-3-(3''-4''methyleneedioxyphenyl) propane, also from the woody trunk of *I. polyanthera* and *I. tricomis*, was published by De Almeida *et al.* The results obtained by us do not agree with those of these last authors but do agree with those published by Morais *et al.* on the synthetic product.

Crystals of compound No. 2 (as well as of two 1,3-diarylpropanes) were obtained from the extracts of the woody trunk of *Iryanthera tricomis* collected in the Amazon region of Colombia. Once the crystals were separated we obtained some transparent, crystalline, needles from a solution of benzene and n-hexane (m.p. $373-374^\circ\text{K}$). We separated a monocrystal of acceptable quality whose dimensions were $0.10 \times 0.66 \times 0.02$ mm and mounted it on the goniometric head of the Enraf-Nonius CAD-4 automatic diffractometer using, as in compound No. 1, $K \alpha$ radiation from Mo.

The parameters were obtained by the same procedure as for the first compound, that is:

$$a = 41.01(1), \quad b = 4.842(3), \quad c = 14.11(3)\text{\AA}, \quad \beta = 93.28(8)^\circ, \quad V = 2.798 (7)\text{\AA}^3$$

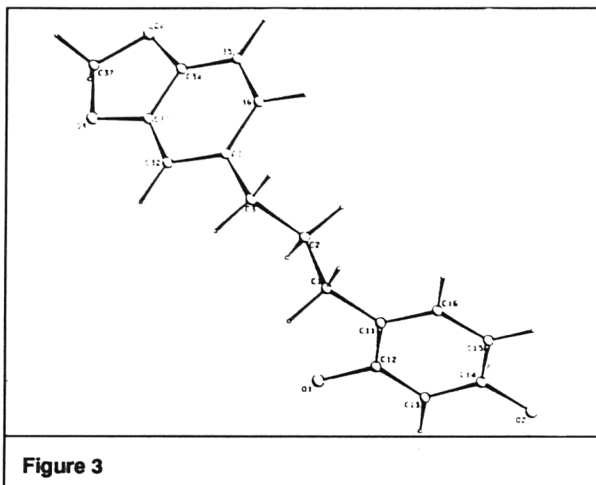
The symmetry is also monoclinic and the spatial group deduced from the systematic extinctions is $C2/c$. Here the number of molecules per unit cell is 8 ($Z = 8$), giving a calculated density of $D_x = 1.33 \text{ g cm}^{-3}$ for the composition $C_{16}H_{16}O_4 \cdot 0.5H_2O$ and molecular weight of 281.3. 2071 reflections were measured using the $\omega-2\theta$ technique of which 863 were considered observed [$I > 2.5 \quad (I)$]. The (h, k, l) range of the measured intensities is:

$$-11 \leq h \leq 11; 0 \leq k \leq 5; 0 \leq l \leq 13; \text{ for a maximum value of } 2\theta = 23^\circ$$

No degradation of the sample was observed during the measurement process. We corrected the intensities for Lorenz and polarization effects but not for absorption, given its low value ($\mu = 0.908 \text{ cm}^{-1}$). The structure was solved by direct methods using the MULTAN program.

The quality of the monocrystals available did not allow enough reflections to enable us to refine the temperature factors anisotropically. The hydrogen atoms of the water molecules were found by a differential Fourier synthesis, but it was not possible to locate the hydrogen atoms of the hydroxyl groups which were finally placed at the expected positions according to the group geometry. The final residual index was $R = 0.090$.

Figure 3 shows a molecule of compound no. 2 formed by pairs of diarylpropane molecules related by a binary axis of symmetry. They are bound through hydrogen bridges to the water molecule situated on the same binary axis of symmetry [$O_w \cdots O_2 = 2.77 (1) \text{ \AA}$]. There also exists a series of other important intermolecular contacts:



$2.67(1) \text{ \AA} \quad O1 \cdots O2' (x, 1-y, 0.5+y)$

$2.74(1) \text{ \AA} \quad O1 \cdots Ow'' (2-x, -y, 2-z)$

The phenyl rings are almost flat and the O_2 , O_4 , O_{37} atoms are found at -0.009 , -0.021 and -0.047 \AA outside the phenyl plane. The angle that gives the general geometry of the molecule, that is to say, the angle between the normals to the phenyl rings, is $100.3(9)^\circ$.

In summary, we have given exact descriptions of two new natural products isolated from plants of the Colombian flora. However, it should be pointed out that this is only a start to the enormous effort that would be involved in the purification and description with structural precision of the enormous quantity of compounds that can be found.

Studies on plant chemistry

***Murraya exotica* (Rutaceae).** A series of 7-methoxy-coumarins with a prenylated chain in different degrees of oxidation located on the 8th carbon of the coumarin nucleus have been isolated and characterized from the leaves of this species (Cuca Suárez and Delle Monache, 1991).

***Petiveria alliacea* (Phytolocaceae).** Two flavenols and a flavenol-ramnoside, dihydroquercetine, dihydrocamphorol and myricetine, and three new flavenoids whose assigned structures correspond to 6-formyl-8-methyl-7-O-methyl-pinocebrine, 6-hydroxymethyl-8-methyl-7-O-methyl-pinocebrine and 6-hydroxymethyl-8-methyl-5, 7-di-O-methyl-pinocebrine have been isolated and characterized from this species. (Delle Monache and Cuca Suárez, in press). In addition an oxygenated sesquiterpene and two new flavenoids have been isolated.

***Cespedezia spatulata* (Ochnaceae).** Terpenic substances such as friedeline, betulinic acid, stigmasterol and two new triterpenes have been found in the bark of this species, while from the leaves metabolites of the sesquiterpene type have been determined and are being studied by gas chromatography. In addition, work continues on the wood.

The genus *Clusia* (Guttiferae). This genus is the least studied of the Guttiferae and this work is to resolve plant chemistry and taxonomic problems. So far, five benzophenones, which are precursors of xanthenes and which present interesting chemical and spectroscopic problems, have been isolated and their structure determined, four of them being new. In addition, three triterpenes and ten sesquiterpenes have been found.

Solanum vestissimum D. (lulo). This is a fruit which is widely commercialised in Colombia and we have started a study of the precursors of the volatile compounds it contains. It seems that some of the main volatile substances of lulo, particularly linalool and some structurally related diole (Suárez *et al*, 1991) are found bonded to glycosides. Thus the phytochemical study of lulo has been orientated towards the isolation and structural elucidation of the glycoside fraction.

Chemical components of marine sponges

Didiscus osceata. Our group has isolated from this sponge the compound (+)- curcuphenol, which is a sesquiterpene, and has shown that it presents antimicrobial activity. It appears that the biological activity of the molecule derives from the presence of functional groups rather than the whole molecule. We have standardized methods of semisynthesis and purification of resulting compounds and have acetylated and oxidized (+)- curcuphenol with a view to carrying out biological tests.

Agelas conifera (Agelasidae). Studies on this species, whose taxonomy has long posed a problem to marine biology, have been focused on sterols and fatty acids, metabolites considered as chemotaxonomic indicators.

Ircinia campana. The chemistry of this species is of interest because of its biological activity and because of its characteristic foetid odour which we believe serves to discourage predators, prevents epibiosis or acts as a mechanism to overcome competition for space. We have thus started work on chemical composition studies with emphasis on the elucidation of the structure of volatile components.

Publications

Cuca Suárez, L.E. and Delle Monache, F. (1991). Constituents of *Murraya exotica* adapted in Colombia. *Revista Latinoamericana Química*, 22/1, 38-40.

Delle Monache, F. and Cuca Suárez, L.E. (1992). Flavanones from *Petiveria alliacea*. *Phytochemistry*, in press.

Matheus, M. de, Martínez, J.C., Rius, J. and Miravittles, C. (1991). Structure of 2',4'-dihydroxy-4',6'-dimethoxy-alpha, beta-dihydrochalcone. *Acta Crystallographica*, C47, 1744-6.

Matheus, M. de, Martínez, J.C., Rius, J. and Miravittles, C. (1991). Structure of 1-(2',4'-dihydroxyphenyl)-3-(3',4'-methylenedioxyphenyl) propane hemihydrate. *Acta Crystallographica*, C47, 1746-8.

Suárez, M., Duque, C., Wintoch, H. and Schreier, P. (1991). Glycosidically-bound aroma compound from the pulp and the peelings of lulo fruit (*Solanum vestissimum* D.). *Journal of Agricultural and Food Chemistry*, 39(9), 1643-5.

6 The extraction of copper by new complexing agents

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Contract number and duration: CI1*/0559, May 1990 to April 1993.

Background

This research project is an extension of the MSc programme in Chemistry established at the University of San Agustín (Arequipa, Peru) in 1984, part of the agreement of collaboration between this University and the University of Surrey (UK) signed in 1984. This academic link has resulted in 41 MSc graduates from different universities and industrial centres in the south of Peru in the 1984-89 period. These MSc graduates are now working in different academic and industrial institutions in Peru. It was thought that a logical extension of the MSc programme was to develop the research capability of a number of MSc graduates by training them in research areas relevant to the needs of the country. Thus, two MSc graduates of 1987, currently members of the academic staff of the Catholic University of Santa María, are carrying out research at the University of Surrey in the area of copper extraction, with the collaboration of the Royal College of Surgeons in Ireland and Imperial Chemical Industries. The Catholic University of Santa María, through consultation with the UK and the copper mining industry in Peru (Cerro Verde), is arranging a suitable infrastructure for the continuation of this research once the training of their staff in Europe is accomplished.

Objectives

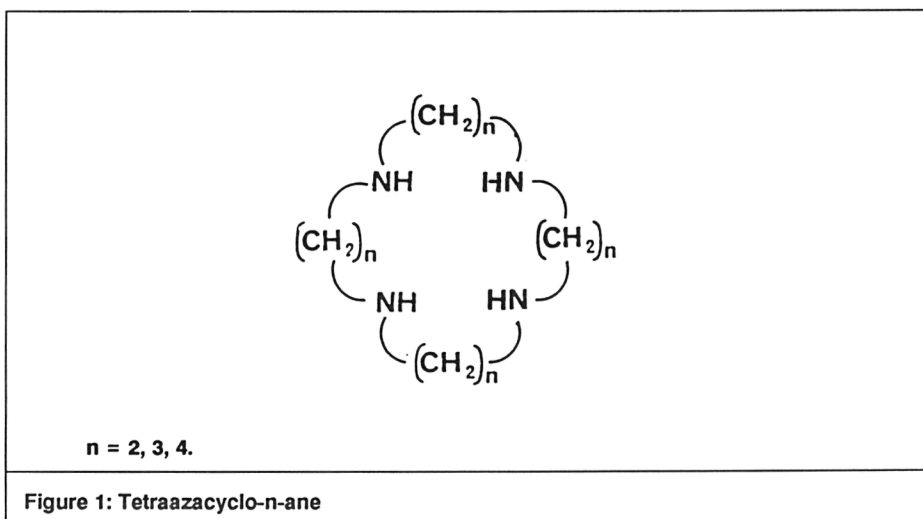
Solvent extraction technology has been successfully applied to the recovery and purification of metals. However, as worldwide pressures increase for reductions in existing limits of chemical pollutants in the environment, extraction processes that require the use of considerable amounts of organic solvents are likely to be replaced by those using new materials that are environmentally safer. Among such materials, special attention is drawn to the development of easily recyclable polymers containing ion-selective ligands as anchor groups. Undoubtedly, the discovery in the last two decades of synthetic macrocyclic ligands has opened the way for the development of a large variety of new materials for extraction purposes.

Methods used for the extraction of copper in Peru are based on the selectivity of oximes for copper. However, these oximes are known to bind aluminium (III), iron (II) and iron (III), all of which are found with copper (II) in ores.

Therefore, the main aims of the project are:

1. To investigate ligands which preferentially extract Cu(II) over Al(III), Fe(III) and Fe(II) from water into non-aqueous media. This step aims to formulate the basis for the selection of ligands to be used as anchor groups in polymers from distribution data for copper and other metal ions (Al^{3+} , Fe^{2+} , Fe^{3+}) between the aqueous phase and an organic phase containing the appropriate ligand (monomer).
2. The development, characterisation and extraction properties of new polymeric materials containing copper selective ligands as anchor groups.

Materials and methods



Complexing agents for copper: Recently a new group of macrocyclic ligands known as polyazas have been synthesised (Figure 1).

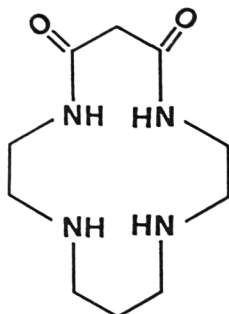


Figure 2: 1,4,8,11-Tetraazacyclotetradecane-12,14-dione

These ligands are able to complex with transition-metal cations. Among the tetraazacompounds, the 1,4,8,11-tetraazacyclotetradecane (Figure 2) shows selectivity for the copper (II) ions. The stability of the complex formed depends on the pH of the solution. By incorporating substituent groups in the external structure of the ligand (in the methylenic group: $-\text{CH}_2-$), the solubility properties can be modified without affecting the complexing properties of the ligand. Thus, the introduction of lipophilic chains in the structure of tetraazacompounds increases their solubility in organic solvents and decreases their solubility in water, making these ligands suitable for extraction purposes. We have recently synthesised the tetraazacompound known as 1,4,8,11-tetraaza-13 [dodecyl] cyclotetra-decane 12,14-dione (Figure 3).

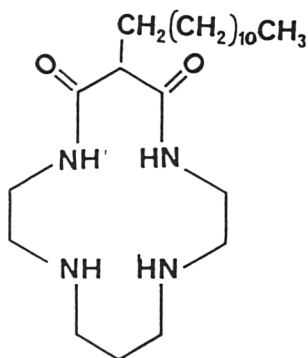


Figure 3: 1,4,8,11-Tetraaza-13-[dodecyl]-cyclotetradecane-12,14-dione

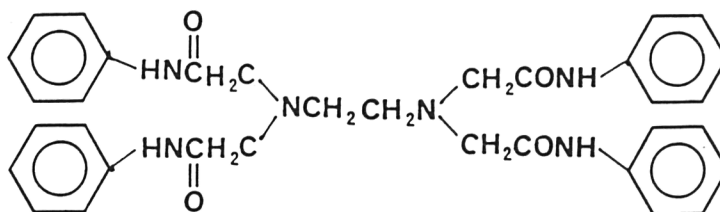


Figure 4: Tetraaniline ethylenediaminetetraacetate

Other ligands that are currently investigated are the amides of ethylenediamine tetraacetate. Figure 4 shows the structure of the ligand recently synthesised following the procedure suggested by Nolan *et al.* The experimental work detailed below is referred to the extraction process using the tetraaza compounds as complexing agents.

Qualitative tests to assess the capability of extraction of the ligand(III) and its recovery:

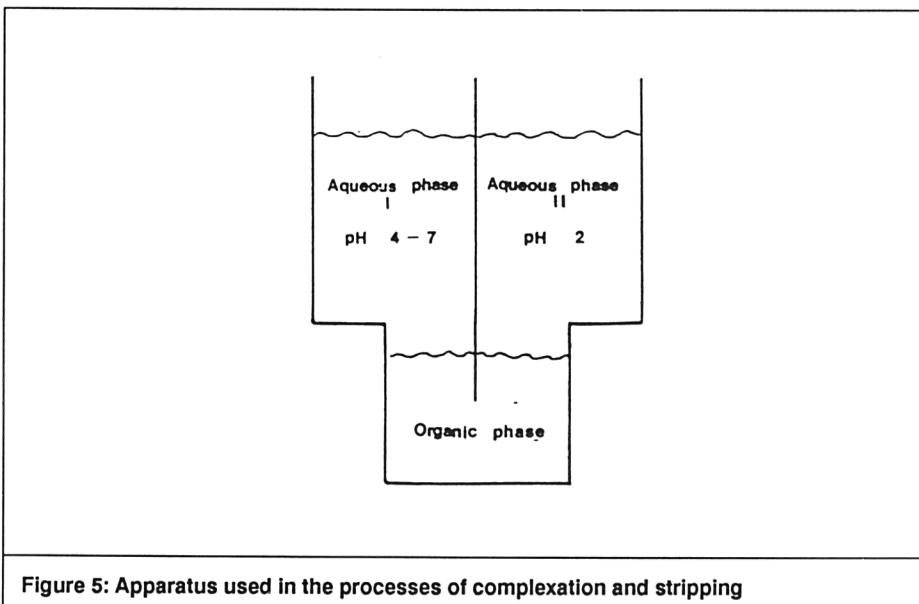
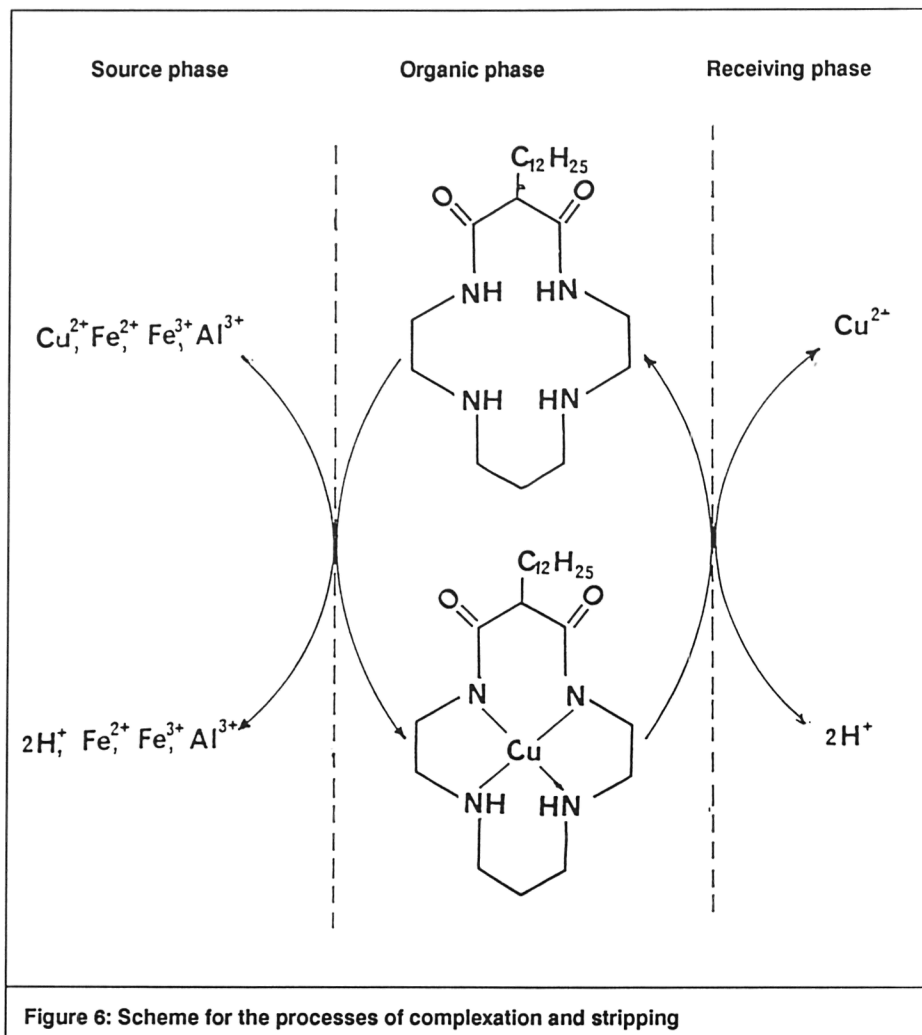


Figure 5: Apparatus used in the processes of complexation and stripping

The apparatus designed for these purposes is shown in Figure 5 in which the source is an aqueous solution of a copper salt at different pH (the range used depends on the ligand). For ligand (III) this range was varied from 3 to 10. The organic phase (different solvents all which are immiscible with water) contained the ligand. The receiver (stripping process) is an aqueous solution at the appropriate pH. The same procedure was used for other cations which are found with copper in ores. A systematic representation of the process taking place is given in Figure 6.



Distribution ratios. For the distribution experiments, equal volumes of water (presaturated with the organic solvent) containing the metal ion and the organic solvent (presaturated with water) containing the ligand were used. The pH of the aqueous solutions was adjusted to the appropriate value with the help of a pH meter. The ionic strength was kept constant in all cases. The mixtures were left to equilibrate at 298.15°K for two hours. Then the phases were separated carefully. Aliquots of the organic and aqueous solutions were taken in order to determine the amount of the metal ion in each phase. The samples were analysed by atomic absorption spectrophotometry and potentiometry using the appropriate ion selective electrode.

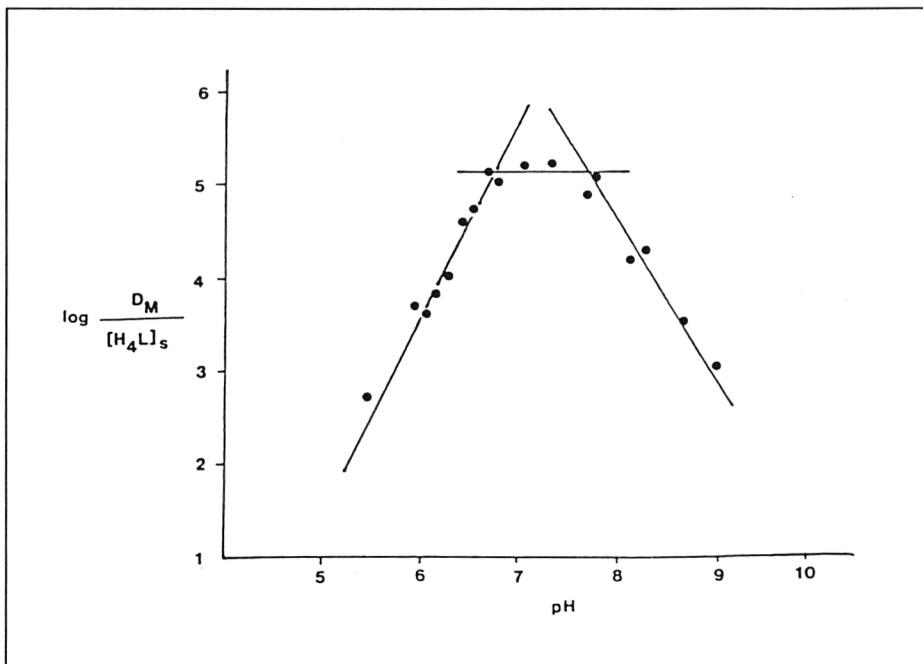


Figure 7: Plot of $\log DM/[H_4L]_s$ vs the pH of solution

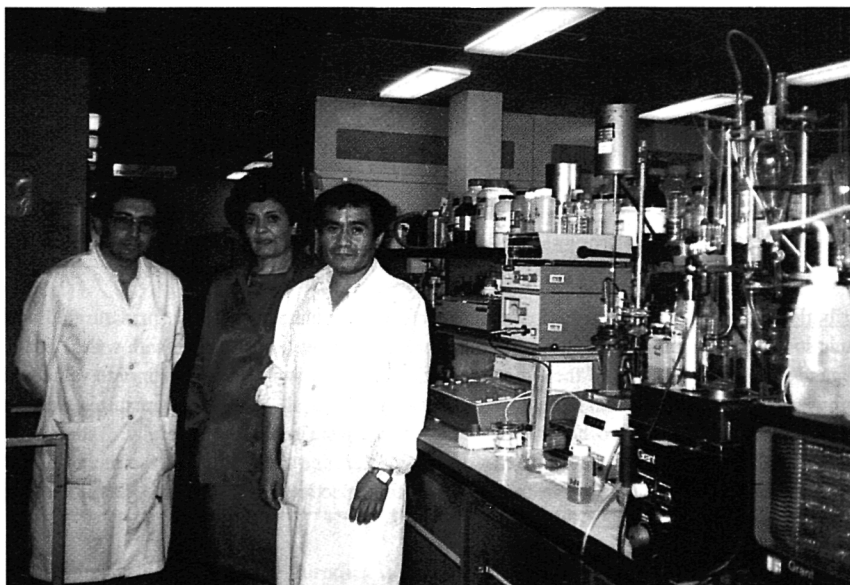


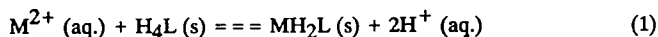
Figure 8: The equipment used for project at University of Surrey. Alongside, (l. to r.) are J.C. Garcia (U.C.S.M.,Peru), A.F. Danil de Namor and J.S. Velarde (U.C.S.M.,Peru)

Results

A representative example is given for the ligand shown in Figure 3, using water-chloroform as the solvent system. The results from qualitative tests indicated that in this solvent system:

1. the ligand is selective for copper;
2. the rate of the extraction process is very fast;
3. the ligand can be easily recovered;
4. the pH range for the optimal extraction of copper was from 6.3 to 6.8. At this pH other cations (Fe^{2+} , Fe^{3+} and Al^{3+}) are not extracted to any significant extent.

From the qualitative tests, the following overall process for the extraction of metal cations (M^{2+}) from water (aq.) to the nonaqueous phase (s) containing the ligand H_4L was suggested. This is represented by the following equation:



The equilibrium constant for process (1), expressed in terms of molar concentration (mol dm^{-3}) is

$$K_{\text{ML}} = \frac{[\text{MH}_2\text{L}] (\text{s}) [\text{H}^+]^2 \text{aq}}{[\text{M}^{2+}] \text{aq.} [\text{H}_4\text{L}]_s} \quad (2)$$

the partition (or distribution constant), D_{M} , for the metal ion is defined by

$$D_{\text{M}} = \frac{[\text{MH}_2\text{L}]_s}{[\text{M}^{2+}] \text{aq.}} \quad (3)$$

Combination of eqs. (2) and (3) leads to the following relationships which expressed in terms of logarithms can be written as

$$\log \frac{D_{\text{M}}}{[\text{H}_4\text{L}]_s} = \log K_{\text{ML}} + 2\text{pH} \quad (4)$$

or

$$\log \frac{D_{\text{M}}}{[\text{H}^+]^2} = \log K_{\text{ML}} + \frac{[\text{H}_4\text{L}]_s}{4} \quad (5)$$

From these equations, K_{ML} can be evaluated from changes in the concentration of the hydrogen ions, eq. (4) or the ligand, eq. (5). Experimental data to assess the validity of eq. (4) are shown in Figure 7 where a plot of $\log D_{\text{M}}/[\text{H}_4\text{L}]_s$ vs the pH of the solution is presented. According to these results, processes are taking place as a result of changes in the pH of the solution. These results are now being analysed and will be published shortly. The data so far obtained are very encouraging. In addition, a method is being considered for selecting the best solvent to be used for extraction, based on enthalpy data for the dissolution of the ligand in each solvent. It is expected that the second stage of the project, which involves the incorporation of highly selective ligands into polymeric frameworks, will be initiated shortly.

7 Study of transfer of electrons and ions at solid-solid and solid-gas interfaces. Application to heterogenous catalysis: study of selective oxidation reactions on supported catalysts

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Contract number and duration: Cl1*/0573, April 1990 to March 1994.

Introduction

Laterites are residual products derived from a wide variety of rocks by intensive chemical weathering under strongly oxidising and leaching conditions. Generally, the main constituents of these residues are Ni, Fe, Ti, Si, V, Zr and Al, present in the form of hydroxides and/or oxides. The nature and chemical composition of laterite depend on its place of extraction and its particle size.

So far, three different types of laterites have been reported: ferruginous, bauxitic and nickel-type. These names are given taking into account the chemical element that predominates in the laterite, e.g. in bauxitic laterites, aluminium. The chemical constituents can be present in the laterites in different crystallographic forms. Iron can be found as haematite (Fe_2O_3) and/or goethite α - FeOOH . Aluminium can be found as gibbsite (α - $\text{Al}(\text{OH})_3$) and/or boehmite (γ - AlOOH) and/or diaspore (α - AlOOH). Silicon can be present either in colloidal form or quartz, which is the most common crystallographic form. Titanium can be found in these lateritic materials as TiO_2 in anatase and/or rutile crystallographic forms.

Being available in abundance in Venezuela, this naturally-occurring material is considered as a potential candidate as a good catalyst. This potentiality can be inferred from the presence of oxides with some surface acidity or basicity, in which catalytic activity is recognised. Another reason to consider the lateritic material as a catalyst is the presence of some chemical elements like iron which is active in hydrodesulphurisation to some extent. In this respect, several researchers have pursued studies to use laterites as hydrocracking and hydrodemetallation HDM catalysts with excellent results.

In the present work, we have studied several samples of laterites extracted from three Venezuelan regions: Los Pijiguao-Edo Bolivar, Corozal-Edo Guaricao, Mérida-Edo Mérida.

The characterisation of these samples, both in their natural state and once they have undergone thermal treatments, is reported here. As is well known, thermal treatments can often modify the catalyst architecture by increasing surface area and pore volume. Thermogravimetric Analysis (TGA), Thermal Differential Analysis (TDA) and X-ray Photoelectron Spectroscopy (XPS) have been

employed for this characterisation. So, a partial study of the analysed samples is presented here and in the future, a more detailed and quantitative study will be undertaken based on a full exploitation of XPS data together with measurements obtained by other analytical techniques.

Materials and methods

A set of Venezuelan laterites (LV) has been chosen for this study. The samples were ground and sieved in order to obtain a particle size of less than 150 microns. Each sample was analysed in the natural state (NS) using XPS and TGA and also after calcination up to 500°C under air (AC), again by XPS.

The thermogravimetric measurements were performed in a Sartorius microbalance with an oxygen-helium mixture as gas vector. The XPS measurements were taken with an AEI ES200 B spectrometer at University of Lille I, France. Non-monochromatic Al K α radiation was used as X-ray source, with a constant power of 300 w during the XPS measurements; the hemispherical electrostatic dispersive analyser was operated in the $\Delta E/E = \text{constant}$ mode. Vacuum in the spectrometer was always better than 10^{-7} torr.

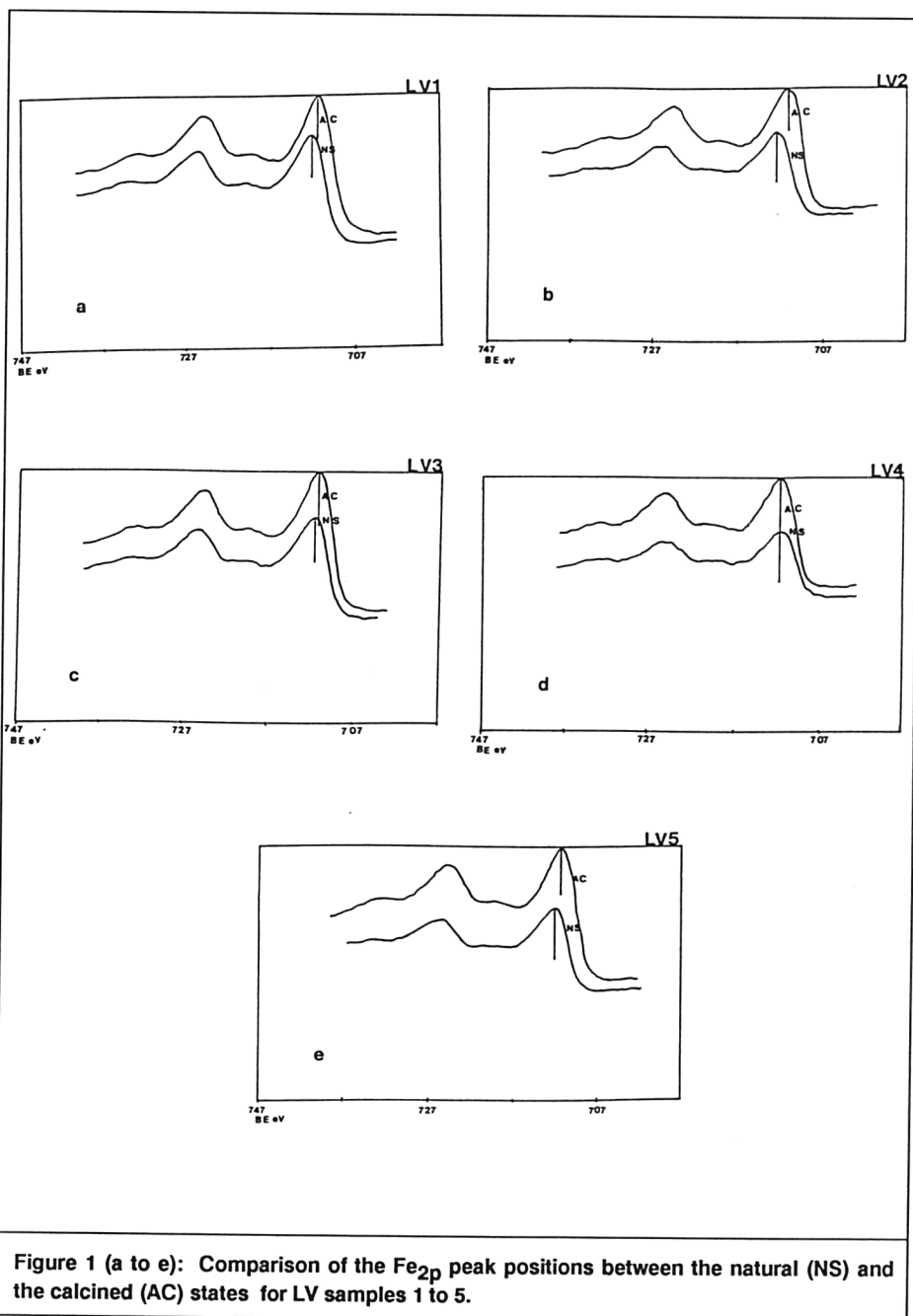
Results and discussion

X-ray photoelectron spectroscopy (XPS): In order to identify the chemical state of the samples that were analysed it is extremely important to determine the binding energies and the possible chemical shifts for different species. To do this, it is mandatory to have a suitable XPS energy reference. The C_{1s} electron binding energy from adventitious carbon is widely used for this purpose. Based on a systematic study of the binding energies for the different chemical elements present in the samples under investigation it was concluded that, for all the LV samples (LV1, LV2, LV3, LV4 and LV5), the binding energy of the C_{1s} level (285.0 eV) from adventitious carbon is a good reference and allows coherence in the interpretation of the data.

However, it has sometimes been found that the use of this reference energy is inconvenient. When one is working on catalysts, the C_{1s} level can present a chemical shift as a consequence of chemical transformations suffered by the carbonaceous species. In general, it is recommended to use an internal reference associated with the sample when this is possible. For the ULA samples, it was decided to take, as a reference, the binding energy of the Si_{2p} level (103.8 eV) since its FWHM of 2.3 eV allows inference of the presence of only one silicon species. On the other hand, the carbon present in the catalyst ULA1 that was used is not from hydrocarbon contamination; thus it is inadequate to assign it a binding energy of 285.0 eV.

The presence of a charge effect greater than 3 eV has been observed when comparing the binding energy of C_{1s} electrons from hydrocarbon contamination on natural lateritic samples and on gold foil. In these samples, however, the charge effect tends to decrease after the sample has been in vacuum and under irradiation for some time. For the calcined samples, the charge effect is greater than 4.5 eV and with the same tendency as natural samples. This large charge effect could be due to the structure or/and to water elimination.

To study the evolution of the chosen samples as a function of the temperature in high vacuum, two samples have been selected: sample LV3 which contains aluminium, iron and silicon and sample LV4 which contains only aluminium and iron. The conductivity of the LV3 sample tends to increase significantly when the temperature increases. The charge effect decreases by 2.5 eV when going from room temperature to 500°C. LV4 sample shows no evolution of the charge effect. This could be correlated to the absence of silicon in the LV4 sample.



Sample	Natural State (NS)	Calcined State (AC)
LV1	$\text{Fe}_{0.52}\text{Al}_1\text{Si}_{0.51}\text{O}_{4.64}$	$\text{Fe}_{0.53}\text{Al}_1\text{Si}_{0.55}\text{O}_{3.91}$
LV2	$\text{Fe}_{0.30}\text{Al}_1\text{Si}_{0.80}\text{O}_{4.47}$	$\text{Fe}_{0.36}\text{Al}_1\text{Si}_{0.85}\text{O}_{4.02}$
LV3	$\text{Fe}_{0.41}\text{Al}_1\text{Si}_{0.48}\text{O}_{4.28}$	$\text{Fe}_{0.56}\text{Al}_1\text{Si}_{0.50}\text{O}_{3.74}$
LV4	$\text{Fe}_{0.11}\text{Al}_1\text{Si}_{0.02}\text{O}_{2.77}$	$\text{Fe}_{0.14}\text{Al}_1\text{Si}_{0.02}\text{O}_{2.08}$
LV5	$\text{Fe}_{0.45}\text{Al}_1\text{Si}_{1.00}\text{O}_{5.25}$	$\text{Fe}_{0.58}\text{Al}_1\text{Si}_{1.02}\text{O}_{4.88}$

Table 1: Surface stoichiometry obtained from XPS results

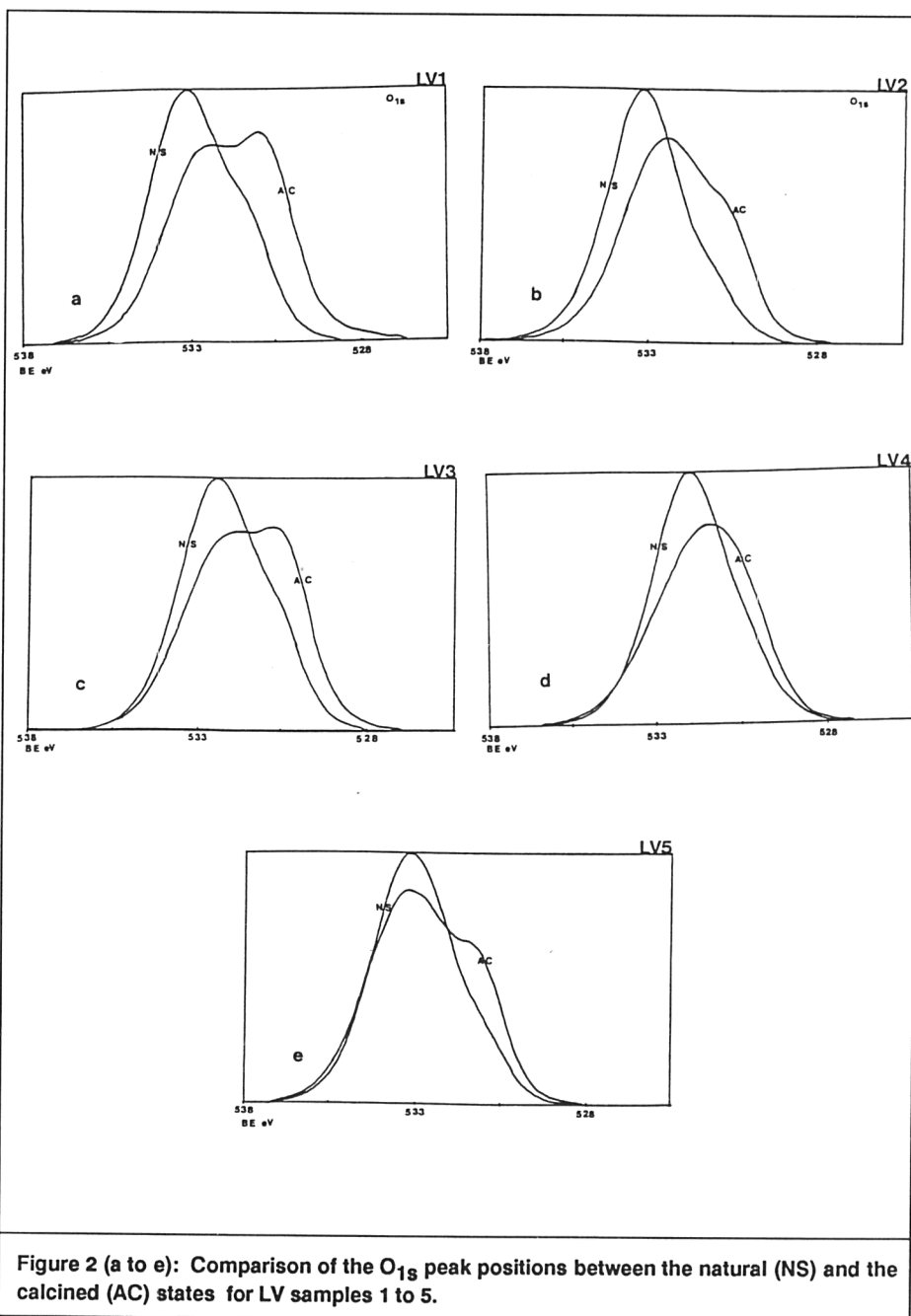
The surface stoichiometry obtained from the XPS results is reported in Table 1 for the LV samples in their natural state and after calcination. Various concentrations of Fe, Al and Si are present in the samples studied. The LV4 sample contains no Si and only a low quantity of Fe. The Si increases according to the following sequence: LV4 < LV3 ~ LV1 < LV2 < LV5; this classification does not vary after calcination. For Fe, the content increases according to the following classification: LV4 < LV2 < LV3 < LV5 < LV1. After calcination, all the samples present a slight increase of the Fe amount except the LV1 sample for which the Fe concentration does not change. It is noted that the O concentration presented corresponds to the total oxygen species analysed. This means that all the oxygen species from O^{2-} to OH^- or H_2O are taken into account. In particular, in the natural state of the samples, some large quantities of H_2O must be present.

In what follows, the XPS data have not yet been fully evaluated and the discussion tends to be qualitative. It was partially supported by results obtained by calculations from first principles. Values were obtained for the binding energies of levels $\text{Fe}_{2p_{3/2}}$, Fe_{3p} , Si_{2s} , Al_{2s} and Al_{2p} for all the samples under study both in their natural state and in the calcined state (see Table 2). The aluminum Al_{2p} , Al_{2s} and silicon Si_{2s} peaks did not shift in energy within the experimental error of about $\pm 0.2\text{eV}$. On the other hand, except for the LV4 sample, a shift towards lower binding energies was observed for the $\text{Fe}_{2p_{3/2}}$ peak after calcination. The shift varied from 0.6 to 1.3 eV (see Figures 1a to 1e). This shift can be explained, as reported by studies of McIntyre, by assuming a dehydroxylation process of FeOOH ($\text{Fe}_{2p_{3/2}} - 711.85\text{eV}$) into Fe_2O_3 ($\text{Fe}_{2p_{3/2}} - 711\text{eV}$): the iron oxidation state remains equal to +3.

In the LV4 sample the $\text{Fe}_{2p_{3/2}}$ level has no shift after the thermal treatment. The binding energy obtained, $711.1 \pm 0.2\text{eV}$, corresponds to the binding energy of the $\text{Fe}_{2p_{3/2}}$ level in Fe_2O_3 -like species according to McIntyre. For this sample it was assumed that Fe is not hydroxylated in the natural state.

Lastly, a general increase of the line width was observed after calcination, that means the samples undergo structural modifications involving the presence of several environments for the different species.

In Figures 2a to 2e the O_{1s} peaks are depicted. For natural samples, the O_{1s} peak maximum is situated around $533 \pm 0.5\text{eV}$, except for the LV4 sample, for which the O_{1s} peak is located at about 532eV . All peaks present a shoulder at 530eV . After calcination a large evolution of the profile of the O_{1s} peaks can be observed, two maxima appear at about 533 and 530eV . Again, LV4 sample behaves differently, since there is only one maximum at 531.2eV . All the peaks exhibit wide profiles indicating the presence of several oxygen species. Based on the composition of the samples (Fe, Al and Si), several types of oxygen species can be proposed: for instance one type bonded to Si under the form of SiO_2 , another type bonded to Al forming, presumably, Al_2O_3 , one type bonded to Fe in the form of



Fe_2O_3 ; the possibility of getting an oxygen bonded to Fe forming FeOOH and another one bonded to water can not be eliminated. The assignment of binding energies for the different species as well as for pure oxides and hydroxides, taken as references, appears in Table 3.

The component at 533 +/- 0.5 eV may be assigned to oxygen either in H_2O or SiO_2 . The decrease of this component after calcination is associated with the loss of water. The remaining component is then assigned to oxygen in SiO_2 -like species, and its strength can also be related to the amount of Si present in the respective samples. The component situated at 530 eV is related to oxygen in iron oxide-like species and agrees with O^{2-} ions of the network. The increasing intensity of the component on the calcined samples is due to the loss of OH^- groups bound to Fe and its relative intensity can be related to the amount of Fe. The position of the intermediate components (531.2 and 532 eV range) is related to oxygen bound to Al, and it must be noted that the energy positions of hydroxyl groups and oxygen bounded to Al are very close. So it is rather difficult, at this stage of the study, to specify the energy positions and relative intensity of these species in laterites. Nevertheless, starting from the particular LV4 sample (without Si) a structural transformation from gibsite ($\text{Al}(\text{OH})_3 \cdot \text{O}_{1s} = 532.2$ eV) to alumina ($\text{Al}_2\text{O}_3 \cdot \text{O}_{1s} = 531.6$ eV) is expected as a shift is observed in the O_{1s} spectra.

Concerning the ULA samples, the first XPS results obtained are presented in Table 2. These samples are characterised by a high Fe content and a low Si content. In the natural state (NS) the binding energy of $\text{Fe}_{2p_{3/2}}$ level is 710.2 eV. This intermediate value obtained between Fe_{3+} (711 eV) and Fe_{2+} (709 eV) as well as the large width at half maximum (6.2 eV) suggest that the ULA samples correspond to either a mixture of these two species or to Fe in an ill-defined environment. If we compare the $\text{Fe}_{2p_{3/2}}$ energy for the natural state with the $\text{Fe}_{2ps_{3/2}}$ energy for the ULA (AT) after being present as a catalyst in a gas synthesis reaction we observe a shift towards 709.1 eV. This value characterises Fe_{2+18} , its environment is still ill-defined as the peak width remains large. If the O_{1s} spectrum is now considered, two species are obtained for the NS: 533 eV (oxygen in SiO_2 -like species) and 530.2 eV (oxygen in Fe oxides). After catalytic test (AT), the former remains unchanged but the latter gives rise to an additional species at 528 eV. At this stage of the study more information is needed to explain this result. A calcination at 700°C under Air Flow (AC) seems to regenerate the initial state (NS) for Fe species as well as for O species.

Thermogravimetric analysis: The weight loss as a function of the temperature under a flow of helium and oxygen is shown in Figure 3 for the different samples of Venezuelan laterite. LV4 sample exhibits the highest weight loss of about 27% in the temperature range from 200°C to 600°C. The other samples experience a weight loss which depends on the Si content in the same temperature range. During this thermal treatment, it can be observed that up to 200°C, laterites dehydrate, losing weight due to loss of absorbed water and without exhibiting significant changes. Around 250°C LV4 compounds shows an appreciable weight loss attributed to the transformation from gibsite to boehmite and from boehmite to χ -alumina. Between 400°C and 500°C an additional weight loss is observed which can be attributed to the complete transformation from boehmite to χ -alumina.

Differential thermal analysis (DTA): The DTA results obtained on the LV2, LV3 and LV4 laterite samples are presented in Figure 4. Two endothermic peaks are observed at 250°C and 450°C attributed to the phase transformation from gibsite to boehmite and from boehmite to χ -alumina respectively. The results obtained are in good agreement with TGA data, but all the samples will be analysed and compared to X-ray powder diffraction studies which are currently being performed.

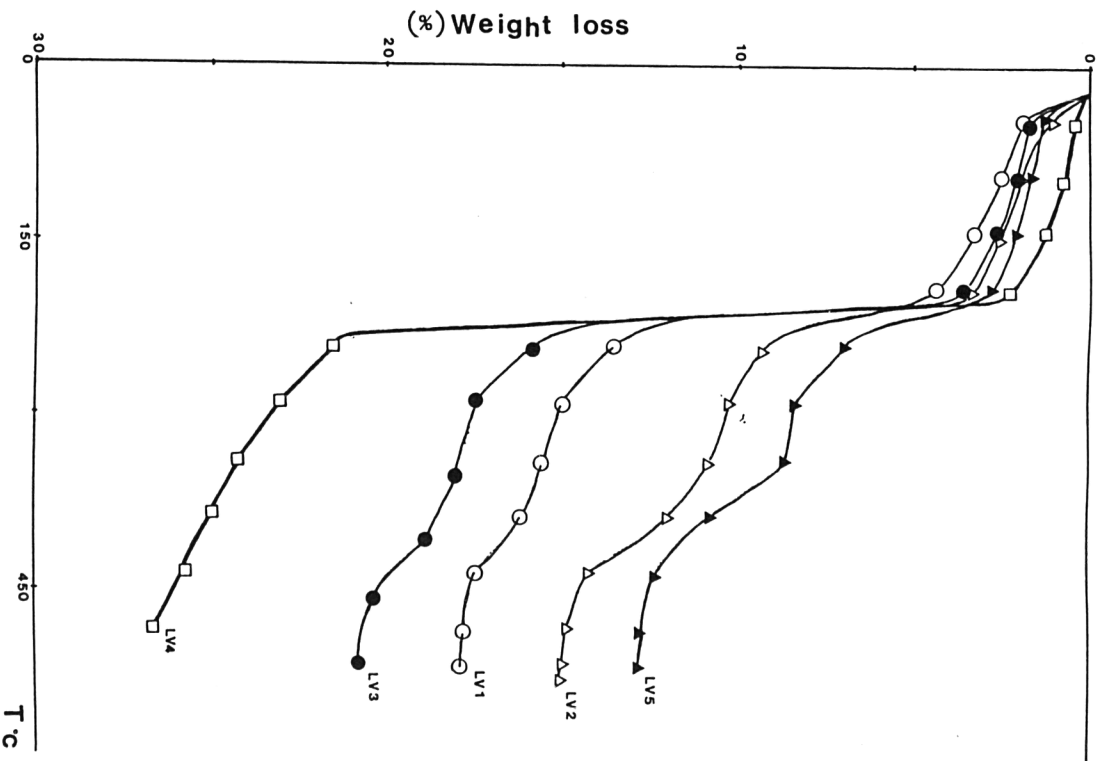


Figure 3 : Thermogravimetric analysis (TGA) of weight loss as a function of the treatment temperature under helium and oxygen for the LV samples.

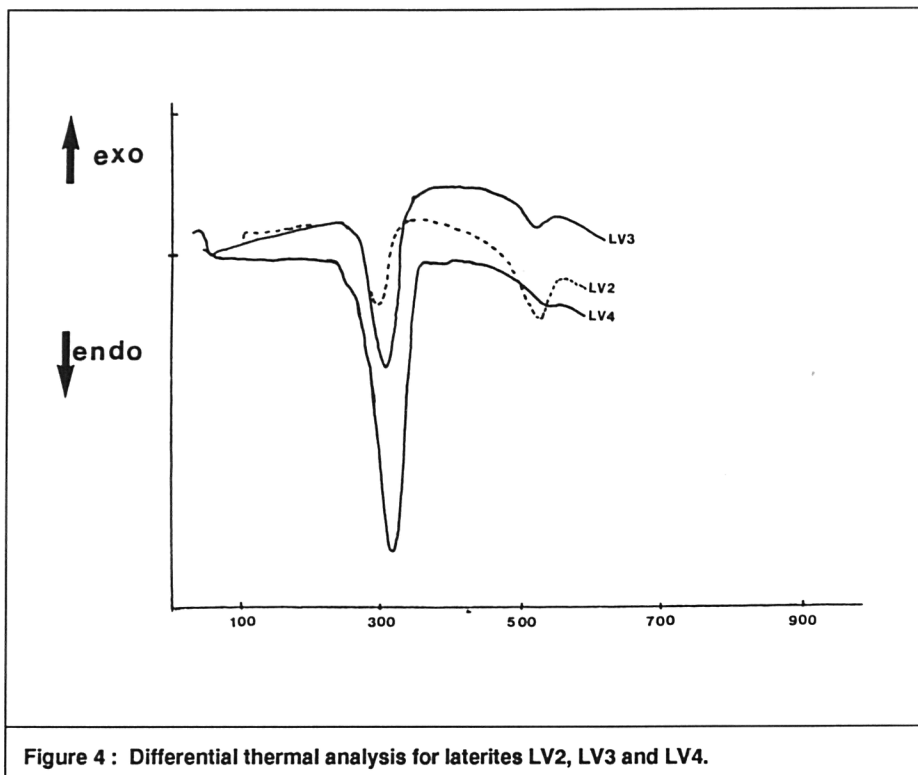


Figure 4 : Differential thermal analysis for laterites LV2, LV3 and LV4.

Conclusion

The XPS results allow characterisation of the surface composition of natural laterites and the structural transformations they undergo during thermal treatment. The surface composition remains unchanged if the Si/Al ratio is considered, but presents a slight increase of the Fe/Al ratio and in a large extent a decrease of the O/Al ratio (loss of water, dehydroxylation). The structural transformations may be put forward considering the $Fe_{2p_{3/2}}$ shift (transformation from goethite to haematite) and the increase of all photoelectronic line widths.

The TGA and DTA results specify the range of temperature for which the transformations occur. Two endothermic phenomena appear at 250°C and 450°C respectively and are related to the weight loss observed in the TGA experiments. In this range of temperatures, according to the literature, phase transformations from gibbsite to boehmite and then to γ -alumina take place.

Further analysis of XPS data is needed in order to obtain more information about the phase transformations that occur in samples subjected to thermal treatments. These results will be correlated with the information obtained by using other techniques like X-ray diffraction.

Sample	Condition	Al _{2p} (eV)	Al _{2s} (eV)	Si _{2s} (eV)	Fe _{2p3/2} (eV)	Fe _{3p} (eV)
LV1	NS (FWHM)	74.5 (2.2)	119.5 (2.5)	154.4 (2.9)	712.2 (6.0)	56.2 (3.3)
	AC (FWHM)	74.7 (2.6)	119.5 (3.1)	154.1 (3.2)	711.1 (6.0)	55.9 (3.5)
LV2	NS (FWHM)	74.8 (2.1)	119.8 (2.5)	154.3 (2.8)	712.2 (6.0)	56.2 (3.5)
	AC (FWHM)	74.7 (2.8)	119.8 (3.1)	154.3 (3.1)	710.9 (6.4)	55.7 (3.6)
LV3	NS (FWHM)	74.6 (2.2)	119.4 (2.6)	154.3 (2.9)	711.5 (5.6)	56.2 (3.4)
	AC (FWHM)	74.4 (2.6)	119.1 (3.0)	153.9 (3.3)	711.0 (6.2)	55.7 (3.6)
LV4	NS (FWHM)	74.3 (2.1)	119.3 (2.6)	- -	711.5 (5.6)	55.9 (3.4)
	AC (FWHM)	74.6 (2.3)	119.3 (2.8)	- -	711.3 (6.0)	55.9 (3.5)
LV5	NS (FWHM)	74.9 (2.2)	119.6 (2.5)	154.2 (2.9)	711.9 (5.8)	56.1 (3.4)
	AC (FWHM)	75.1 (2.8)	119.9 (3.2)	154.0 (3.2)	711.1 (6.2)	55.3 (3.4)
ULA2	NS (FWHM)	- -	- -	154.6 (3.1)	710.2 (6.2)	55.2 (4.1)
ULA1	NS (FWHM)	- -	- -	154.6 (2.9)	709.1 (6.2)	53.9 (4.0)
	AC*	- -	- -	154.6 (2.9)	710.1 (7.0)	55.1 (4.0)
	(FWHM)	- -	- -	(2.9)	(7.0)	(4.0)
NS : Natural Sample; AC : After Calcination at 500°C; AC* : After Calcination at 700°C. AT : After Test; FWHM : Full Width at Half Maximum.						
Table 2: Binding energies for different levels on the LV samples with respect to C_{1s} (285.0 eV) from adventitious carbon and on the ULA samples with respect to Si_{2p} (103.8 eV).						

Sample	O _{1s} (Si) (eV)	O _{1s} (Al) (eV)	O _{1s} (OH) (eV)	O _{1s} (Fe) (eV)
SiO ₂	533.2 533.1	- -	- -	- -
Al ₂ O ₃	- -	531.7 531.6 +.2/- .1	- -	-
Fe ₂ O ₃	-	-	531.4	529.8 +.5/- .5
Al(OH) ₃	-	-	532.2	-
Á-FeOOH	-	-	531.4	529.3
Fe(OH) ₃	-	-	531.5	-
Fe ₂ O ₃ + Al ₂ O ₃	-	531.5	-	530.0

Figures in italics are literature results, from various sources, for comparison with the present work.

Table 3 : Binding energies for different types of oxygen O1s for the samples containing Fe, Al, Si.

8 Insertion of reactive intermediates in tellurium and selenium compounds

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Contract number and duration: Cl1*/0574, May 1990 - April 1993

Background and objectives

This project is based on research work developed over a period of ten years by the group of L. Torres on compounds that contain selenium and tellurium. In particular it is concerned with:

1. The reactions of insertion of carbenes, dihalocarbenes, diacetylides and dinitrides in the bonds Se-Se and Te-Te of dialkyl and diarylditelluride and the corresponding compounds of selenium.
2. The reactions of insertion of carbenes and dihalocarbenes in the bonds Te-X and Se-X (X=halogen) of dialkyl and diaryldihalotelluride and dialkyl and diaryldihaloselenide.

The project also involves the crystallography group of the Autonomous University of Barcelona. Since its formation, this group has worked on the characterisation of new compounds through the use of spectroscopy and X-ray diffraction techniques. The third participating group, directed by G. Germain, has specialised in the determination of crystalline structures.

In this synthetic-structural complex the first objective of the project is to synthesise and to characterise new compounds which contain two atoms of Se or of Te in their molecules. The compounds with which the project will start are the bis(aryltelluro)ethyne (some of which have already been obtained). Later the synthesis of the corresponding derivatives of selenium will be tackled.

A second objective of the project is an understanding of the reactivity exhibited by these compounds.

Materials and methods

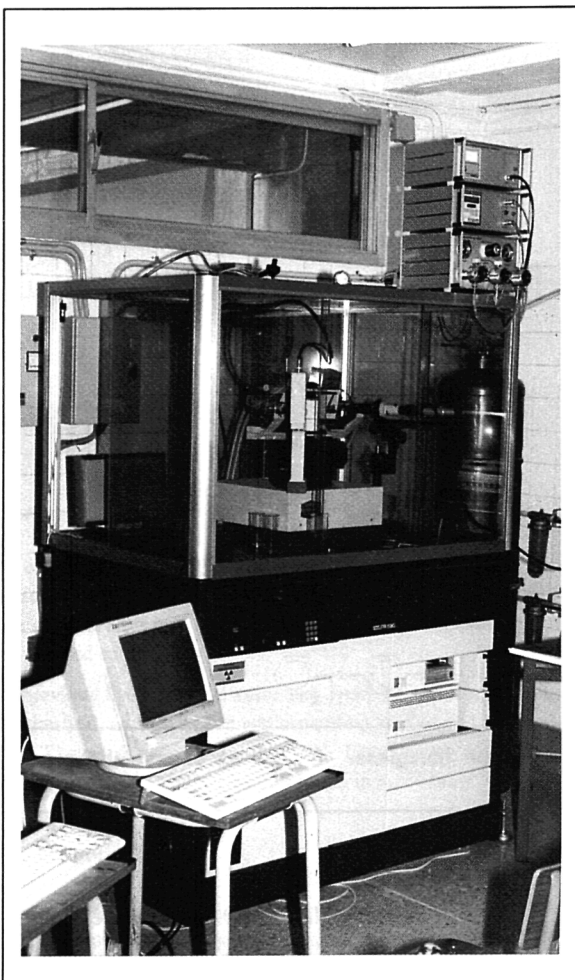


Figure 1: General view of automatic four-circle diffractometer

The synthesis work is carried out initially in Colombia and uses the materials typical of a synthesis laboratory. Techniques employed include, particularly, those which use vacuum lines to work with products that are sensitive to air. In the future it is intended to do part of the synthesis work in Barcelona. The start-up of that activity will coincide with the planned visit of L. Torres to Barcelona in 1991.

The spectroscopic part of the project will be carried out in Barcelona, the principal technique to be used is nuclear magnetic resonance. This will be done on a 400 MHz Bruker machine able to stimulate resonance in selenium and tellurium.

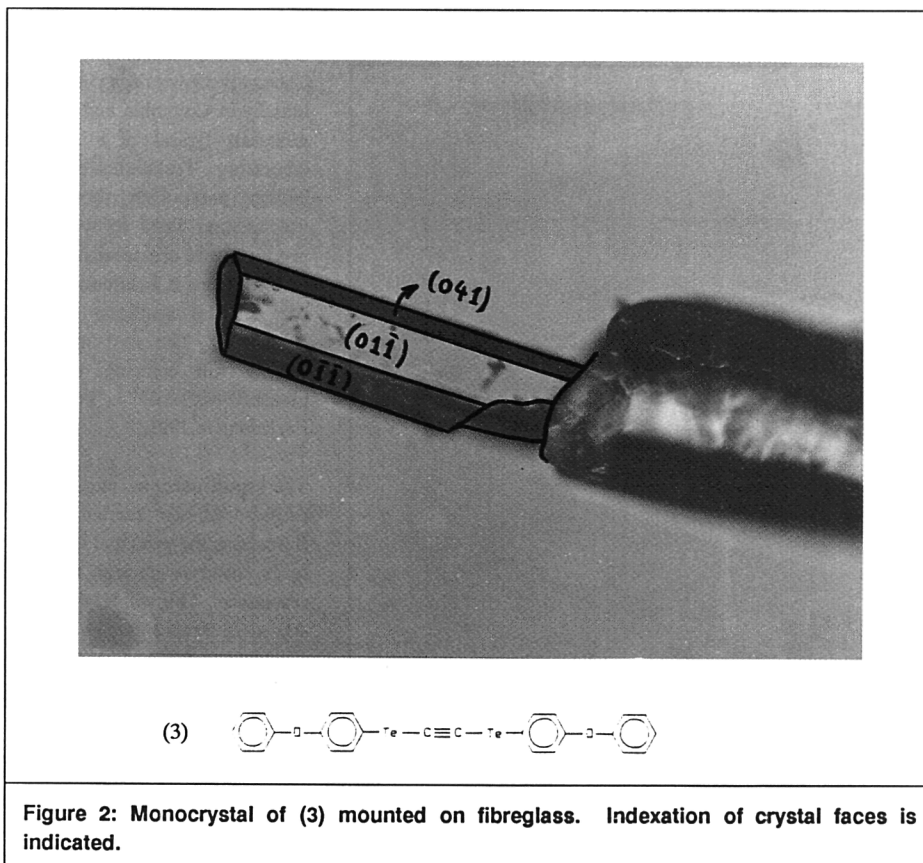
For the crystallography part, monocrystals will be obtained in Bogota, or exceptionally in Barcelona. The X-ray diffraction spectra will be recorded principally on an automatic diffractometer, Enraf-Nonius CAD4, of the University of Barcelona (see Figure 1).

The crystal structures will be resolved mainly in Belgium using a RISC-type IBM workstation operated by G. Germain.

Results

So far the following Te compounds of the form Ar-Te-C \equiv C-Te-Ar have been synthesised; Ar represents C₆H₅-, p-Me₂NO₆H₄-, p-C₆H₅OC₆H₄-, p-MeC₆H₅-, 2-C₁₀H₇-, 1-C₁₀H₇-.

From the point of view of characterisation, work is aimed at obtaining and studying spectroscopic data. On the crystallographic side, there are problems in obtaining monocrystals of these compounds which are suitable for study by monocrystal X-ray diffraction (see Figure 2). So far four monocrystals have been tried of which only two have been acceptable. The X-ray diffraction spectrum has been recorded and one crystal structure has been resolved.



Publication

de Matheus, M.; Torres, L.; Cabiativa, C.; Fuertes, A. and Miravittles, C. (1991). Structure of Bis-(*p*-methoxyphenyltelluro)ethyne. *Acta Crystallographica C* 47. 977-80

Postdoctoral fellowships**E. Castellanos Borrero**

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Catalysis for gasoline production with zeolites

Fellowship period: June 1990 - May 1991

Summary

In cooperation with the German Institute of Petroleum Research a de-asphalting method has been tested which eliminates asphaltenes and resins with only one solvent. The metals and basic nitrogen are eliminated from heavy oils and atmospheric distillation residues so that these de-asphalted oils can be cracked catalytically.

A previous hydrothermal screening (part I) optimised the testing of the best zeolites Y and ZSM-5 synthesised, as well as the corresponding zeolite Y with lanthanum. In this way the microactivity test (MAT) was done with 2 zeolites Y, 12 zeolites ZSM-5 and one LaY zeolite. For this purpose an automatic MAT reactor was developed in accordance with ASTM norm D-3907-80.

Publications

Castellanos, E.; Melo, F. and Perez, P.J. (1991). Catalytic cracking of petroleum deasphalted fractions. Part I: Catalysts synthesis and characterisation. *Ingeniería Química*, in press.

Castellanos, E. and Melo, F. (1991) Catalytic cracking of petroleum deasphalted fractions. Part II: Catalysts testing and reaction optimisation. *Ingeniería Química*, in press.

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Crystallisation of plant products and selenium/tellurium compounds

Fellowship period: December 1990 - November 1991

Summary

The objective of this study is to transform the quasicrystalline (paracrystalline) compounds derived from Colombian natural products and from carbonic and di-halocarbonic derivatives containing selenium or tellurium atoms, generated by projects 5 (page 34) and 8 (page 56), respectively, into monocrystals in order to resolve their crystal structure.

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Crystallochemical study of natural products and organometallic compounds

Fellowship period: April 1989 - July 1990

Summary

This work led to two joint research projects, no. 5 (page 34) and no. 8 (page 56).

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**Studies of metallic coordination compounds and other solids as
oxidation catalysts.**

Fellowship period: September 1990 - August 1991

Summary of work by E.A. Páez-Mozo

The work of this fellowship is concerned with the synthesis of encapsulated phthalocyanines in zeolites; cobalt and iron phthalocyanines have been synthesised within the cavities of Zeolite-Y.

In the literature there is no conclusive evidence for the formation of the metal phthalocyanine inside the cavities, so emphasis has been put on the characterisation of the encapsulated compounds by using: elemental analysis, X-ray diffraction, infra-red spectroscopy, BET analysis, UV-Vis spectroscopy, Mossbauer, EPR and XPS spectroscopy. So far we have found evidence for the formation of cobalt phthalocyanine inside the cavities and work is under way to characterise fully the encapsulation of the complexes.

Synthesis of encapsulated phthalocyanines of Mn, Fe, Ni and Co is in progress.

Summary of work by N.A. Gabriunas de Páez

In studies of synergism between phases, several catalysts have been prepared by mechanical mixtures of metallic phthalocyanines and molybdenum oxide. Synergism has been studied between the catalyst phases in the following oxidation reactions: of isobutylene to methacrolein; of cyclohexane and cyclohexene; and of ethanol to acetaldehyde.

Preliminary results indicate that there might be some degree of synergism. The oxidation of isobutylene shows a very strong increase in activity toward oxidation but without selectivity. Oxidation of cyclohexane and cyclohexene shows synergism principally in the selectivity toward dehydrogenation. Since it has to be established if there is some degree of decomposition of the catalyst, the fresh and used catalysts are being characterised by several techniques.

Publication

Páez-Mozo, E.; Gabriunas, N.; Lucaccioni, F.; Acosta, D.; Patrono, P.; La Ginestra, A.; Ruiz, P. and Delmon, B. Cobalt phthalocyanine encapsulated in Y-zeolite: a physico-chemical study. Submitted to *Journal of Catalysis*.

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Stabilisation and immobilisation of plant proteases, ficine and bromelaine

Fellowship period: May 1989 - April 1990

Summary

Ficine (EC 3.4.22.3) obtained from the fig (*Ficus carica*) and bromelaine (EC 3.4.22.4) extracted from the pineapple (*Ananas comosus*) are plant proteases. They are widely used as biocatalysts in the agro-industry; in brewing, for example, for the colloidal stabilisation of beer. These native enzymes are also very sensitive, which makes their recovery after industrial use very difficult.

In this research different methods of stabilisation and immobilisation of the enzymes on soluble and insoluble supports have been tried in order to obtain enzymes that are more resistant to temperature, pH and the other factors that affect native enzymes.

For stabilisation, the method most used was that by statistical chemical modification. This is done by coupling covalently the native enzymes (F, Br) to a polysaccharide (Dextran T 70), oxidised with sodium periodate in the presence of sodium cyanoborohydrate, in an alkylation reduction reaction giving FS and BrS.

The preferred method of immobilisation of the native or chemically modified enzymes was that on xenolyte, an inorganic support activated by APTS. The native enzymes are bonded covalently to the support by a bifunctional bridge via glutaraldehyde giving F_{in} , Br_{in} . Chemically modified enzymes are linked via free aldehyde functions still present on the soluble support by reduction alkylation, giving FS_{in} , BrS_{in} .

All these conjugates, as well as the native enzymes, were submitted to different tests such as resistance to temperature over the range 37° to 74°C, thermal stability as a function of time, stability over the pH range 5.0 -9.5, effect of urea, etc. Enzyme activity was determined using casein as substrate.

The residual activities obtained were: FS (56%), BrS (46%), F_{in} (98%), Br_{in} (98%), FS_{in} (41.6%), BrS_{in} (37.5%).

The conjugates F_{in} and Br_{in} gave the best resistance to high temperatures (68°C), thermal stability as a function of time (68°C, 30 min.), pH optimum at 8.0, one unit higher than the native enzymes, and the activity was less sensitive to change in pH.

These immobilisation techniques are of important economic concern to industry because they allow the use of continuous flows which can be automated. Easy recovery of the biocatalyst enables it to be reused as well as its separation from the products formed and, in certain cases, to proceed to a modification of the properties of the enzymes.

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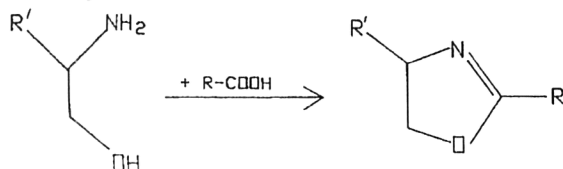
Synthesis and fixation on polyacids of products of biological interest.

Fellowship period: April 1990 - June 1991 (including 3 month gap)

Summary

Synthesis and characterisation of cyclic imidic ester: Two oxazoline rings were obtained i.e. 1-naphthylmethylene-2,2-dimethyl-2-oxazoline and 3-pyridine-2,2-dimethyl -2-oxazoline, respectively. The active species are naphthalene acetic acid and nicotinic acid, which are used as plant growth regulators. The synthesis of these two compounds was accomplished by the reaction of the corresponding carboxylic acid or their acylchloride with 2-amino-2-methyl-propanol-1.

General equation:



R' = methyl

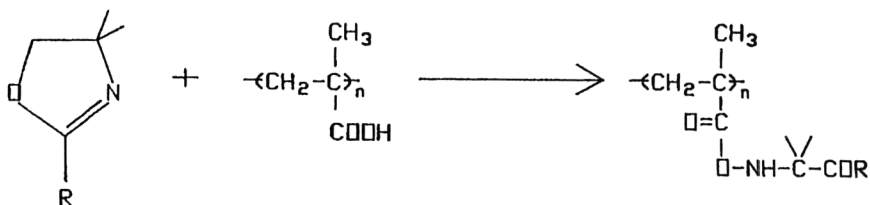
R = naphthalene or 3 pyridine

The 2-oxazoline derivatives obtained were analysed by different techniques such as NMR, IR, GPC, micro-analysis.

Preparation of poly: The poly (methyl acrylic acid) was prepared by the reported method in the literature. The average molecular mass Mw is 150,000.

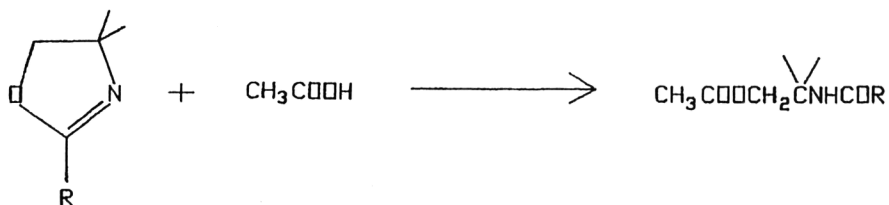
Modification of poly (methyl acrylic acid) with 2-oxazoline derivatives: The addition reaction of 2-oxazoline derivatives on poly (methyl acrylic acid) PMAC was investigated under various conditions i.e. solvents such as methanol, dimethyl-formamide, different molar relation [catalyst]/[acid], temperature and time. The modified polymeric systems obtained were analysed by analytical and spectroscopy techniques, i.e. NMR, IR, microanalysis and GPC. The percentage of addition of the bioactive compounds was evaluated.

General equation:



R = naphthalene or 3-pyridine

Addition reaction between acetic acid and 2-oxazolines: The addition reaction between acetic acid and the 2-oxazolines mentioned above was also carried out under different conditions of reaction according to the equation.



The products of the reaction were characterised by spectroscopic and analytical techniques.

Current activity: At the present time work is on progress on the kinetics of the model reactions.

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Crystallographic studies of legume lectins

Fellowship period: July 1991 - June 1992

Summary

The lectins are a diverse group of proteins that share the common property of the ability to agglutinate cells in a highly specific manner, based on the composition of cell surface carbohydrate. Their possible use as drug targetting molecules, i.e. to carry cytotoxic agents to specific cells such as tumours, makes a full molecular understanding of their specificity a high priority in order to lay the foundation for new clinical treatments based on modern biotechnology.

Lectins have been detected in more than 1000 plant species, as well as in micro-organisms, viruses, invertebrates and vertebrates. They are composed of between 2 and 20 subunits and have molecular weights in the range 8.5 to 400 kD. The best characterised are legume lectins, of which South America affords a rich variety with a wide spectrum of specificities. The only way fully to understand the molecular specificity of a lectin is to determine its molecular structure in three dimensions using X-ray crystallography. This has been carried out for only a few, namely concanavalin A, pea lectin, *Faba* lectin, ricin, *Erythrina corallodendron* lectin, wheat germ agglutinin and influenza haemagglutinin. ConA is additionally interesting in that it undergoes an unusual cyclic permutation of its sequence post-translationally.

A research project has been set up in Bogota by the Fellow, in collaboration with G. Perez, to study the structures of lectins. So far, three legume lectins have been purified and crystallised, from *Erythrina rabinervia*, *Dioclea lehmanni* and *Erythrina costarricense*, although only the first has yielded high quality crystals. Perez, working with M. Richardson at the University of Durham, UK, has determined the sequence of the *Dioclea* lectin, but the others are unknown at present. The *Dioclea* sequence is homologous to that of ConA, and probably undergoes a similar post-translational cyclic permutation.

The primary objective of the fellowship is to pursue the crystallography studies of the three lectins, taking advantage of the excellent facilities at Leeds, in particular, to optimise the crystallisation of the *Dioclea* lectin and carry out a full three-dimensional structure determination of *Erythrina rubrinervia* lectin. This is a necessary prelude to detailed analysis of their sugar-binding specificities by crystallographic and other analyses.

Other objectives are to gain further experience in protein crystallography to help in setting up the laboratory in Colombia and to forge strong links with the laboratory at Leeds and with the University of Durham, with a view to joint research projects, exchange of personnel etc. in the future.

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The extraction of copper: new complexing agents.

Fellowship period: February 1990 - January 1991

Summary

The main objective of this project is to investigate ligands that preferentially extract Cu(II) rather than other cations which are usually found combined with Cu(II) in ores, in order to develop extraction techniques with a higher degree of efficiency than those currently used in Peru. To achieve the preferential extraction, it is planned to use macrocyclic amides such as dioxocyclam(I) in which some 'grease' will be incorporated to reduce aqueous solubility. The following activities are being pursued:

Synthetic work to make the ligand shown in Figure 1 and also to incorporate some grease into the ligand in order to reduce its aqueous solubility to an absolute minimum - for the synthesis of this ligand procedures analogous to those previously described will be used. This part of the work will be carried out partly in Ireland (with Nolan).

Determination of stability constants (therefore free energy of complexation and enthalpy data) for the complexation reaction between these ligands and Cu (II) in solvents potentially suitable for extraction processes.

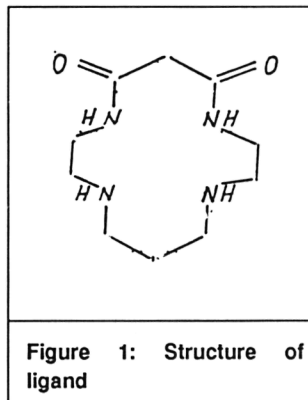


Figure 1: Structure of ligand

These thermodynamic parameters will be obtained by the use of titration calorimetry, a technique which offers the advantage that stability constant and enthalpy data can be obtained simultaneously enabling study of the contribution of enthalpy and entropy to the free energy (therefore stability) of the complexation process. Interpretation of the thermodynamic data will allow the selection of the best conditions for the extraction process. This stage will be carried out at the University of Surrey under the supervision of A.D. de Namor.

Studies of complexation equilibria across boundaries involving the appropriate solvents to be used in extraction processes. This step will be carried out at ICI with Cox and the Mining Chemical Group since ICI is well equipped for practical studies of extraction work.

This fellowship is linked with joint research project no. 6, page 39.

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Study of the weathering steel protective layer under a high SO₂ corrosive atmosphere: influence of the wet-dry cycle.

Fellowship period: February 1991 - January 1992

Summary

It is well known that weathering steel is resistant to corrosion because of the protective layer grown in the early stages. When the materials are in a corrosive atmosphere, the formation of the protective layer and the progress of the corrosion are determined by the relative humidity (RH) and the concentration of the different corrosive pollutant substances (NO₂, SO₂, etc.) and some minor alloying elements. The protective quality of the layer depends on its texture, uniformity and adherence.

The intention of the study is to determine the corrosion products grown in the early stages which form the protective layer. The weathering steel used is of the type COR-TEN B with a chemical composition: 0.17% C, 1.00% Mn, 0.27% Si, < 1% P, 0.018% S, 0.30% Cu, 0.52% Cr, <0.001% Ni, 0.07% Al and <0.02% V. For comparative study a commercial iron specimen was also used. It had a maximum impurities limit of 0.25% C+P+Cu+Mn+Si. Two different atmospheres were used, the first one with 98% RH and 0.22% SO₂, named HA, and the second, LA, with 15% RH and 0.22% SO₂.

The investigation began with a wet-dry cycle of 1 hour HA (wet period) and 3 hours LA (dry period). After three such wet-dry cycles both samples exhibited a semi-transparent and electrically isolating layer.

Another type of cycle used was a 6 hour HA (wet period) and 18 hour LA (dry period). In this, at the end of the wet period the samples presented a homogeneous black colour and both samples had a high weight increase. After the dry period the samples showed a dry grey surface. In the full wet-dry cycle the net gain in weight was less than for the wet period only.

Mossbauer spectroscopy, in transmission (TMS) or reflection (CEMS) modes, is a useful technique for studying the corrosion products of iron-based materials. The reflection measurements can be carried out by Integral Conversion Electron Mossbauer Spectroscopy (ICEMS) or by Differential Conversion Electron Mossbauer Spectroscopy (DCEMS). This report presents the preliminary results for TMS and ICEMS measurements on the samples discussed above.

The corrosion products of all the samples studied were identified as FeSO₃·3H₂O, FeSO₄·4H₂O, an unknown Fe²⁺ compound and a poorly crystalline ferrihydrite compound, in agreement with previous results of Gancedo *et al.*

CEMS and transmission spectra were obtained for outer surface and the descaled bulk products, respectively, of the 6 h HA:18 h LA samples. These measurements show that the relative concentration of the Fe^{2+} species is similar for outer surface and bulk in the weathering steel. On the other hand, in the iron sample the Fe^{2+} species ratio is higher in the bulk than in the outer surface rust.

It is considered that the dry period stabilises the Fe^{2+} species because their oxidation to Fe^{3+} by dissolved O_2 is hampered by the decrease of the aqueous layer which modifies the solute concentration, and also leads to the formation of ferrihydrite, as has been indicated by others.

Conclusions from the work of the first semester of the fellowship are that:

1. Mossbauer spectroscopy in CEMS and TMS allows identification of surface and bulk components of early corrosion products in iron and weathering steel samples.
2. The Fe^{2+} compounds $\text{FeSO}_3\cdot 3\text{H}_2\text{O}$, $\text{FeSO}_4\cdot 4\text{H}_2\text{O}$ and the Fe^{3+} compound ferrihydrite are identified as initial phases of the corrosion reaction.
3. The cycling reduces the oxidation of Fe^{2+} to Fe^{3+} due to the aqueous medium decrease during the dry period.

4 EARTH SCIENCES

Summary

Two of the studies described in this chapter deal with mineral resources; one (page 71) demonstrated the presence of rare metals in Bolivian ores and, therefore, the possibility of adding value to the traditional mining products of the country, while the other (page 88) was essentially a desk and laboratory study of Peruvian coal deposits.

Earthquakes are of regular occurrence in many parts of the Andean Pact region and another of the studies in the earth sciences (page 82) deals with seismicity with a view to improving seismic hazard evaluation, a subject of obvious social and economic importance.

The final project in this chapter (page 85) concerns remote sensing of natural ecosystems. Focussing on the development of methodology for the assessment of environmental effects on natural ecosystems, this study is particularly relevant since it offers the possibility of covering large areas with difficult access whose future is of increasing regional and worldwide concern.

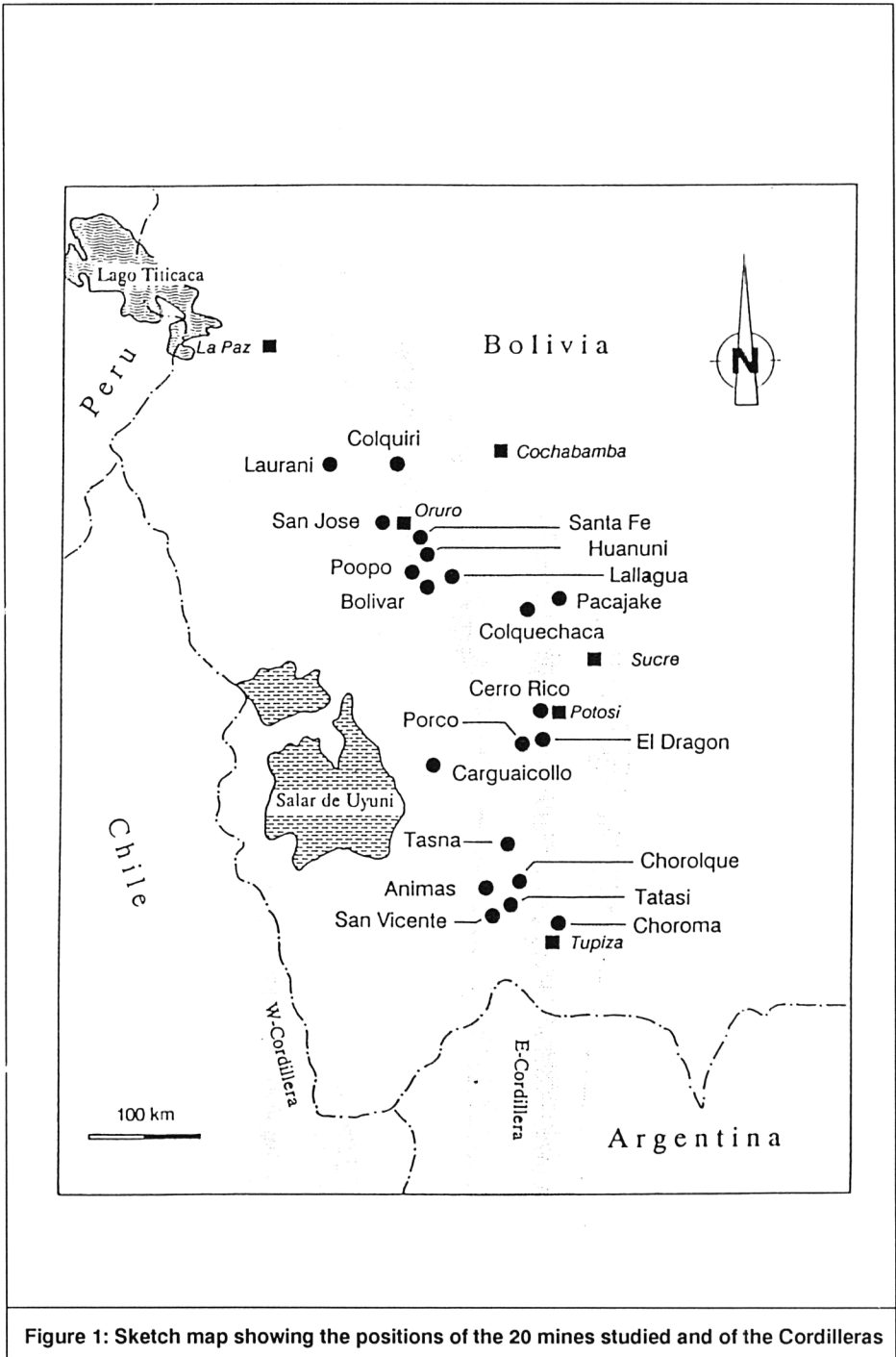


Figure 1: Sketch map showing the positions of the 20 mines studied and of the Cordilleras

Joint research projects**9 Rare metals in Bolivian ores****G. Morteani**

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Contract number and duration: Cl1*/0128, September 1987 to February 1990.

Background and objectives

The mining industry in Bolivia, which is one of the classic mining countries of South America, was mostly oriented towards tin production. It therefore experienced major difficulties in the second half of the nineteen-eighties as a result of dramatically decreasing prices for tin in consequence of the breakdown of the so-called Tin Council.

This project was an attempt to improve the economically bad situation of the mining industry in Bolivia, by exploring the possibility of an additional source of income from the recovery of rare metals like Ga, Ge, In, Se, Te, and Tl from Bolivian ores. At the time the project started, the rare metal contents of these ores were mostly unknown.

These metals belong to the so-called "electronic metals" of groups III to VI of the periodic system. The term "electronic metals" as used in the specialised literature originates from the fact that these elements are essential for the production of semiconductors.

The individual objectives of the project were defined by the geologists of the Technical University of Munich and the University of Trento in cooperation with the Bolivian partner "Instituto de Investigaciones Minero-Metalúrgicas" (I.I.M.M.) in Oruro as follows:

1. **Compilation and critical evaluation of all data on the subject available at the I.I.M.M.**
2. **Sampling of ores and concentrates in selected mines.**
3. **Chemical analyses of the main element and rare metal contents in minerals, ores and concentrates.**
4. **Microscopic study with reflected light for the determination of opaque ore minerals.**
5. **Electron-microprobe chemical analyses of selected minerals in order to determine their rare metal contents.**

6. Chemical analyses for rare metal contents of slags and dust precipitated from the fumes of selected metal refineries.
7. Evaluation of all data, identifying the mines with the most promising rare metal contents.
8. Suggestion possible changes in the preparation plants or smelters so as to increase or decrease specifically the content of rare metals in individual products.

From the first evaluation of the available information and from basic geochemical considerations the most promising targets for such a study were the subvolcanic-hydrothermal polymetallic deposits, found within the central and southern part of the Bolivian tin belt.

Based on a careful evaluation of the geological and metallogenetic setting of the different individual mines and considering, too, the possibility of getting samples constrained by access to the surface and underground workings, the following 20 mines were selected for a detailed investigation in close cooperation with the director of the I.I.M.M. and the COMIBOL district manager:

Animas, Bolivar, Carguaicollo, Cerro_Rico de Potosi, Chorolque, Colquechaca, Colquiri, El Dragon, Huanuni, Laurani, Llallagua/Siglo XX, Pacajake, Poopo, Porco, San Jose (Oruro), San Vicente, Santa Fe, Tasna, Tatasi.

Figure 1 shows a sketch map of the location of the mines and of the Cordilleras.

At the time of the project the selected mines produced mainly tin, lead, zinc, silver, antimony and bismuth ores and/or concentrates. Sampling took place in Bolivia in two periods, the first from September to October 1987 and the second one in September 1988.

Materials and methods

In addition to the "electronic metals", Cd, Bi and the precious metals gold and silver were considered in this study. The reason for doing this was that from geochemical considerations an enrichment of these metals has to be expected in the ores of the mines mentioned above. Such a study was never previously done for Bolivian ores, and it was seldom done elsewhere. Wellmer *et al.* state that such an investigation: "..... is a task for economic geologists in the future, a contribution to the optimisation of programming of mining operations and the smelting process as a maximum utilisation of the ores."

One of the fundamentals of geochemistry is that elements tend to occur in associations. Main elements (also called in this context carrier or collector elements) have a high abundance in the crust and are found enriched in their own deposits. The rare elements, on the contrary, are too rare to form their own deposits and they remain associated with the main elements in minerals and ores, due to similar atomic radius and valency.

The determination of rare metals in a concentration range of some ppm in base metal ores and minerals needs particular care and therefore different analytical methods had to be used, such as DCP (direct coupled plasma spectrometry), FADCP (fire assay direct coupled plasma spectrometry), ICPMS (inductively coupled plasma mass spectrometry), GFAA (graphite furnace atomic absorption), and FAA (flameless atomic absorption). The main elements were analysed by standard XRF (X-ray fluorescence spectrometry). In order to determine the distribution and zoning of main elements and of rare metals individual grains were analysed by electron microprobe. The modal composition and grain size distribution of ore concentrates was determined on polished grain mounts by image analysis methods and subsequent statistical evaluation. All data were evaluated graphically and statistically by computer.

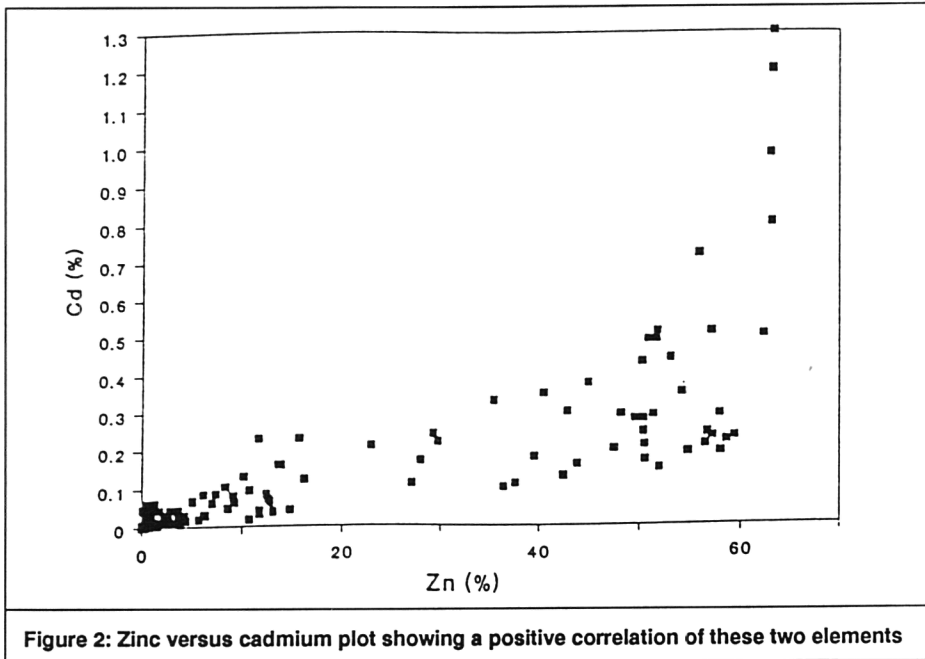


Figure 2: Zinc versus cadmium plot showing a positive correlation of these two elements

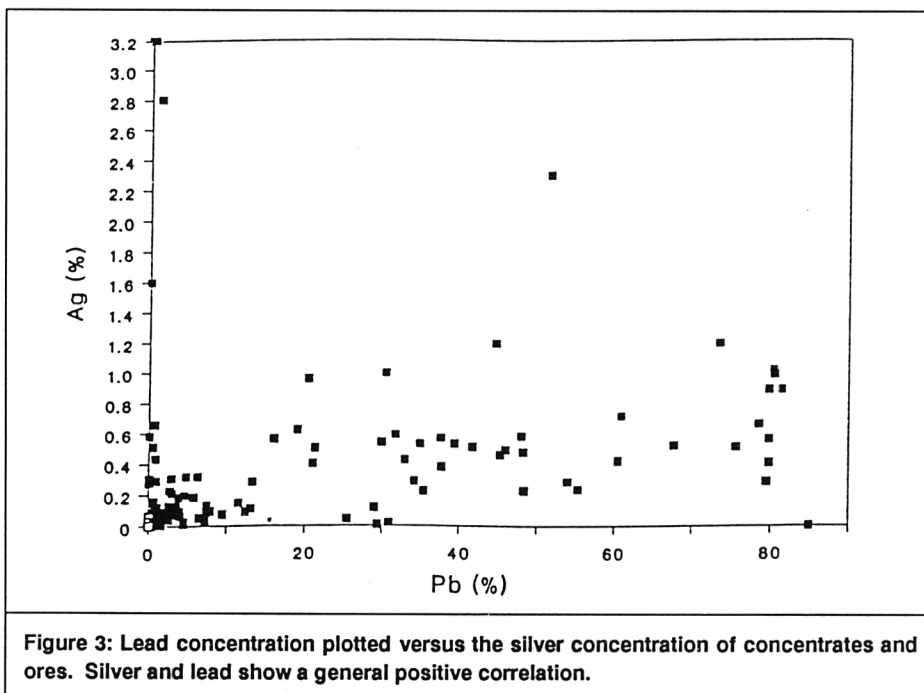
Results

The final report was organised in such a way that, for every mine, the fundamental geological facts were presented first, then the analytical data are given and discussed and finally an evaluation of all the data, including the recommendations for future activities, are given. The most important results are summarised in Tables 1 and 2 appended to this report.

Table 1 gives the rare metal content or concentration ranges, respectively, including gold and silver, of the different concentrates produced by the individual mines at the time of sampling. (All values are in ppm, except when defined as %.) If at the time of sampling at the mine site no concentrates were available, a representative sample of the bulk ore produced was taken for further analysis. In this case the mineral name has the addition (VE). Table 1 therefore gives an idea of the rare (and precious) metal potential of the mines at the time of sampling with the given preparation plants and preparation schemes operating.

Table 2 gives the rare metal and the gold and silver concentrations of the most important carrier minerals separated carefully by hand picking from the ores. If no pure minerals could be separated the respective mineral is marked as MI. The data from Table 2 allow the mine staff to plan a production of individual mineral concentrates, i.e. of potential products, with maximum (or minimum) rare metal concentrations.

From the analytical data given in Tables 1 and 2 some basic correlations and information result. As an example Figure 2 shows the good correlation of Cd with Zn in all the concentrates analysed. This gives a clear indication that Cd is prevalently bound to sphalerite.



In Figure 3 the silver concentrations of all the concentrates and ores analysed are plotted against Pb concentrations. This diagram shows that there is a general tendency of the silver concentrations to increase with the amount of Pb, i.e. with the amount of galena, in the concentrates, but some very high Ag concentrations at Pb values of about zero show that in the concentrates there are other Ag carriers than galena. Microprobe analyses showed in fact that silver is found in high concentrations not only in galena, but also in complex minerals like franckeite and in the tetrahedrite-tennantite-freibergite series.

Discussion

The investigation showed that in the products of some of the mines studied there are interesting concentrations of rare metals. In addition interesting concentrations of gold were found in selected ores of the mines of El Dragon (selenide ores), Chorolque (cassiterite ores), Laurani (copper sulphide ores), Tasna (arsenopyrite ores) and San Jose (galena, pyrite and franckeite ores). In selected ores of the mines of Tatasi (galena ores), Santa Fe (galena, pyrite and sphalerite ores), San Jose (complex sulphide and galena ores), Cholquechaca (galena and sphalerite ores) and Bolivar (complex sulphide and galena ores) interesting silver concentrations could be detected.

Considering the large number of mines studied the number of analyses was rather limited. The study has therefore a pilot character. Nevertheless the data encourage further investigations in order to optimise the mining operations and the preparation plants.

In view of the economic target it must be kept in mind that elevated concentrations of rare metals in the ores and concentrates do not automatically mean an additional income for the respective mines. The market for rare metals is fundamentally a buyers' market and the bonus for the rare metal concentrations of the ores and concentrates is negotiated by the refineries to whom the concentrates are sold. These refineries are predominantly found outside Bolivia.

Conclusions

The project demonstrated for the first time the presence of noticeable enrichments in rare metals and, in some instances, in gold and silver in the products of 20 polymetallic deposits of the central and southern part of the Bolivian tin belt. The data produced in this study can form the basis for a negotiation of better prices for some of the concentrates and ores by the mine owners.

The study may also encourage the Bolivian side to consider its own production of rare metals, but profitable production will require high-technology processing and consequently high investments. It is the processing technology, in fact, that gives the greatest additional value and the market is consequently controlled by those companies possessing the necessary technology. In the investment planning one has to consider furthermore that, in terms of price, the risk for rare metals is higher than for precious metals and much higher than for non-ferrous metals.

If in the short term a follow-up to this project should be considered, then this should focus on additional analyses and the optimisation of the mining and milling processes of those mines showing the most promising rare metal contents in order to assure the maximum recovery of the rare (and precious) metal contents of the ores.

Acknowledgements

We want to thank explicitly the tireless driver Jeronimo Padilla from the I.I.M.M., in Oruro who took the European geologists safely and on time to the most lonely mines in the high Andes. The following people cooperated in the field and laboratory work: From the Technical University of Munich, Christine Preinfalk, Renate Jordan, Gunter Gundmann, Gerhard Lehrberger, Fritz Gruner, and Axel Zwicker; from the University of Trento, Franca Bazzanella; and from the I.I.M.M., A. Ortiz C., Carlos Garron and Tito Novillo. Without their energetic help the tasks of the project could never have been performed.

Publication

Grundmann, G.; Lehrberger, G. and Schnorrer-Köhler, G. (1990). The El Dragón mine, Potosí, Bolivia. *Mineralogical Record*, 21, 3/4. 133-46.

Mine\Element	Ga	Ge	In	Se	Te
Animas	169	<10	471	0	0
	100	<20	510	21	4
	91	<10	462	5	0
Bolivar	28	-	-	1	<0.02
	35-44	<20	440-468	<1	8
	174-250	<20	544-660	<1	<1
Carguaicollo	-	-	-	-	-
Cerro Rico de Potosi	49	5	0	4	3
	70-90	20-50	970-1450	1	3-6
Chorolque	57-350	<20	45-75	<1-140	<1-21
Choroma	5	10	<10	10	3
Colquechaca	380-650	<20	60-150	<-16	1
	10	<20	140-420	<1	4-8
Colquiri	24	<10	-	7	0
	58	<10	268	1	0
El Dragon	<10	<20	<10-90	42-43%	1-14
Huanuni	65-230	<20	55	82-100	0-2
	10	<20	95-479	<1-37	0-2
Laurani	<10	<20	20-490	11	2-660
Llallagua	160-320	<20	50-110	1-12	2-87
Pacajake	<10	<20	160	7500	7
Poopo	620	20	3500	<1	8
Porco	12	20	266	8	1
	26	<10	275	1	1
San Jose	9	5	187	68	2
	25	<20	270	37	<1
	32	5	0	180	1
	9	20	167	40	1
San Vicente	20	20	1460-2200	1	0.02-7
Santa Fe	15	<20	85	40	14
	10	10	-	170	0.02
	8	<10	<10	220	<0.02
Tasna	9	<10	60	280	110
	<10	<20	202-360	280-350	3-75
	35	<10	-	3	6
	4	<10	155	190	78
	<10	<20	<10-110	1	<1-5
Tatasi	6-10	<20	181-340	2	<0.02-1
	13-85	<10-100	426-800	<2	<0.02-13

Table 1 : Rare element, silver and gold concentrations in the different concentrates

Tl	Bi	Cd	Ag	Au	Minerals
18	300	160	4012	0.16	Cassiterite
4-15	6890	1300	3170	0.41	Complex sulphides
6-9	800	3300	3173	0.18	Sphalerite/Pyrite
4	19	1309	69	0.03	Cassiterite
10-45	25-1300	940-1030	5330-5816	0.04	Complex sulphides
9	22-110	3000-3490	580-821	0.05	Sphalerite
-	-	-	-	-	
21	400	81	260	0.57	Pyrite/Cassiterite
17-30	450-3000	1000-1100	870-3070	0.11-0.32	Sphalerite
<1.5	190-4600	<1-15	3-690	0.45-33	Cassiterite
<1	600	2	228	0.05	Pyrite/Rock fragments (VE)
<10	65-85	39-114	36-70	<0.1	Cassiterite
10	85-160	470-696	0.22-12%	<0.1	Galena/Sphalerite
6	25	71	129	0.08	Cassiterite
5	72	1800	109	0.05	Sphalerite
5-15	1900-6900	29-69	258-918	20.5-47	Selenides (VE)
10-11	160-290	11-29	3-10	0.02	Cassiterite
<10	85-260	1210-2400	1089-7180	0.16-0.18	Sphalerite/Galena
<10	40-5700	10-182	653-5800	2.2-26.6	Copper sulphides (VE)
<10-25	12-5200	18-29	7-150	<0.1	Cassiterite
10	<10	<1	23	0.17	Selenides (VE)
<10	290	5120	520	<0.1	Sphalerite (VE)
47	900	630	4199	0.07	Galena/Sphalerite
11	110	1900	585	0.04	Sphalerite
20	1400	610	73	2.20	Galena/Pyrite
<10	360	373	6200	1.30	Galena/Sulphosalts
6	130	120	520	2.00	Pyrite/Cassiterite
15	700	400	5008	2.70	Pyrite/Franckeite
13	1100-1200	3500-3760	2889-4330	0.19-0.3	Sphalerite/Chalcopyrite
<10	110	300	4930	0.12	Galena
33	89	300	4261	0.39	Pyrite
24	110	290	4790	0.26	Sphalerite
80	2.9%	40	162	12.13	Arsenopyrite/Pyrite
29-35	1.29%	57-64	603-1018	3.3-5.1	Bismuthine/Chalcopyrite
1	250	1	2	0.03	Cassiterite/Pyrite
55	7.7%	48	498	7.20	Chalcopyrite
<10-20	3100-4200	2	25	0.16-0.37	Wolframite
10-24	220-260	620-822	4603-5130	<0.1	Galena
<10	78-80	1700-2930	895-1303	<0.1	Sphalerite

produced by the preparation plants and in bulk ores (VE) of the mines studied.

Mine\Element	Ga	Ge	In	Se	Te
Animas	30-280	-	90-280	-	-
	260-350	20-540	140-270	-	-
	-	-	120	-	-
	-	-	210	-	-
	-	-	1200	53	-
	25-31	-	-	4-13	-
Bolivar	140-10	-	730-1200	25-26	-
	35	-	45	-	-
	350	-	740-780	-	-
	30	-	110	-	-
	(Antequera)	123	-	64	18
Carguacolollo	-	-	55	-	-
Cerro Rico de Potosi	60-230	30	820-3400	-	-
	40	-	100	-	-
	650	-	-	-	-
	-	-	260	-	-
	30	-	160	-	-
	-	-	770	-	-
	25-39	-	-	-	-
Chorolque	-	-	-	95-200	11-29
	6-43	-	-	27-160	2-40
	11-42	-	-	27-50	0-21
	56	-	-	48	6
Colquechaca	90	-	1300	-	-
Colquiri	9-110	-	610-900	17	-
	400	-	100	-	-
	-	-	110	-	-
	-	-	30	17	-
Huanuni	180	-	-	-	-
	-	-	-	-	-
	50	-	1300	-	-
Laurani	-	-	25	-	660
	-	-	490	-	2
	-	-	20	-	100
Pacajake	-	-	-	65.8%	190
Poopo	-	-	1400-1900	-	-
	310-620	-	650-3500	>1-38	<5-8
	-	-	60	34	-
Porco	-	-	50	13	-
	30-110	-	270-290	-	-
	-	-	26	7	2

Table 2: Rare element, gold and silver concentrations of minerals, tailings and slags of
The symbol "MI" means that the mineral was not very pure due to problems in mineral

Tl	Bi	Cd	Ag	Au	Minerals
-	-	0.5-12%	228-742	-	Sphalerite
-	-	0.98-135	99-2770	-	Wurtzite
-	-	33-2100	2380-5600	-	Galena
-	-	-	100	0.13	Pyrite
-	2.0%	-	5100	0.15	Stannite
-	45-91	-	57-173	0.16-0.31	Tailings
-	5-70	4300-8000	186-847	0.08	Sphalerite
-	20	400	2880	0.27	Galena
-	-	2200-2300	5600-9600	0.07	Complex sulphides
-	15	290	46	0.12	Pyrite
-	-	350	211	0.12	Slag
-	-	430	190	0.52	Teallite
-	5-180	1600-2400	626-2820	0.07	Sphalerite
-	60	-	274	0.26	Pyrite
-	25	-	-	-	Cassiterite
-	1.1%	-	3.2%	1.6	Fahlore
-	210	-	9.9%	0.13	Galena
-	45	421	5.7%	-	Complex sulphides
-	51-230	-	54-97	0.07-0.37	Tailings, Pyrite
-	400-450	-	12-34	0.06-0.17	Pyrite (MI)
-	28-270	-	2-74	0.03-7.10	Arsenopyrite (MI)
-	-	-	1-50	0.02-0.13	Quartz, carbonate (MI)
-	200	-	18	0.42	Tailings, Pyrite
-	-	2900	376	0.08	Sphalerite
-	25-60	2800	12-55	-	Sphalerite
-	55	-	45	-	Cassiterite
-	290	110	36	-	Arsenopyrite
-	-	180	2860	-	Galena
-	75-760	-	17-32	-	Cassiterite
-	>5-55	430790	5100-6600	0.08	Galena
-	10	5100	1540	0.12	Sphalerite
-	40	-	653	4.7	Enargite
-	2300	182	5800	26.6	Fahlore
-	5700	-	3040	2.2	Famatinite
-	110	-	255	5.0	Penroseite
<5-150	25-95	264-830	5300-5900	<0.1-0.52	Cylindrite
<5-38	10-290	5120-7200	292-520	<0.1-0.08	Sphalerite
-	-	320	5400	-	Sulphosalts
-	-	-	2840	-	Galena
-	5-100	1490-2900	910-1460	-	Sphalerite
-	65	140	123	0.1	Tailings

the mines studied. The minerals are pure hand-picked ore fractions.
separation.

Mine\Element	Ga	Ge	In	Se	Te
San Jose	-	-	530-1000	10	25
	-	-	130-390	16-77	-
	-	-	45-60	27-62	-
	6-70	-	5-140	18-320	-
San Vicente	-	-	110	15	-
	20-60	-	670-2900	-	-
	-	-	440-1300	-	-
	50	-	66	-	-
Santa Fe	-	-	15-25	18-19	-
	-	-	80	25	-
	60-100	-	70-790	16-31	-
Tasna	-	-	55	107	90
	-	-	85	14	5
	-	-	30-70	850-1100	1400
	-	100	220	11	-
	-	-	80	-	-
(Quechisia)	-	-	301	-	-
	<10-250	-	<10-230	36-280	<1-24
Tatasi	-	-	10-670	-	-
	-	-	65-130	-	-
	30-60	250-380	75-220	-	-
	-	-	33	-	-

Table 2 (continued): Rare element, gold and silver concentrations of minerals, tailings and
The symbol "MI" means that the mineral was not very pure due to problems in mineral

Tl	Bi	Cd	Ag	Au	Minerals
<5-95	15-35	290-430	0.4-1.2%	0.06-0.30	Franckeite
-	110-1200	450-600	0.2-1.0%	2.0-7.9	Jamesonite
-	5-75	-	16-52	0.08-0.95	Pyrite
-	95-120	-	215-289	0.96-2.8	Tailings
-	-	330	2280	-	Galena
-	3-50	2100-2300	282-910	-	Sphalerite
-	370-6400	218-2300	0.7-3.2%	0.01-0.1	Tetraedrite/Chalcopyrite
-	26	66	64	0.05	Tailings
-	-	200-370	5200-8900	-	Galena/Sphalerite
-	30	-	23	0.10	Pyrite
-	75-360	4400-4900	1190	<0.05-0.16	Sphalerite
40	3900	-	8	25.2	Arsenopyrite
-	1200	-	22	6.9	Pyrite
90-510	58-68%	31-46	2090-2270	1.5-7.8	Bismuthinite
-	1800	-	288	0.47	Chalcopyrite
-	360	-	13	0.31	Wolframite
-	28.8	400	296	9.33	Fume dust
9-260	0.2-2.4%	9-33	17-220	0.24-5.4	Tailings
-	<5-20	22-250	0.4-1.02%	-	Galena
-	150-760	-	0.02-1.6%	<0.05-0.7	Pyrite
<5-30	5-170	1700-2300	420-741	-	Sphalerite
-	18	110	71	0.1	Tailings

slags of the mines studied. The minerals are pure hand-picked ore fractions. separation.

10 Seismotectonics of northern Venezuela. The contact between the Caribbean and South American plates

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Contract number and duration: Cl1*/0459/0460/0461, October 1989 to September 1992.

Introduction

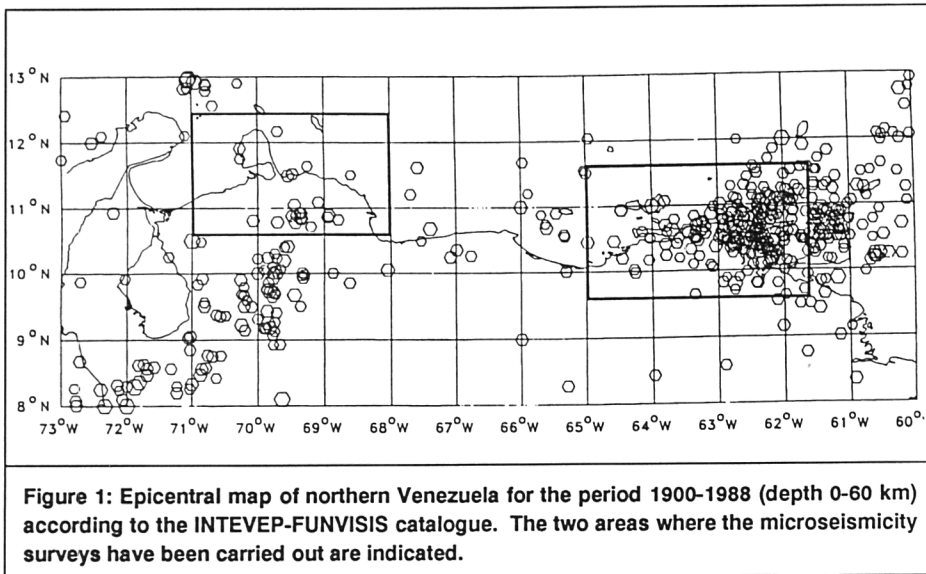
Northern Venezuela is within the interaction zone between the Caribbean and South American tectonic plates. This plate boundary displays a wide zone of deformation, where tectonic activity is accompanied by moderate to high seismicity, with several destructive earthquakes within historical record.

Two microseismicity studies, in northwest and northeast Venezuela respectively (Figure 1), were performed within the project to evaluate seismology parameters and to improve the knowledge of the present-day tectonic regime of these regions. Since both have social and economic interest, data gathering to improve the seismic hazard evaluation is one of the most important objectives of these surveys.

Seismicity

Northern Venezuela is characterised by a high to moderate seismicity. The occurrence of several destructive earthquakes is known from historical chronicles, since the arrival of the Spanish in the American continent.

The historical record from the beginning of this century has been complemented with a compilation of instrumentally recorded earthquakes, obtained from international data files and seismic bulletins from local and Caribbean seismological stations. This data base is not homogeneous in time, since technological advances have progressively increased the detection capability and reliability of seismic networks. Nevertheless, it provides an excellent picture of the seismic pattern of northern Venezuela.



The seismicity distributions for the two areas involved in this study are quite different. The instrumental record for the western area contains only 101 events. Most of them are shallow, with magnitude (m_b) mainly between 4 and 5. The maximum magnitude for the instrumentally-recorded data is 6.5 (M_c) and five destructive earthquakes, with MMI intensities of 7 or more, are documented to have occurred within the limits of this area since 1610.

The eastern area is much more active and at least thirteen destructive earthquakes are documented within the historical record since 1530. The instrumental file for this area contains 1388 events with few earthquakes exceeding 6.0 m_b magnitudes. Seismicity here is distributed over a wide range of depth, with many earthquakes occurring from 0-40 km and around 100 km depth. A high concentration of epicentres north of the Paria peninsula is associated with the southern end of the convergent margin between the Atlantic and the Caribbean plates (Figure 1).

Methodology

The microseismicity studies have been carried out using 60 portable seismological stations deployed in both surveyed areas. The equipments used were the 3C-LOBS model developed by the Institute of Geophysics of the University of Hamburg. They consist of a masterclock, a three-component geophone and a recording device. A coded time signal and three seismic channels are recorded in analog form on magnetic tapes, at three different gain levels. The power is supplied by 27 1.5 V dry cell batteries, enabling a recording capacity of about 250 hours.

Every recording site was previously selected according to criteria such as spacing, background noise, known seismicity and the presence of possible seismogenic structures. In general terms, the spacing between stations varied from 10 to 15 km, covering an area of about 28 700 km² in the west and 26 000 km² in the east. The actual time during which the complete set of stations was operational was 14 and 13 weeks for the western and eastern arrays respectively. The field headquarters were located within each of the studied areas.

A playback system was installed in the field headquarters and a shift schedule was established in order to operate 24 hours per day. Event detection, correlation and digitization began as soon as the first set of cassettes was recovered. Preliminary hypocentre determination and focal mechanisms were computed for some events of the western area. Due to technical difficulties, only one playback unit could be used in the field, which limited the processing capacity during the field work period. Later a second playback system was incorporated.

The stations' maintenance was carried out every nine days. Instrument performance was checked, batteries and cassettes were replaced and the internal time difference was compared with a well-controlled master-clock. Four field maintenance groups were established for this task. They were provided with four-wheel-drive vehicles and with a radio-communication system that permanently linked the maintenance group with the field office. Each group was responsible for the servicing of 15 stations which took an average period of four days.

Data processing and preliminary results

The data processing is currently being performed on an IBM-compatible PC-AT and two IBM PS/2, after conversion of the data from analog to digital form. The software used is a collection of several programmes from the Institute of Geophysics of the University of Hamburg and other institutions, but several new algorithms were developed to meet the specific data set requirements. Most of the programmes are written in C, to guarantee a high performance on personal computers.

The processing steps can be summarised as follows:

Detection: Analog tapes are analysed to evaluate the occurrence time of possible events.

Correlation: The detection times are correlated for the stations of the network. Correlation improved when sub-class networks were defined.

Storage: The digitised data files are stored in IBM 3090 cartridges.

Phase picking: P- and S-phases are picked on the screen and hard copies are made.

Hypocentre location: Hypocentres, magnitudes and first motion patterns are calculated using well-known algorithms such as HYP071, HYPOLAYR and JHD (Joint Hypocenter Determination) for clusters of earthquakes.

The processing for the western network (Estado Falcon) has just started. However, even considering the few final hypocentre locations available it is worth mentioning that an average of 10 events per day could be correlated. The most seismically active area is around Churuguara, responsible for about 50% of the events correlated. The peninsula and the western part of the array seem to be less active.

The processing for the eastern network has also been started. Here the number of events per day is higher than that of the western network. Seismic activity appears to be concentrated at the north-eastern part of the network, near the villages of El Pilar and Casany.

It is considered that data processing will take a further few months. The first reliable results are therefore expected to be available by the end of 1991.

11 Remote sensing of ecosystem dynamics

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Contract number and duration: CI1*/0630, December 1990 to November 1993

Background and objectives

The project is a study of the radiometric behaviour of natural and cultivated plant formations in tropical surroundings. It is concerned with the application of satellite remote sensing to monitoring the influence on ecosystems of certain characteristics of the environment.

Its main objectives are:

Spectral characterisation of the principal natural coastal ecosystems (mangrove swamps, submarine phanerogams, sectors of algal invasion of coral reefs), forests and areas of essentially tropical cultivation (coffee, sugar-cane, cotton, sorghum, soya, rice) by means of radiometric and spectro-radiometric measurement *in situ* and/or helicopter borne.

Evaluation of the influence on spontaneous and on cultivated vegetation of substrate characteristics (nature, salinity, drainage, mineral element concentrations, etc.) in order to establish a series of relations between the components of the spectral response due to these factors and the physiology of the species concerned.

Estimation of the spectral changes due to variations in water quality (for under-water ecosystems) and atmospheric effects, particularly air humidity, mist and cloud cover in tropical conditions, with the objective of modifying and/or establishing algorithms for atmospheric corrections.

Determination of the directional response of vegetation in order to correct satellite data acquired from an oblique view (for the SPOT earth observation satellites and the NOAA meteorological satellites). Most of the data on reflectance have been obtained from vertically-oriented images.

Evaluation of the effects of the orientation and slope of the terrain on the spectral response of the vegetation in mountainous zones. As well as the effects due to variations of the pedological conditions, two different types of problem can be distinguished: effects linked to the relative geometry of illumination and the directional effects due to the non-Lambertian response of the plant cover.

Inversion of the proposed models so that the parameters that characterise the state of the vegetation can be extracted from satellite data. Experimental analysis of the directional and temporal spectral variations of the radiometric response is likely to contribute to a better understanding of the physical phenomena that affect the spectral response of the vegetation. This will enable advances to be made in the interpretation of satellite imagery of coastal, wooded and agricultural environments.

Finally, a diachronic evaluation of the satellite data acquired from the selected test zones (c.f. infra-red) and further research on the applicability of the proposed models to other zones and geographic regions.

Geographical location

The research will be carried out simultaneously in three different regions of Colombia, each offering one or several sites characteristic of the several ecosystems being studied.

The first region selected corresponds to the Caribbean coast where amphibious forests are well developed, mangroves (*Rhizophora mangle*, *Avicennia nitida*, *Conocarpus erectus* and *Lagularia racemosa*), the meadows of underwater phanerogams (*Thalassia testudinum*, *Syringodium filiforme*, *Halophila vailonis* and *Halodule wrightii*) accompanied or not by algae (*Avranvillea*, *Halimeda*) and where one can find an actual spread of the algal invasion of coral reefs (called "verdigris" by the Carib indians). These plant environments are under heavy attack from human causes; the effects of pesticides used in cultivation of bananas, degradation in the mangrove swamps through logging for timber or the opening of ponds for shrimp farms, hyper-salination of the soil due to the closure of lagoon entrances, deterioration of under-water ecosystems linked to the disequilibrium in the sedimentary flows produced by realignment of water-courses, not to mention mechanical destruction from fishing with dynamite. The areas selected for this research are the national parks "Isla de Salamanca" and "Corales de Rosario" along with the sector Bay of Barbacos - Gulf of Morosquillo.

The second region is situated in the valley of the river Cauca and is characterised by a relatively flat topography and a technically advanced agriculture, with cultivation of cotton, sugar cane, maize, rice, soya and sorghum. This region has several areas of poor drainage and local problems of soil salinisation. The two principal zones selected for pilot studies are the experimental farms of the Centre International d'Agriculture Tropical (CIAT) and the Instituto Colombiano Agropecuario (ICA).

The third zone selected is known as the "Coffee zone". Its economic importance to the country is, on its own, enough to justify its inclusion in the programme of research. Its characteristics are mountainous, with altitudes varying between 800 and 2,500 metres. Scientific investigators can rely on the cooperation of the Federacion Nacional de Cafeteros (FEDECAFE).

Approach

The proposed approach has several innovative aspects, as follows:

An interdisciplinary approach to the different ecosystems studied (physical, bioclimatology, botany, agronomy, geomorphology).

The compilation of a spectro-radiometric data base on the spectral behaviour of the different intertropical areas noted; currently, the radiometric signatures of these areas are known only partially if at all.

The establishment of physico-mathematical models that take account of incident solar radiation, directional aspects of reflectance, the natural environment and the biotic parameters of vegetation in intertropical climatic conditions.

Benefits

The principal benefits expected from the research are:

A greater possibility of applying remote sensing to the compilation and monitoring (for production estimates) of an inventory of natural plant formations, land use and cultivation in the intertropical zone.

The applicability of the methodology developed to other intertropical regions; whether under-water near the coast, in amphibious areas or in continental zones these are all essentially difficult of access, cover large areas and lack reliable cartographic data.

Postdoctoral Fellowship**E. Dunin Borkowski**

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Peruvian coals: characteristics and relations with regional geography.

Fellowship period: June 1990 - May 1991

5 ENVIRONMENTAL SCIENCES

Summary

The research projects reported here are at an early stage of development. The first of them (page 90) deals with an examination of the occurrence of toxic heavy metals and micronutrients in tropical soils, which have been studied much less intensively than soils of the temperate zone. The second study (page 92) concerns an environmental inventory of the Orinoco basin which has been designed in an integrated manner to include not only the natural environment but also the extent and nature of human intervention in the region. Finally, a fellowship study (page 93) has focussed on the relationship between pollution sources and the benthic communities of mangrove swamps and flats on the Colombian Pacific coast.

Joint research projects**12 Inorganic trace pollutants and micronutrients in soils of tropical ecosystems with special reference to Venezuela****B.E. Davies**

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Contract number and duration: Cl1*/0554/0572, September 1990 to April 1993.

Background and objectives

In contrast with developed countries and the temperate regions, little is known about the occurrence, chemical behaviour and bioavailability of trace metals in tropical soils and ecosystems of developing and underdeveloped countries. This project aims at providing such information for toxic heavy metals and essential micronutrients in soils and typical plants of representative ecosystems in Venezuela.

Materials and methods

An initial survey is designed to provide baseline information on soils and plants in tropical habitats, about which little is known in terms of trace elements. In the first phase (12-15 months) samples of surface soil and selected plant species will be collected along two transects. One is west-east for 700 km in northern Venezuela from the Lake Maracaibo basin to Maturin in Monagas. The other runs 700 km north-south from the Caribbean to the frontier with Brazil. At least 200 samples will be collected, from sites well away from roads, and analysed for total trace metals and other constituents. The sample interval is approximately 7 km. Two general samplings will be undertaken. One will be during a dry season, the second will take place in a wet season. Care will be taken to ensure that the second set of samples will be taken at locations identical to those of the first.

Following this, soils, soil profiles and plants will be sampled in greater detail in major ecosystems; cactus forest, semi-deciduous forest, savanna grassland and Amazonian rain forest. These ecosystems have analogues elsewhere in the tropics. From the results of the first surveys it is proposed to identify areas where metal contents of soils are relatively high, possibly because of contamination, and low because of the nature of the deeply weathered regolith. In each principal ecosystem a 'high' and 'low' soil metal area will be selected and extensive soil and plant samplings undertaken to provide new data. Each area will present its own challenge in terms of sampling design. Appropriate internationally-recognised sampling procedures will be used.

During the early months of the project a computer-aided literature survey will be undertaken. This will form a database of a) published information concerning trace elements in tropical soils and natural plants and b) information concerning soils and ecology of specific habitats of Venezuela.

All material will be analysed for heavy metals and micronutrients by inductively coupled plasma emission spectrometry or by atomic absorption. The schedule of metals will include As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Zn (priority pollutants and micronutrients). In addition, determinations of pH, cation exchange capacity, organic content and major nutrient status will be provided using conventional techniques.

After the routine analysis, soils will be selected for study by these specialised methods in order to give information concerning the speciation, dynamics and bioavailability and retention characteristics of the elements in the soils. The soils will also be characterised in terms of mineralogy and humus composition. This work will include studies of the composition of the soil solution (saturation extracts) together with computer modelling of the chemical species present.

Work assignments

The sampling will be undertaken by Venezuelan personnel with additional assistance from Bradford as appropriate. Sample preparation will also be undertaken by Caracas staff. Thereafter, the work is divided according to the special skills and facilities available at each centre.

Activities in Caracas laboratory:

Routine analysis for soil pH, organic content and exchangeable soil cations;

Plant fresh weight determinations and nitrogen assay;

Soil Hg determinations by AAS.

X-ray diffraction analysis and NMR investigations;

Part of the soil fractionation studies;

Preliminary data appraisal using PC computers.

Activities in Bradford laboratory:

Multi-element assays (ICP) on all soil and plant material;

Part of the soil fractionation studies;

Extraction and analysis of soil solutions. (Selected samples will be passed to a cooperating laboratory in Aberystwyth for work on iodine and stable isotopes);

Comprehensive data evaluation using advanced cartography systems (Uniras) and statistical packages (SPSSx or BMDP).

13 Environmental inventory of the upper Orinoco.

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Contract number and duration: C11*/0768, September 1990 to August 1992.

Objectives

The general objective of this research is to contribute to knowledge on the Amazon basin as an environmental system and as an area of scientific, economic, historical and political integration in Latin America.

The more specific objectives are: to identify and study situations of environmental competition and synergism which are found in the Alto-Orinoco; to establish from an overall, integrated and systematic point of view, general guidelines which would permit environmental synergism between the different activities developed in the area; and to contribute to the establishment of methodological proposals appropriate to the study of the tropical environment.

Methods

The study will begin with a review of documentation and maps of the area and with interviews with other specialists on the subject.

Characteristics of the natural environment of the Alto-Orinoco and of land use in the region will then be considered, placing emphasis on the use of remote sensing systems and climatic data.

Finally, case studies will be made using the "Guri" model developed by Rabinovich at IVIC. These will cover the Casiquiare river catchment and selected areas showing different types of human intervention in the natural environment.

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Community structure of benthos of mud flats and mangroves in the Colombian Pacific coast in relation to pollution.

Fellowship period: October 1989 - September 1990

Summary

The principal objectives of this study were to identify the main benthic communities associated with the mudflats and mangroves of Buenaventura and Malaga bays of the Colombian Pacific Coast; to study the general environmental biology of these bays; and to try to establish a relationship between these communities and pollution sources.

6 HEALTH AND BIOMEDICAL SCIENCES

Summary

Health and biomedical sciences are the most important subject area of International Scientific Cooperation with the Andean Pact countries with 9 research projects and 5 fellowships reported here. This reflects the priority given to the subject and particularly to diseases of public health importance in the programme. The studies range from the molecular genetics of variation in human populations and susceptibility to disease (pages 100, 117 and 133), immunology (page 131) and cellular biochemistry (page 126) to drug chemistry and design (pages 105, 115), the interaction between nutrition and the effects of high altitude (page 109) and disease (page 139) and include social factors affecting the use of pharmaceutical products and a consideration of community-based disease control (page 127).

The principal diseases covered by these studies include malaria, schistosomiasis, leishmaniasis, Chagas' disease, tuberculosis, human papilloma virus, and cervical and stomach cancer.

Joint Research Projects

14 Patterns of distribution, prescription and consumption of pharmaceutical products in Peru and Bolivia

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Contract number and duration: Cl1*/0210/0210-01, April 1988 to January 1991.

Objectives

To identify the patterns of distribution, prescription and consumption of pharmaceutical products in Peru and Bolivia and to show to what extent an educational intervention could change people's irrational use of drugs to a more rational one.

Study design

The study was carried out in five areas in Peru and two in Bolivia. In Peru, these were two marginal urban areas on the coast in Lima and Chimbote; one marginal urban area in the highlands (Cajamarca 1); one middle-class urban area in the highlands (Cajamarca 2); and one area with small towns in the Oxapampa province, eastern Peru. In Bolivia there was one marginal urban area in the highlands (El Alto) and one rural area in the tropical part of the country (Alto Beni).

The methods used were a formal household survey with questionnaires in a sample of 5391 households; qualitative studies in the general population; "simulated client interviews" in 22 drugstores and different health units; and bacteriological studies of antibiotic resistance. There was also an educational campaign about the irrational use of drugs in three study areas. In each of them a control group was maintained without intervention in order to be able to measure the impact of the campaign before and after and control versus intervention group. A separate study on the validity and quality of interview data showed the research was of high consistency.

The demographic and socioeconomic situation in the study areas

The demographic structure in the study populations: In the population pyramids, the typical triangular shape could be identified of populations with a high fertility rate, relatively low life expectancy and some indication of migratory movements. The proportion of children was between 33% and 53%. The median number of persons per household was 5.6; an expression of the traditional cohesion of families and the scarcity of houses.

Socioeconomic characteristics of the study populations: The level of schooling was relatively high in the middle class population of Cajamarca 2 and, unexpectedly, in the urban marginal area of Lima (probably due to the young age of its population). By contrast, in the rural area of Alto Beni and the urban marginal area of La Paz the level of school attendance was low.

The commonest occupations were self-employed (hawkers, market sellers, artisans, etc.) and employees (particularly workers in factories).

Morbidity and risk groups

Magnitude of morbidity: Between 22% and 53% of the persons living in the different study areas had suffered from some illness during the two weeks preceding the interview, which is within the range of results found in other studies in Latin America with a similar methodology.

The populations in our study which had a high disease prevalence are similar to those living under difficult conditions in deprived rural areas. The other populations, with relatively low disease prevalences, showed similar features to other Latin American metropolitan areas where the standard of living is usually better.

Health risks according to age and sex: As could be expected, we found the highest morbidity rates in under-fives and in women.

Health risks according to economic situation: The relative risk of contracting a severe disease was particularly high (5.8 times) in the rural areas of Alto Beni compared to the middle class population of Cajamarca 2; it was moderate (between 1.9 and 2.8 times) in the other study areas. An inverse correlation was found between socioeconomic level and severe morbidity.

The therapeutic response

"Therapeutic response" is defined as all those actions taken by the sick and their families in order to be cured.

Self treatment: Self treatment was the most frequent therapeutic response in all study areas. Typical behaviour was to do nothing: 13% (range 4% - 30%) of all the ill people; to use traditional medicine: 15% (range 9% - 27%) of all ill people; or self treatment with drugs: 42% (range 25%-47%) of all ill people.

Explanatory factors for the use of health services: The accessibility of the health services and their attractiveness or appeal play an important role in their acceptance. In the middle class population of Cajamarca 2, a better utilisation of the health services was observed (particularly of the private services) than in the other areas. The use of health services was relatively frequent for infectious diseases, chronic diseases and diseases perceived as being severe. The under-fives were almost always taken to the health services if diseased; in the other age groups, the use of health services was less frequent. There were no gender differences in the use of health services.

The consumption of drugs

Results of the qualitative study in a marginal urban population of Lima: The methodology consisted of group discussions in mothers' clubs and interviews with heads of households who had taken their sick children to the health centre. People's opinions about essential drugs (*medicamentos básicos*) were usually negative. They thought that these drugs were produced only for the poor and were thus of poor quality.

It was noticed that the large drug companies had a real impact on people's opinions because the quality of a drug was associated with its presentation and wrapping. Furthermore, the irrational use of drugs and the use of unnecessary or even dangerous drugs was related to an exaggerated concern for a disease and to the underestimation of side-effects. There was a general lack of knowledge about the indications for, and potential dangers of, some potent medicines.

Results of the quantitative study: Two types of irrational drug use could be identified: first, an inappropriate use of specific drugs for certain diseases and second, use of drugs with an irrational blend of components. The inappropriate use of drugs can be shown by the frequent consumption of drugs to combat the common cold, coughs, diarrhoea and lack of appetite. These drugs have to be considered irrational because they do not have any proven therapeutic efficacy. The irrational consumption of drugs is principally conditioned by the pharmaceutical supply in the private market. Self treatment often leads to irrational drug use but this is also due to senseless professional prescription. It was observed that most unnecessary drugs, such as multivitamins and appetizers, had been prescribed by physicians. Furthermore, the analysis of drugstores showed that the sale of medicines without any control and the drug advice given by the drugstore staff were generally inappropriate.

The use of antibiotics and bacterial resistance: The study of *E. coli* strains (isolated from stool specimens) provided general information about the prevalence of resistance to widely-used antibiotics. It enabled the existence to be demonstrated of a direct correlation between the consumption of antibiotics and the prevalence of strains resistant to them.

The impact of the educational campaign

One of the major objectives of the study was to show to what extent an educational campaign could change people's irrational use of drugs into a more rational one. The educational campaigns were designed, on the one hand, for volunteer health workers in the communities and, on the other, for the general population. Different didactic methods were used, such as didactic games, posters, flannel boards, role playing, megaphone announcements, handouts, marches, meetings, workshops, discussion groups, etc.

By means of these activities it was hoped to decrease self treatment; decrease the use of drugs whenever they were not indicated (common cold, diarrhoea, loss of appetite); and decrease the use of those drugs identified as being not essential or even dangerous.

The measurement after the campaign, by means of the second household survey, showed a general change in people's behaviour; there was less self-treatment and there were more treatments without drugs. However, no significant change was found in the actual consumption of some drugs. In other words, those who had used non-essential drugs for common diseases (diarrhoea, common cold, loss of appetite) before the campaign continued to do so afterwards. Use of metamizol (dipirone) decreased for non-visceral pain and it was less frequently found among the drugs stored at home. However, its use for fever was unchanged.

Direct and indirect results of the study

On the basis of the results of the study results and the experience gained in health education during the campaign, teaching materials have been developed which will be used in the universities for improved training of medical and pharmaceutical students in the prescription of drugs. The results of these activities will be measured in another phase of the study. The wide dissemination of the project results among the Ministries of Health, medical associations, universities, health services and study populations showed very positive responses. Members of the research team have been invited by the Ministry of Health and the Pan-American Health Organisation to help in the design of a new drug policy in Peru and to support other research projects in this field.

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15 Etiological studies on cervical cancer

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Contract number and duration: Cl1*/0371, April 1989 to March 1992.

Background and objectives

Cervical cancer is an important public health problem. Worldwide, it is the second most common cancer in females and it is the most common cancer in the developing world. Areas at high risk include the majority of countries in Latin America. The disease in these areas frequently develops during the reproductive age, in which women mostly both contribute to the labour force and to the support of families. The etiology of cervical cancer as a possible consequence of a venereal infection is currently one of the most active research areas in cancer epidemiology. The role of herpes virus type 2 has been postulated for about 30 years without definitive evidence. Recently, evidence of a strong statistical association with certain types of human papillomavirus (HPV) has been reported but because of methodological limitations of the available assays used to assess exposure to HPV, the nature and magnitude of the association with this virus remains uncertain.

Three research projects aimed at shedding light on the causes of cervical cancer, and which use populations with contrasting risks for this cancer, are being undertaken. These projects have been designed by the Unit of Field and Intervention Studies (FIS) of the International Agency for Research on Cancer (IARC) in Lyon, France. The FIS Unit is also coordinating and supervising the implementation of the projects in conjunction with the appropriate institutions in the various collaborating countries. The first two research projects are being carried out in Spain and in Cali, Colombia; the third involves countries in Europe, Latin-America, Africa and Asia.

I. Case-control study of cervical cancer in Spain and Colombia

Background

The incidence of cervical cancer is 10 times higher in Colombia than in Spain. This collaborative study was set up to explain the reasons for this large difference and to evaluate the role of infection with human papillomaviruses in the development of cervical cancer.

Materials and methods



Figure 1: Over 3000 questionnaires from Spain and Colombia were completed and analysed in Lyon for the case-control study on cervical cancer

A population-based case-control study was conducted in nine provinces of Spain (Alava, Gerona, Guipuzcoa, Murcia, Navarra, Salamanca, Sevilla, Vizcaya and Zaragoza) and in Cali, Colombia, including 406 women with incident invasive cervical cancer, 416 population controls; 526 women with high-grade CIN lesions and 512 controls. In addition, 1,108 husbands of cases and controls also participated in the study to evaluate the role of the male in the transmission of the potential risk factor(s) for cervical cancer.

The investigation included completion of a questionnaire, collection of serum and of exfoliated cells from the cervix of case and control women and from the penis of their husbands. Questionnaires have been analysed using the standard methods of case-control studies. Multivariate statistical techniques have been used to adjust the estimates of the relative risk for potential confounders.

The assays for STD markers were selected because of their documented high sensitivity and specificity and they were performed in several European laboratories (J. Orfila, Laboratory of General Bacteriology and Immunology, Amiens, France; I. Lind, Statens Seruminstitut, Copenhagen, Denmark; B. Wahren, National Bacteriological Laboratory, Stockholm, Sweden). Three different methods of detecting HPV-DNA have been used in aliquot samples of the specimens. These were the ViraPap^R, Southern blot and polymerase chain reaction (PCR L₁ and generic).

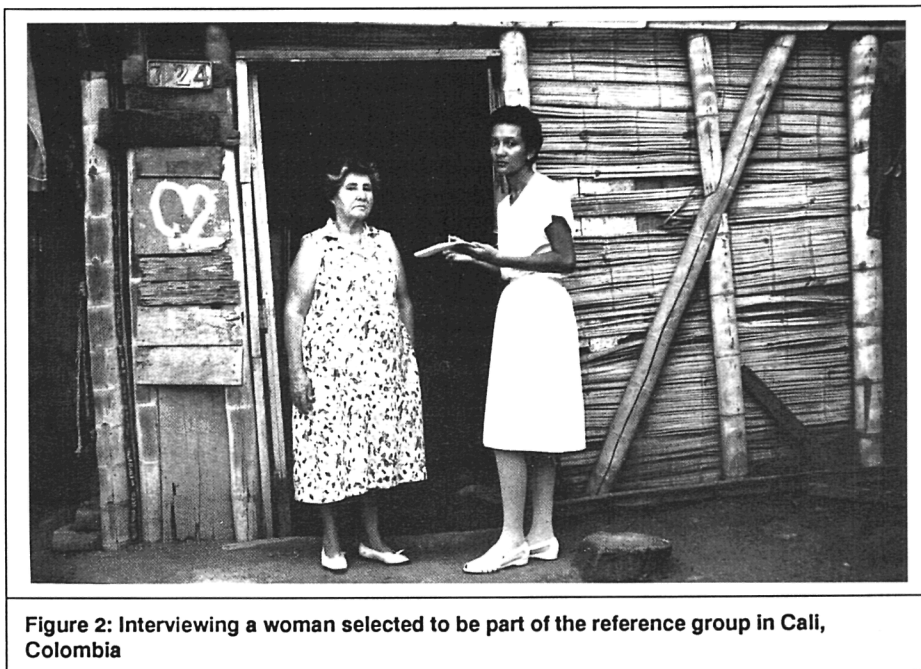


Figure 2: Interviewing a woman selected to be part of the reference group in Cali, Colombia

Results

Analysis of the questionnaire indicated that the main risk factors in women were: early age at first sexual intercourse or at first birth, high number of sexual partners, low level of education and practice of prostitution. Among husbands, a high number of lifetime sexual partners and contacts with prostitutes were risk factors in Spain but not in Colombia.

Concerning HPV, the adjusted relative risks (RR_a) and (95% confidence interval, CI) for the two countries for a positive *ViraPap*^R, Southern blot and PCR were as follows:

	Spain RR_a (95% CI)	Colombia RR_a (95% CI)
<i>ViraPap</i> ^R	26.2 (9.3-73.5)	77.5 (10.6-568)
Southern blot	10.1 (4.3-23.6)	10.4 (3.8-28.6)
PCR	45.3 (17.9-114.8)	14.6 (6.4-33.2)

Type-specific analysis indicated that HPV-16 was the predominant HPV type in both countries. For *in situ* cases and controls only the *ViraPap*^R was used showing a RR_a of 27.2 (9.7-76.1) in Spain and 73.8 (10.1-539.0) in Colombia.

For HSV-2, significantly increased risks were observed for invasive cancers in Spain and CIN lesions in Colombia. Seropositivity for *Chlamydia* was associated with an increased risk for CIN lesions in both countries and seropositivity for *N. gonorrhoeae* with an increased risk for invasive cancer in Spain only.

Discussion

These results provide, for the first time, quantitative estimates of the association between HPV-DNA and cervical cancer which is method-independent. It has also demonstrated that the contribution of HPV is consistent in two countries at high and low risk from cervical cancer. One striking finding of this project results from the comparison between the prevalences of HPV-DNA among the population controls in both countries. Using the PCR results, the HPV prevalence was slightly higher in Cali (15%) than in Spain (5%), but the difference is not large enough to explain the differences in incidence of cervical cancer. It thus seems that other factors might be necessary to promote HPV lesions into neoplastic lesions. Amongst the likely candidates are HSV-2 and other sexually-transmitted agents.

Extension of the study to other areas of the world

Following the completion of this case-control study new questions arose. Specifically, it remains to be clarified why male sexual behaviour is a risk factor in Spain but not in Colombia.

It was therefore decided to prepare a new version of the questionnaires in which these aspects are more extensively addressed and to initiate studies in areas at high risk from cervical cancer in which a wider range of exposures/practices could be found. These areas were identified in Brazil, Paraguay, Mali, Morocco, Thailand and the Philippines. These studies are at different stages of implementation but no results are available.

II. Prevalence survey of CIN lesions among prostitutes from Spain and Colombia

Objectives

The purpose of this survey is to verify if the population of prostitutes could be considered as a high-risk group for Cervical Intraepithelial Neoplasia (CIN) and also as a reservoir of the viral infections associated with cervical cancer.

Methods

The prevalence of CIN lesions among prostitutes and non-prostitutes was determined by reviewing the cytology records of various centres in Cali, Colombia and in Oviedo, Spain. In Cali, 805 prostitutes and 1,957 non-prostitutes were included in the study. In Oviedo, 758 prostitutes and 1,203 non-prostitutes who have had a first cervical cytology during 1985-90 were included. Data on the prevalence of various sexually transmitted diseases (STD) among prostitutes was available in Spain only.

Results

The prevalence of CIN lesions among prostitutes was 2.5% in Spain and 1.9% in Colombia, while among non-prostitutes it was 1.2% in Spain and 1.0% in Colombia. These results indicate that the prevalence of CIN lesions is higher among prostitutes than among non-prostitutes in both countries but that this difference is less than expected. There is no difference in the prevalence of CIN lesions in these two groups between Colombia and Spain. This is in great contrast with the 10-fold difference in the incidence of cervical cancer which exists between these two countries. This might suggest that the differential in risk is mainly due to factors acting in the late phases of the carcinogenic process.

Concerning the prevalence of STD among prostitutes in Spain, the most common infections were *Trichomonas vaginalis* (36.5%) and syphilis (25.1%) followed by chlamydia (14.6%), mycoplasma (12.2%), gonorrhoea (11.4%), *Candida albicans* (11.0%), HIV (4.9%), hepatitis B (4.6%) and HSV-2 (0.7%). The prevalence of CIN lesions was significantly higher among the HIV-positive (15.2%) than among the HIV-negative prostitutes (1.4%) ($P < 0.001$).

III. The international biological study on cervical cancer

Background and objectives

The purpose of this project is to assess at the international level the prevalence of several biological markers of sexually transmitted diseases among cases of invasive cervical cancer. Particular attention is devoted in this project to assess the prevalence of different types of human papilloma-viruses.

Materials and methods

A standard protocol and questionnaire were developed. Nineteen countries have agreed to participate in this effort and are currently at different stages of data collection. These are Algeria, Argentina, Benin, Bolivia, Brazil, Canada, Chile, Colombia, Cuba, Guinea, Mali, Panama, Paraguay, Rwanda, Spain, Sudan, Tanzania, Uganda, and the United Kingdom. Collaborators have been asked to identify a minimum of 50 consecutive cases of invasive cervical cancer attending one (or several) health facilities in their area. For each person agreeing to participate the following are obtained: a biopsy slide in which the histology of the lesion is documented, a frozen biopsy or tissue specimen, a serum sample, a brief questionnaire on the most relevant risk factors for cervical cancer, and a questionnaire on clinical stage, means of diagnosis and macroscopic appearance of the neoplastic lesions.

The tests for HPV will all be conducted by one laboratory under strict conditions of non-contamination. The most sensitive and specific methods will be used, namely methods based on DNA amplification techniques (PCR).

Results

As of March 1991, ten countries have completed the study and all material has been safely received at IARC. The remaining countries are expected to finalise data collection within the first semester of 1992 at which time the laboratory component of the study will commence.

16 Synthesis and characterisation of transition metal complexes with n-donors ligands. Application to chemotherapy of Chagas' disease, leishmaniasis, cancer and to catalytic reactions

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Contract number and duration: Cl1*/0450 January 1990 to December 1992.

I. The application of transition metal complexes as potential drugs against tropical diseases.

Background and objectives

Chagas' disease is a particularly harmful illness, affecting at least twenty million people in South America and for which there is no known effective therapy. The agent responsible for the disease is *Trypanosoma cruzi* which appears to be sensitive to radical producing agents. The activity of some transition metal (TM) complexes as drugs and chemotherapeutic agents has also been reported. The typical classical drugs for Chagas' disease (and leishmaniasis) are nitroheterocycles (nitroimidazoles, nitrofurans, etc.) but even the most active ones are only active during the early stages of the illness.

The objective of this part of the project is the application of transition metal complexes as potential drugs against tropical diseases (essentially Chagas' disease and to some extent, leishmaniasis and malaria). Eventually some complexes will be tested in cancer chemotherapy.

Materials and methods

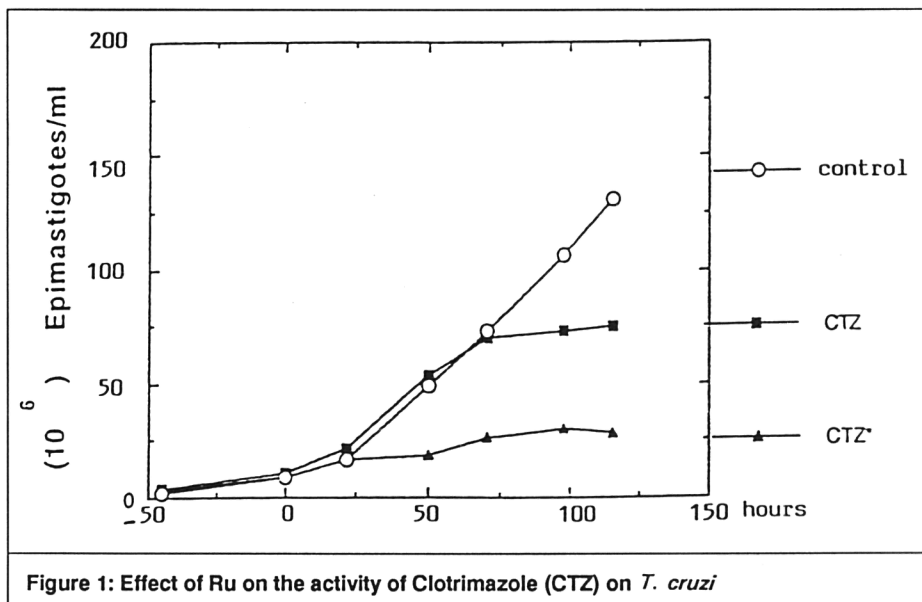
The first stage of the project was based on the synthesis, by the Venezuelan laboratory (IVIC), of TM-complexes (Ru, Cu, Pt) of commercial drugs and on the synthesis of nitroheterocyclic ligands in the University of Liege (ULg). The ligands will be tested in IVIC as well as the corresponding TM-complexes of the most promising ones.

Complexes were synthesised by contacting in a solvent (eg: CH_2Cl_2) metal salts or complexes (e.g., RuCl_3 , RuCl_2 , $(\text{DMSO})_4$, $\text{RuCl}_2(\text{NCMe})_4$, CuCl , $\text{Cu}(\text{NCMe})_4\text{PF}_6$, PtCl_2 , L_2PtCl_4) with the ligands (e.g., 2- and 4-nitroimidazoles, metronidazole, clotrimazole, ketoconazole, mebendazole, quinoline, cloroquine, primaquine, etc.). The resulting complexes are investigated by elemental analysis and classical spectroscopical methods.

The biological tests are run on the epimastigote form of *Trypanosoma cruzi* in LIT medium, according to the protocol reported by Urbina (Universidad Central de Venezuela - UCV). The proliferation of the epimastigote was measured as a function of time (numbers of epimastigotes/ml at different times) in the presence of the compounds to be tested.

Results

During 1990, a number of new compounds were prepared by interaction of appropriate Cu, Ru and Pt complexes with organic molecules known to have some antiparasitic activity, such as nitroimidazoles, substituted imidazoles and quinolines. The new compounds were characterised by NMR and analytical data, and those that appeared adequate were used in biological tests (*in vitro*) against the epimastigote form of *Trypanosoma cruzi*.



Clotrimazole (CTZ) complexes. The most interesting results were obtained from the interaction of clotrimazole with $\text{RuCl}_2(\text{NCMe})_4$, leading to a new organic molecule CTZ*, not yet fully characterised. As shown in Figure 1, at a concentration of 10^{-5} M, this compound displays a remarkably high lethal effect on the parasites; preliminary tests show it not to be toxic towards mammal cells. CTZ* was obtained with $\text{CTZ}/\text{Ru} = 2$, although it is noted that with $\text{CTZ}/\text{Ru} = 5$, the activity obtained was more or less the same as for CTZ alone.

Various complexes were isolated but did not display any significant biological activity (e.g. $\text{K}_2\text{PtCl}_2\text{-4-}$ or 2-nitroimidazoles, $\text{K}_2\text{PtCl}_2\text{-metronidazole}$, $\text{PtCl}_2\text{-2-methyl-nitroimidazole}$, $[\text{Cu}(\text{NCMe})_4 \times (\text{clotrimazole}) \times] \text{PF}_6$, $\text{PtCl}_2\text{-biotine}$).

Some complexes are highly insoluble and are not suitable for biological tests; they were, therefore, not investigated further.

Quinoline complexes. Four Ru-quinoline complexes (including one chloroquinine - an antimalaria drug) and six cuquinoline (including chloroquine and primaquine) have been prepared. None of these complexes are active against Chagas' disease but will be tested against malaria (in collaboration with H.Perez at the Microbiology Center of IVIC).

Ligands. Various ligands (nitroimidazole derivatives) were synthesised in ULg. Complexes will be prepared in IVIC and they will be tested (as well as their complexes) for biological activity.

Conclusions

The investigation of a promising biologically-active compound against Chagas' disease, prepared during the initial stage of this research programme, constitutes the main objective of the next period (structure determination and full therapeutic evaluation).

The synthesis of novel complexes from novel ligands (potentially active such as nitroimidazoles) and their screening will be continued with further development directed toward the synthesis of such ligands connected to biochemically significant entities (sugars, nucleosides, lipids, etc.) that could act as a "pilot" for the potential drug.

These ligands as well as some of their complexes will also be tested. A particular extension will consist in synthesising nitro-derivatives of selenoheterocycles (e.g., nitroselenazoles) and their complexes. Screening within the context of leishmaniasis (and eventually cancer) chemotherapy is also foreseen.

A second aspect will be the extension of the programme to malaria by investigating transition metal-quinoline complexes.

II. Application of some complexes to olefin metathesis reactions.

Background and objectives

Metathesis of olefins is a very important industrial reaction applied *inter alia* to the preparation of polymers (e.g., polypropylenamer) and to the redistribution of olefinic fractions. In fact, an investigation of Ru(II) complexes by an IVIC student in Liege led to the discovery of a catalytic activity for cross-metathesis and metathesis of olefins such as norbornene and styrene.

Further investigation of this catalysis system was continued during the first year of the present project and the screening of some of the complexes prepared by IVIC is one of the objectives of the next years together with some applications in organic synthesis (e.g: carbene chemistry).

The problem of the metathesis of functionalised olefins remains partly unsolved and constitutes a difficult problem. Extension of the investigation to the metathesis of functionalised olefins is now the objective of a novel stimulation (twinning) project within the EC involving four European institutions.

Materials and methods

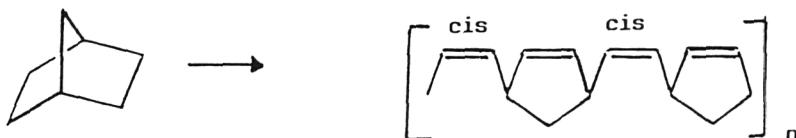
The reaction was run in the presence of Ru salts (halides, carboxylates) activated by a carbene precursor (ethyl diazoacetate) in chlorobenzene as solvent at room temperature. The tested olefins are norbornene, styrene and cyclo-octene.

The analyses were performed by GLC and the products were identified by MS and ^1H and ^{13}C -NMR techniques.

Results

The Ru-based catalyst (Ru(II) salts + carbene precursor such as diazoesters) resulted from an initial collaboration between IVIC and ULg: cross metathesis of norbornene-styrene was observed.

Further studies have been devoted this year to Ru(II) carboxylates (particularly the trifluoroacetate) as catalyst for norbornene polymerisation. Some cis-selectivity (+/- 70%) is observed in the polymer. Some Ru acetates and carboxylates required a Lewis acid as cocatalyst (in addition to the carbene precursor).



Styrene and cyclo-octene also polymerize under these conditions.

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Fontaine, M.; Hubert, A.J.; Noels, A.F.; Demonceau, A. and Teyssie, P. (1991). Cobalt-catalyzed dimerization of styrenes under syngas. *Journal of Organometallic Chemistry*, 417 (1-2).

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17 Effects of nutrition and physical activity on physical capacities of prepubertal children living at altitude

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Contract number and duration: CI1*/0507, January 1990 to December 1993.

Background and objectives

The major part of the activities covered by this report were carried out during the scientific visit to Bolivia which took place from 27 June to 7 September 1990. Four people from the University of Clermont Ferrand I and three people from the University of Amsterdam took part. Most of the visit took place in La Paz (3700 m), in the premises of the Bolivian Institute for Biology at Altitude (IBBA). Members of ORSTOM-Nutrition, established in Bolivia, and researchers and technicians of IBBA also attended.

The objective was to study the effects of nutrition and physical activity on the physical capacities of prepubertal children living at altitude. With the written agreement of their parents and with the consent of the children, 139 boys, from 10 to 12 years old, were studied. These boys came from three groups:

1. 32 boys from the Franco-Bolivian College, from comfortable homes at a high socio-economic level;
2. 46 boys, from the Hernando Siles College in the Villa Fatima area, from poor families at a low socio-economic level;
3. 41 boys, from the Jesus Obrero college in the Alto quarter, from families at a medium socio-economic level.

Overall, the children were of cross-bred ancestry with a preponderance of Aymara. They were for the most part born in La Paz and had always lived there, at altitudes between 4100m (in the upper town) and 3200 m (the lowest part of town).



Figure 1: The Bolivian Institute for Biology at Altitude, La Paz.

Data collection

The following data were gathered for all the children:

Anthropometric data. Weight, height (standing and seated), skin folds with determination of fat content, biacromial, bi-iliac and thoracic antero-posterior diameters, circumference of thorax, of the arms, the thighs and the calves with determination of total and muscular volumes of the lower members and the thighs. These data were completed by a full clinical examination, with determination of the Tanner stages from the secondary sexual characteristics, volume of testicles (orchidometry) and by taking saliva samples to determine for proportion of testosterone in the saliva. A survey was made at the same time of personal antecedents, family, ethnic origin and weekly physical activity.

Spirometric data. These comprised measurements of pulmonary volumes (current volume, inspiratory and expiratory reserve volumes, vital capacity, residual volume (helium method) with calculation of residual functional capacity), the maximal expiratory debit and the breathing rate in repose.

Bioenergetic data. These were collected on a cyclo-ergometer adapted for children, equipped with an electronic revolution counter with recording of the curves of pedalling speed as a function of time. Initially, after a learning phase, the maximum oxygen consumption ($V O_2 \text{ max}$) was measured by a direct method. The proportion of the maximum value of blood lactates, sampled at the earlobe, was measured immediately by an enzymatic micromethod (Analox). Several days later a force-speed test (for determination of the maximum anaerobic power) was held and a 30-second Wingate test (for determination of "anaerobic capacity") with sampling of expired gases during the test, to measure the aerobic share. After each of the tests the concentration of blood lactates were measured.

Haematological data. The following measurements were made: haematocrits, concentration of haemoglobin, blood count, and blood type, evaluation of mean globular volume, of the content and mean globular percentage of haemoglobin. Measurements were also made of the erythrocytic concentration of protoporphyrin.

Biochemical data. These were collected from the 103 children who agreed to the taking of blood samples from a vein. Data comprised the proportion of albumin and of all proteins (colorometric method), of pre-albumin and of orosomucoids (radial immunodiffusion)

Evaluation of energy balance. This was carried out on a limited number of subjects.

1. Evaluation of energy expenditure. This was done for 52 boys (10 from the Franco-Bolivian college, 24 from Villa Fatima and 18 from the Alto). The calculation was made from the heart rate, continuously recorded for 24 hours (Sport Tester). The relationship between heart rate and oxygen consumption was previously established in the laboratory. The study was completed by an evaluation of the physical activity of children by the interview method. The data obtained are expressed in equivalent metabolic units (MET): low activity corresponds to an energy expenditure lower than 7 MET, medium activity to values between 7 and 10 MET, intense activity to values greater than 10 MET.

2. Evaluation of food intake. Of the 52 boys considered above, 47 were studied (7 from the Franco-Bolivian, 23 from Villa Fatima and 17 from the Alto). The evaluation rested on the technique of interviews in the families together with weighing the food intake and analysis of its composition.

Impedance measurement data. These were obtained with the Thomasset equipment (Impedancemetre EMI) with implantation of needles under the skin and the use of two frequencies (5 kHz and 1 MHz).

Results

Before all the analyses of the data, one fact must be underlined. The children from the Franco-Bolivian and the Alto colleges gave the impression of being in good health whilst the boys from the Villa Fatima school appeared to be in a rather miserable state; clothes dirty and tattered, health deplorable with many cases of scabies, often infected. Equally it is necessary to emphasise that the children of the Alto are on average older (12 +/- 0.7 years) than those of the Franco-Bolivian (11.2 +/- 0.8) and of Villa Fatima (10.9 +/- 0.7). In this report, therefore, there is an insistence on the comparison between the Franco-Bolivian and the Villa Fatima being made for groups of children having roughly the same age. The statistical analysis rests on the analysis of variance of a single factor (Single factor ANOVA Test).

Anthropometric data. These clearly bring out the difference between the Franco-Bolivian College boys (FB) and those from Villa Fatima (VF). For the same age the children of VF are shorter (10 cm less), lighter (7 kg less) and have less fat (16.5% body fat against 20.2%). Taking into consideration the circumference of the upper arm and the cross-sectional area of the thigh, good criteria of nutritional state, a significant difference is noted between the groups (arm circumference of 18.9 cm for VF and 21.5 cm for FB, cross sectional area of the thigh 132 cm² for VF and 164 cm² for FB). The gonadic maturity of the two groups is identical, the mean for VF as well as for FB children was prepubertal.

Spirometric data. These do not differ between the children of VF and FB. It is noted that the values of residual functional capacity (RFC) and of residual volume (RV) are, for these groups at altitude, higher than those that have been described for children of the same age at low altitude. This fact has already been described for adults resident in the high Andean plateaux: is it an ethnic characteristic or is it a question of a phenomenon of adaptation to long term living at high altitude? Experimental data collected in animal species would seem to favour the second hypothesis.

Bioenergetic data. Maximum oxygen consumption ($\dot{V} O_2 \text{ max}$) was high in all three groups; although lower in absolute terms for VF compared with FB, the values are not significantly different when expressed in relative terms (by kg of total body mass or by kg of lean mass). As has already been described, maximum heart rates are lower at high altitude (11 to 13 beats per minute less in La Paz) than at low altitude. As has already been observed for prepubertal children, the maximum values of blood lactates ($[L]V O_2 \text{ max}$) are low for all three groups. No significant differences are noted between VF and FB.

Force-speed tests revealed significant differences between the groups, particularly between VF and FB. Whether expressed in absolute values (maximum anaerobic power in watts) or in relative terms (maximum anaerobic power per kg of total body mass or per litre of muscular volume or per cm^2 cross-sectional area of the thigh) the values for VF boys are significantly lower than those of the FB boys.

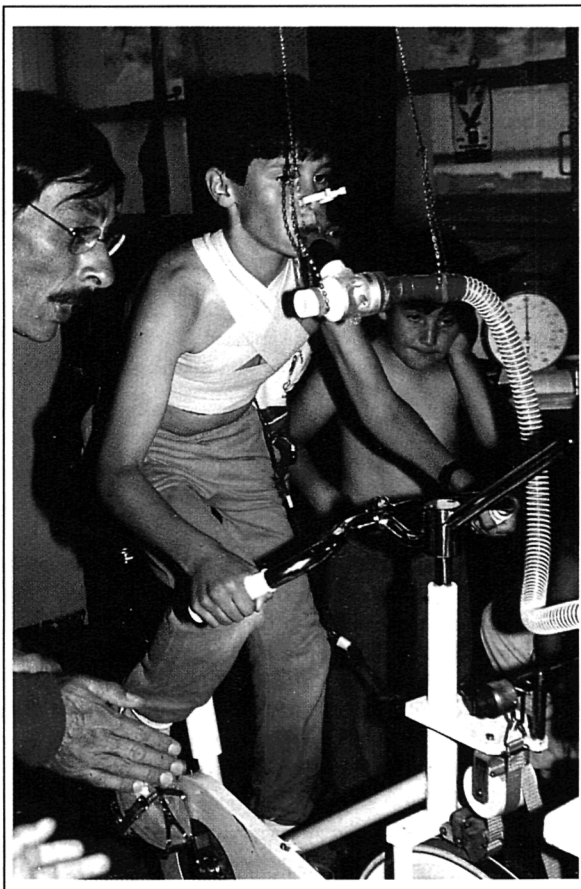


Figure 2: $\dot{V} O_2 \text{ max}$ determination on a highland boy with cycle ergometer.

Wingate tests showed the same; the VF values are significantly lower than those of FB for peak and median power, whether expressed as absolute values or in relative values. The maximum values of $[L]$ obtained during these tests are not significantly different between VF and FB.

Haematological data. Over all the groups, only three cases of mild anaemia were observed. The median values of haemocrits and of blood concentrations of haemoglobin are higher than those of children of the same age studied at sea level; this is explained by the erythropoietic response induced by chronic hypoxia at altitude. This response was of the same size for all the groups and no significant differences can be noted between VF and FB. The values for erythrocytic protoporphyrin lie within the normal limits for sea level.

Biochemical data. No significant difference was observed between groups.

Data concerning energy balance: energy expenditure. Comparison of the profiles of activity established by the interview method shows no significant differences for sleeping time, low activity (less than 7 MET), and medium activity periods (between 7 and 10 MET). Periods of high activity (more than 10 MET) are greater for VF children than for those of FB.

Food intake. No significant difference was observed between the groups studied.

Impedance measurement data. These were obtained for only 44 children (23 boys from VF and 21 from Alto). Impedances corresponding to intracellular liquids (Z1) and extracellular liquids (Z5) do not differ between the two groups. The relationship Z5/Z1 is, however, higher for VF (1.41 +/- 0.03) compared with AL (1.37 +/- 0.05). Normally, between 10 and 12 years, this relationship lies around 1.37 +/- 0.05. It is a biological constant which indicates a good hydroelectrolytic balance in an organism. The abnormally high value for group VF could be an indicator of a hydroelectrolytic imbalance amongst these children.

Preliminary conclusions

The totality of the data on the low socioeconomic group (VF) compared with that for the high-level group (FB) shows the following:

A net retardation of height/weight development is associated with a lower percentage mass of body fat and lower muscular mass of the thighs and arms. It should be noted that, despite this retardation, the thoracic cage and pulmonary volumes have developed in the same way as in the group from the high socio-economic level. The phenomenon of "adaptation" to the chronic hypoxia of altitude is thus maintained amongst these underprivileged children.

There is no impairment of the aerobic metabolic pathway expressed in relative terms (by kg of body or muscular mass). There is a great diminution of the maximum anaerobic strength and capacities, in absolute terms as well as relatively. This bears witness to a qualitative impairment of the anaerobic muscular metabolic pathway. The haematological and biochemical data provide no obvious evidence of malnutrition; in particular, there was no abnormally high proportion of anaemia (only 3 mild cases reported). Total proteins, albumins and orosomucoids lie in their normal zones. Pre-albumin, even though low on average, is not significantly different from that of other groups. Food intakes also lie within normal limits; it must be emphasised, however, that the interview method used to establish them is coarse and very approximate.

Lastly it should be noted that it is the underprivileged group (VF) that had to undergo the greatest bioenergetic constraints (intense activity is more marked in this group than amongst the other children); the calculation of energy expenditure from heart rate is in process (H.C.G. Kemper, Amsterdam). The impedance measurement data, whose use in the evaluation of child nutrition is still little developed, provide some evidence of an anomaly in the hydroelectrolytic equilibrium in the organisms of the VF children.

Our earlier studies had shown that at La Paz (3700 m) boys from a good socio-economic level show no retardation of height/weight development compared with their equivalents at low altitude (Clermont-Ferrand, 300 m). They are characterised by a development of the anaerobic metabolic pathway which is comparable or even more precocious. The underprivileged group (VF), despite the absence of evident biological criteria of malnutrition, are characterised, in comparison with the privileged group, by a net retardation of height/weight development and a collapse of anaerobic physical aptitudes, even though their aerobic aptitudes are relatively well conserved.

1991 Work programme

Analysis of data gathered during previous mission. This phase is planned for February - March 1991. It will include completion of the quantitative analyses not yet done:

Salivary testosterone (by the Clermont-Ferrand team)

Reactive protein, iron and ferritic sera and if possible vitamins A and D3 (by ORSTOM-Nutrition, Bolivia)

Determination of the alimentary contributions of iron, calcium and other vitamins.

The actual processing of the data will be completed by a study of the relationships of the different magnitudes, measured or calculated. This work is being undertaken by Philippe Obert, a student preparing his doctoral thesis on the theme "Physical Aptitudes and Nutrition in Children".

Validation of methods used during the study. The use of heart rate for evaluation of the daily energy expenditure of a child will be correlated with the calorimetric chamber method (indirect respiratory calorimetric chambers of Vermorel at the Institut National de Recherche Agronomique at Theix Clermont-Ferrand). This correlation should allow a reduction in the errors corresponding to physiological situations such as rest and muscular exercise of less than 50% maximum oxygen consumption, after-meal periods and, above all, sleep.

The impedance measurement method will similarly be correlated with data on children collected by other authors.

Mission 1991. This visit is planned for July and August 1991. It will take place mostly in Santa Cruz de la Sierra (420 m). Accommodation will be provided by the National Centre for Tropical Diseases (CENETROP), built with Belgian cooperation and directed by Benjamin Ribero. The study will be conducted on two groups of children.

From a high socio-economic level; children from the private "De La Salle" school with the collaboration of Alfredo Romero Davallos, Director of the Institute of Cardiology at Santa Cruz.

From a low level; pupils from the state school of the zone called "Plan 3000". This is a poor quarter of the town in which are housed 300 broken families, victims of floods in the zone of Santa Cruz.

The materials, methods and personnel will be identical with those of the previous mission. The parasitological study will be entrusted to the CENETROP personnel. Costs of the mission will be particularly high because of the need to charter an aircraft to transport material which could not survive without damage being transported on Bolivian tracks; because of the need to lodge, feed and pay a large party of Bolivian personnel based in La Paz; and because of the need to pay for the physical and scientific support of CENETROP. Because of these high costs the duration of the visit, initially planned for two months, will most likely have to be reduced to match the available finance. Medicines will be supplied by "*Pharmaciens sans frontières*", a charitable group based in Clermont-Ferrand, in order to combat the infectious and parasitic diseases which are very prevalent at low altitude in Bolivia.

This study will provide a low altitude reference which should enable better interpretation of the data collected at high altitude in July-August 1990 (La Paz, 3700 m).

18 Mechanism of action of polyene antibiotics. *In vitro* studies for the improvement of the therapeutic index against leishmaniasis

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Contract number and duration: Cl1*/0517, May 1990 to April 1993.

Background and objectives

Leishmania are obligate intracellular parasites that cause a spectrum of diseases called leishmaniasis, ranging from self-healing skin ulcers to a fatal visceral infection. Leishmaniasis is one of the six diseases, important in tropical countries, that the World Health Organisation selected for inclusion in its special programme for research and training in tropical diseases. As many as 20 million people are thought to be infected and more than 400,000 new cases appear each year, 4,000 of which are in Venezuela. Only a few drugs are available for treatment of leishmaniasis. The need for several intravenous treatments, the toxicity of the drugs used and the frequent relapses make the present treatment possibilities less than satisfactory.

Consequently, there is a need for new drugs. Among them the polyene antibiotic Amphotericin B (AmB) can be useful in the treatment of visceral and mucosal leishmaniasis, although very few studies have been devoted to this question. It is the most common drug for the treatment of fungal diseases and recent studies have shown that its therapeutic index, limited by renal toxicity, could be increased by the use of liposomes (lipid vesicles in which the antibiotic is encapsulated) as a delivery system.

The aim of this research is to determine whether the liposomal formulations proposed for fungal diseases can be applied to leishmaniasis and, if so, whether they can be improved. A study will be carried out on the mechanism of action of AmB against the pathogenic strain *Leishmania mexicana*, taking into account that *Leishmania*, found in the promastigote form in the gut of the infected sandfly transmitting the parasite, is in the amastigote form in the macrophages (host cells). The activity will then be studied of liposomal AmB preparations which will be designed taking into account the results obtained on the mechanism of action of the free form of the drug. The penetration of AmB and liposomal AmB into the macrophages and how they reach their target will be analysed. From these results it is hoped to be able to propose an improved formulation of liposomal AmB.

Materials and methods

AmB is generally assumed to act at the plasma membrane level of the parasite, by perturbing the membrane permeability. Therefore our study will determine the binding of AmB to plasma membranes of *Leishmania* in promastigote or amastigote form, or macrophages or tubular renal cells, and its consequences for the transmembrane permeability and the viability of the cells.

The following specific methods will be applied. Heat-transformation will be applied to obtain amastigote-like *Leishmania* from promastigotes. The influence of AmB on cell viability will be determined by incorporation of ethidium bromide and measurement of its fluorescence. Binding of AmB to isolated membranes will be measured by circular dichroism, which will also give information on the conformational change in the drug when incorporated in the membrane. Permeability to protons, sodium, potassium, calcium and small electrolytes will be measured by different methods: rapid kinetics methods (stopped-flow), specific electrodes, absorption and fluorescence spectroscopies of fluorescent probes. Compartmentation of pH in *Leishmania*, macrophages and *Leishmania* parasiting macrophages will be obtained by microspectrofluorimetry. Different types of liposomal formulations will be used: large unilamellar vesicles, multilamellar vesicles and mixed micelles.

Results and discussion

At the present time the study is only in its initial stages. It has been possible to demonstrate an enhancement of AmB action on *Leishmania mexicana* resulting from heat transformation and to relate it to a modification of AmB binding. Heat transformation modified the binding from non-cooperative to cooperative, decreasing the amount of antibiotic bound to the membrane. Thus, the increased rate of ethidium bromide incorporation into transformed cells was not related either to the amount of AmB bound or to an increased amount of ergosterol in the membrane (the ergosterol/lipid ratio was four times smaller after heat-shock). An increase in the magnesium content of the external aqueous solution was able to prevent the AmB-induced incorporation of ethidium bromide into *Leishmania* promastigotes to a greater extent than it was into heat-transformed cells, suggesting that there were significant changes at the *Leishmania* cell surface on heat transformation.

Conclusion

The project is just at the stage of start-up, however it can already be said that the collaborative approach will be fruitful. It allows the European partner to gain knowledge on the biology and the biochemistry of the parasite whilst the Venezuelan partner will gain access to sophisticated equipment such as microspectrofluorimetry and circular dichroism.

It is thought that the cooperative study, which aims at an understanding of the physiochemical basis of the differential selectivity of AmB and liposomal AmB to promastigote and to amastigote-like forms of *Leishmania*, may have important applications for the improvement of the therapeutic index and treatment strategies against leishmaniasis.

Publications

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Cohen, B.E.; Benaïm, G.; Ruiz, M.C. and Michaelangeli, F. (1990) Increased calcium permeability is not responsible for the rapid lethal effects of amphotericin B on *Leishmania* sp. *FEBS Letters*, **259**, 286-8.

19 Studies on etiology and prevention of stomach cancer in Venezuela

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Contract number and duration: CI1*/0555, June 1990 to May 1994.

Background and objectives

In 1980, cancer of the stomach was the most frequent cancer worldwide, although its incidence is declining. In Latin America, the high risk areas are the Andean regions of Venezuela, Colombia, Ecuador, Chile and Peru, as well as in the central mountains of Costa Rica. It is known, particularly from migrant studies, that the risk of stomach cancer is established early in life. This implies that in the high risk countries, it will be many decades before stomach cancer ceases to be an important public health problem. It is important, therefore, that practical preventive measures are identified, to be applied at the population level.

Primarily prevention demands knowledge of factors which either increase or decrease the risk. Previous studies have suggested that, as might be expected, the most important are elements of lifestyle, particularly diet. Since it is very difficult to change dietary habits, a more feasible approach is to seek to supplement the diet with agents which decrease risk. Certain micronutrients have been shown in epidemiological studies to have a protective effect. The biological basis of this may well be their action in interrupting the natural course of the disease. For gastric cancer, the progression is thought to start with intestinal metaplasia of the mucosa which progresses through dysplasia to cancer. There is experimental and epidemiological evidence to show that β -carotene and perhaps other antioxidants are able to block progression from metaplasia and dysplasia. They have the potential, therefore, for preventing the onset of invasive disease.

A further approach to prevention is to detect invasive disease at an early stage, which then permits treatment to be more effective. Early detection in a population requires mass screening; most experience of this has been with photofluorographic examination of the stomach, particularly in Japan, where a large proportion of gastric cancer is found in an early stage. However, it is essential to be certain that the outcome of early detection is avoidance of advanced disease, and therefore death, in the population that is screened.

This joint research project embraces three related studies, as follows:

1. A case-control study of etiological factors
2. Case-control studies to evaluate effectiveness of screening
3. Chemoprevention trial of gastric dysplasia

1 A case-control study of etiological factors

300 patients with histologically-diagnosed gastric cancer will be recruited from the two principal hospitals in San Cristobal, which together treat approximately 60% of cases in Tachira State. 300 matched controls from the same hospitals, and 300 neighbourhood controls, will be investigated by questionnaire, orientated towards lifestyle and detailed dietary habits. In addition, markers for exposure to carcinogenic substances, and levels of protective factors (micronutrients) will be sought in specimens of blood and urine. The degree of association between the different etiological factors and the two principal histological subtypes of gastric cancer ("intestinal" and "diffuse") will be investigated. The various laboratory assays will be carried out in San Cristobal (sodium and creatinine in urine), at the IARC laboratory (DNA adducts in lymphocytes and 3-methyladenine in urine) and at selected European laboratories (levels of micronutrients in blood). Statistical analysis of the results will be done at IARC with the collaboration of the Venezuelan epidemiologist who will be the local field coordinator.

2 Case-control studies to evaluate effectiveness of screening

Case-control study with deaths from gastric cancer as the "case" group. Deaths in residents of Tachira State certified as gastric cancer (part I of the death certificate) for 1987 and 1988 (approx. 250) will form the case group. For each case, three live controls of the same sex and age group (+/- 3 years) who are the nearest neighbours to the cases will be selected from the electoral register. Cases and controls will be compared in terms of their screening histories, using the computerised records of the screening programme in the Cancer Control Centre. The relative protection in relation to the number of tests and to the time since the last test will be estimated.

This study can be completed rapidly (first year of project). It has the disadvantage, however, that possible selection bias can only be minimised by the close residential matching, not by controlling for possible confounding factors during the analysis.

Case-control study of advanced gastric cancer cases (living). To overcome the defects of the preceding case-control study, a case-control study of advanced gastric cancer cases (living) will be undertaken. The cases and their corresponding controls will be a subset (probably about 75%) of those enrolled in the etiological study. All of the cases and controls in the study will have been interviewed for relevant etiological factors (see above). In addition, they will be asked about behaviour in relation to preventive medicine and other "providential" behaviour (to determine whether this relates to screening attendance, and/or to disease status), and their history of gastric cancer screening. Screening histories will also be determined from the records of the Cancer Control Centre computer. Again, the protective effect of screening will be evaluated as the relative protection associated with different numbers of examinations and the time elapsed since the last test. Possible confounding by relevant etiological factors and, if found to be important, health behaviour, can be controlled for during the analysis, a method of minimising the selection bias. The latter is inevitable in any non-randomised study of the effectiveness of screening. It is the consequence of the fact that, in general, it may well be low risk individuals who elect to be screened.

Analysis of the two studies of screening efficacy will be carried out at IARC in collaboration with the Venezuelan investigators.

3 Chemoprevention trial of gastric dysplasia

This study will be a double-blind randomised trial to determine whether certain antioxidants (β -carotene, ascorbate) can interrupt the gastric carcinogenic process by blocking the progression from intestinal metaplasia to dysplasia. A total of 800 subjects, 35-65 years of age, with histological diagnosis of intestinal metaplasia will be recruited from the early detection programme of gastric cancer in Tachira state. These subjects will have to be in good health, give their consent to participate in the trial and will have to be residents of the Tachira state. On entry to the trial all subjects will be interviewed, a physical and endoscopic examination performed and gastric biopsies taken. Blood and urine specimens will also be collected and then they will be randomised into two groups; one will receive the treatment (β -carotene and vitamin C) and the other will receive a placebo. The treatment and placebo pills will be distributed at monthly intervals when compliance with the treatment will also be recorded. One and two years after the initiation of the treatment, a physical examination will be performed and blood and urine specimens will be collected in a subsample. At the end of the treatment period (3 years after initiation), the interview, physical and endoscopic examination with gastric biopsies, collection of blood and urine specimens will be performed on all subjects. At the end of the study, the code of the treatment will be broken by the statistician at IARC and analysis of the results will be done jointly by IARC and the Venezuelan investigators. Several end points will be used to evaluate the effect of the treatment: regression or progression of histologically-confirmed precursor lesions and changes in the expression of sulphomucins and relevant oncogenes.

20 The anticoagulant factor(s) present in the saliva of vampire bats (*Desmodus rotundus*)

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Contract number and duration: Cl1*/0575, May 1990 to October 1991.

Objectives

The main objective was the study of the anticoagulant factor(s) present in vampire bat saliva with the purpose of establishing its site of action in the coagulation cascade, as well as its structure. This knowledge is relevant to a better understanding of the phenomena of blood coagulation and the discovery of new anticoagulants with potential clinical use.

Materials and methods

Animals. Vampire bats (*Desmodus rotundus*) were regularly captured from wild colonies living in a cave in the northwest part of Venezuela (State of Falcón). Twenty vampires were currently kept in captivity, in individual cages of the metabolic type, under controlled light and temperature (25°C). The animals were maintained on bovine blood anticoagulated with 3.2% sodium citrate at a ratio of 1:9. Food was given every 24 hours, always in the late afternoon. Water was given *ad libitum*.

Saliva collection. For saliva collection, the vampires were anaesthetised and, once anaesthetised, pilocarpine was placed in the mouth in order to stimulate salivation (see Figure 1). Saliva was collected in plastic microcentrifuge tubes and placed in ice. The collection period was 15 to 20 mins, with a regular yield of about 1 ml/animal. Individual samples were kept at -30°C until use. Routinely, after thawing, each individual sample was centrifuged at 12000 g for 5 minutes, pooled and dialysed in the cold for 24 hours against 10 vol. of deionised water. The dialysate was centrifuged at 48000 g for 20 minutes, in order to discard any insoluble material.

Depending on the particular experiment, the dialysate was stored in aliquots, at -30°C or, lyophilised and re-dissolved in 1/10 the original volume. Repeated freezing and thawing or lyophilising of the dialysed saliva did not affect its anticoagulant activity.

Most experiments to be described in this summary were done with partially purified saliva (PPS), corresponding to material pooled from the active fractions (anticoagulant activity) obtained after gel filtration of dialysed saliva through Sephacryl S-200.

Coagulation assays: Whole Blood Coagulation Time (WBCT). This was measured in 1 ml samples. The first 1 ml to 2 ml of blood, after venipuncture, was discarded. Coagulation was allowed to proceed at 37°C, in glass tubes containing 50 µl of EACA (114 mg/ml). Under these conditions, the coagulation time of controls was 180 seconds.

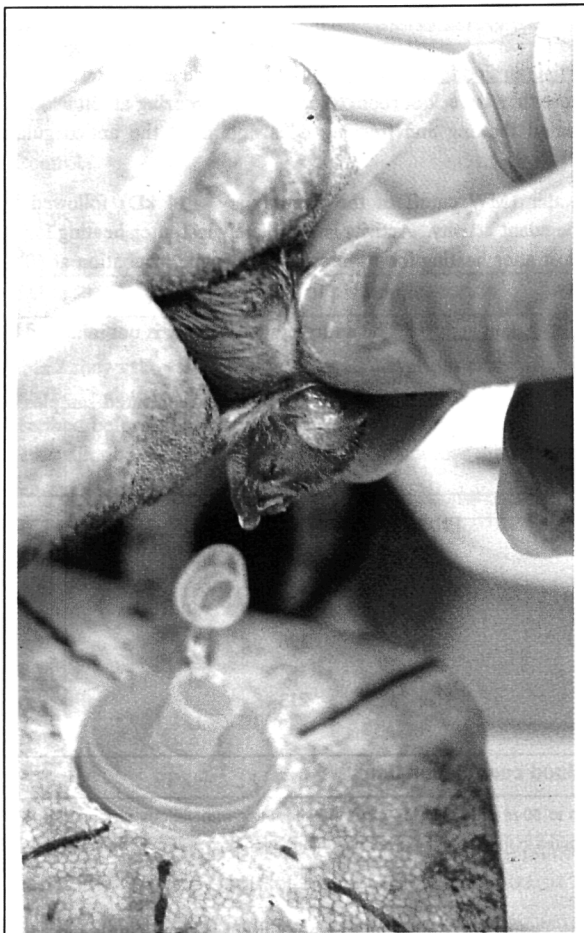


Figure 1: A salivating vampire.

Activated Partial Thromboplastin Time (APTT). This was measured using a commercial partial Thromboplastin, which contains elagic acid as activator. Since the saliva or partial purified fractions (S-200) contains an activator of plasminogen, all the coagulation assays were performed in the presence of EACA. A typical assay contains: 100 μ l of citrated plasma; 25 μ l of EACA (128 mg/ml); 80 μ l distilled water; 20 μ l Thrombofax. After 3 mins incubation at 37°C, coagulation was triggered by the addition of 100 μ l of 20 mM calcium. Final volume of 325 μ l is always maintained at the expense of water. Under these conditions, control APTT is 45-47 seconds.

Prothrombin Time (PT). This was measured using a commercial Brain Thrombo-plastin/Calcium chloride reagent. For assay, 100 μ l of citrated plasma is maintained at 37°C for 2 mins., and 200 μ l of prewarmed thrombo-plastin /calcium reagent is added to initiate coagulation. Control PT was 13.4 seconds. For some experiments, the commercial reagent was appropriately diluted.

Thrombin Time (TT). This was assayed with bovine thrombin. Experimental conditions were chosen in order to obtain a TT of 30 seconds.

Factor Xa activity. This was assayed using purified FXa and the chromogenic substrate S-2222. The reaction was followed at 405 nm in a Hemker Photometer and all relevant parameters were calculated by dedicated software developed for and along with the photometer in the Instrumental Development and the Biochemistry Departments of the University of Limburg.

Protein determination. This was done using the method described by Bradford.

Results

Properties of the crude saliva or the partial purified fractions: Stability. The crude saliva, as well as the partial purified fraction, is stable for more than a week at room temperature. Freezing at -30 or -80°C does not make any difference. Repeated thawing and freezing does not affect the anticoagulant activity.

Extensive dialysis against deionised water (MW cutoff of the membrane = 14 kD) followed by lyophilisation does not affect the anticoagulant activity. Activity is completely lost after heating for 10 mins at 80°C. However, only 40% is lost after heating for 10 mins at 60°C while incubation at 60°C for 30 mins produces 80% loss of activity.

Effect of protease inhibitors on the anticoagulant activity. The anticoagulant activity is not inhibited by PMFS (1 mM) or DFP (2 mM).

Presence of heparin-like components. Uronic acid was not detectable in 10x concentrated samples of saliva.

Effect of PPS on coagulation assays.

PPS ($\mu\text{g/ml}$)	Prolongation of WBCT (sec.)
2.5	56
5.0	126
10.0	213

Table 1: Effect of PPS on whole blood coagulation time.

One ml of whole blood was taken directly on to 50 μl of EACA (114 mg/ml), without or with various amounts of PPS. Mean WBCT of controls was 180 seconds. Figures in the Table are given as prolongation of WBCT over the controls.

Effect on whole blood coagulation time. The coagulation time of whole blood is significantly increased in the presence of PPS, in a dose-dependent manner (Table 1). Clot retraction is not apparently affected by the presence of PPS.

Effect on Activated Partial Thromboplastin Time (APTT). PPS prolongs the APTT in a dose-dependent manner. This effect shows a saturation behaviour and after a certain concentration linearity is lost and plasma becomes incoagulable. The effect of PPS on the APTT is markedly decreased if saliva is added to plasma already activated with thromboplastin (3 mins, 37°C), followed immediately by addition of calcium. Increasing the incubation time of plasma and saliva, prior to activation, does not influence the inhibitory effect. Activation of plasma with Thromboplastin for 10 mins, in the presence of saliva, does not correct the APTT.

Effect on Prothrombin Time (PT). Incubation of plasma with PPS, at concentrations that prolong the APTT > 10 mins., produces prolongation of the PT by 3 seconds.

Effect on Thrombin Time (TT). This is not affected by PPS even at concentrations that produce an almost infinite prolongation of the APTT. This is in agreement with preliminary experiments using the chromogenic substrate S-2238, which show no effect of PPS on thrombin activity.

Effect of PPS on plasma coagulation triggered by diluted thromboplastin.

Thromboplastin Dilution	0	1/10	1/100	1/500	1/1000
Control	14	25.5	51.5	90.6	102.5
+ PPS	18	39.2	145.9	238.0	480

Table 2: Effect of PPS on PT of plasma, seconds

Table 2 shows the values obtained for PT at various rabbit brain thromboplastin dilutions. As has been described by others, PT increases with increasing thromboplastin dilution. In the presence of PPS, as described before, there is a prolongation of PT of 3-4 seconds with undiluted thromboplastin and, at 1/1000 dilution, the prolongation of PT is about four times the control PT.

Effect of PPS on the APTT of FVIII-deficient plasma.

Addition	APTT (sec)
None	128
PPS	750
1.3 U FVIII/ml	31
PPS + 1.3 U FVIII/ml	90

Table 3: Effect of PPS on APTT of FVIII-deficient plasma. PPS was used at a concentration that produced an APTT of normal plasma = 77 sec.

Table 3 shows that incubation of FVIII-deficient plasma (FVIII <0.5% of control plasma) with PPS produces a marked prolongation of the APTT. This prolongation of APTT is only partially corrected by addition of purified FVIII. Similarly, addition of purified FVIII to normal plasma does not correct the inhibitory effect of PPS.

Effect of PPS on the APTT of FIX-deficient plasma.

Addition	APTT (sec)
FIX deficient plasma	130
FIX deficient plasma + PPS	1440

Table 4: Effect of PPS on APTT of FIX-deficient plasma. Amount of PPS as in Table 3.

Table 4 shows that incubation of FIX-deficient plasma with PPS results in a dramatic prolongation of the APTT, which is more pronounced than that observed with FVIII-deficient plasma.

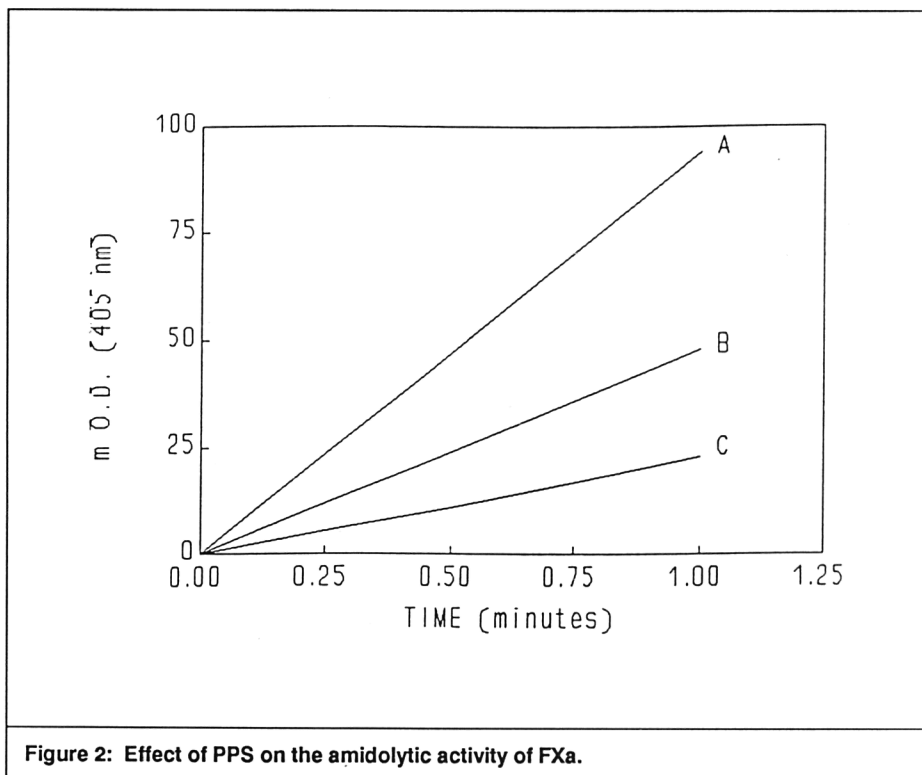
Effect of PPS on the amidolytic activity of purified FXa.

Figure 2 (Curve A) shows the progress of colour generation due to the amidolytic activity of FXa on the chromogenic substrate S-222. Curves B and C correspond to colour generation, under the same conditions, in the presence of two arbitrary concentrations of PPS. These results clearly indicate that PPS directly inhibits, in a dose-dependent manner, the amidolytic activity of FXa.

Discussion of results and direction of future work

The presence of anticoagulant factor(s) in the saliva of blood-feeding bats (vampires) has been suggested by several authors. While a potent plasminogen activator with properties somewhat similar to the tissue plasminogen activator (tPA), have been reasonably characterised biochemically, studies of the presumed anticoagulant factor(s) have been so far inconclusive. The results presented in this summary yield compelling evidence for the existence in vampire saliva of at least one component, different from the plasminogen activator, that is capable of inhibiting blood coagulation. It is clear that this anticoagulant compound(s) is not an antithrombin or a heparin-like substance. The results obtained of the effect of partial purified saliva on the different coagulation assays strongly suggest that a major site of action must be at or below the molecular step dependent on the activity of factor IXa. At present, the precise mechanism of action at this level is not clear. The observation that the anticoagulant effect is also seen on the extrinsic pathway suggested that not only the intrinsic generation of factor Xa was affected, but also the extrinsic and/or the common pathway. This was corroborated by experiments in which the inhibitory effect of PPS was directly demonstrated on the amidolytic activity of purified factor Xa on a specific chromogenic substrate. Besides the obvious

relevance of this observation, it provides a method of assay for the anticoagulant activity that is, at the same time, highly specific and sensitive.

At this point, the immediate effort will be centred in the purification and structural characterisation of the factor involved in the specific inhibition of factor Xa. Preliminary experiments indicate that the molecular weight of the compound that inhibits FXa is in the range of 20 to 50 kDa. With the chromogenic assay described, which will be adapted to an ELISA Microplate Reader, the follow-up of the various purification procedures to be tested will be greatly facilitated. Once a purified inhibitor is obtained, assays will be implemented to find if the same factor is responsible for the inhibition of TenAse. In any event, detailed studies on the kinetics and mechanism of the inhibition are planned.

The obtaining of a highly purified material with the anticoagulant properties described necessarily leads to the possibility of obtaining it by modern biotechnology techniques, which can supply enough quantities for further studies related to its potential clinical use.

21 Effect of stress on the localisation of the glucose transporter in cells

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Contract number and duration: CI1*/0765, January 1991 to December 1993.

Objectives

To determine the intracellular localisation of the glucose transporter protein in tissues of experimental animals and in blood cells of man, and to study the effect of stress, insulin and other treatments on this location.

Materials and methods

Animals. Mice will be subjected to two kinds of stress initially: hyperthermia (e.g. 20-60 minutes at 40-45°C) and virus infection (e.g. Semliki Forest virus infection); fed, starved and experimentally diabetic mice will also be examined, before and after injection of insulin. Other inducers of cellular stress (e.g. sodium arsenite; anoxia) may also be tested, once the initial experiments have been performed.

Preparation of tissue sections. When mice are killed, tissues will be rapidly dissected out and samples frozen and sectioned in a cryostat. After fixation and permeabilization (ice-cold acetone or triton X-100), sections will be incubated with an affinity-purified preparation of anti-glucose transporter antibody (raised in rabbits). After washing, sections will be incubated with an FITC-labelled goat anti-rabbit antibody (commercially available). Stained sections will be examined with a fluorescence microscope and photographed as appropriate.

Blood cells from human volunteers. Blood smears of healthy, starved, fed, diabetic and stressed (post surgical trauma; sepsis) volunteers will be prepared and stained as described above. Plasma insulin (and other hormones, interleukins etc) will be measured simultaneously.

Co-ordination of research. The research work in this project is shared in the following manner. Work in Venezuela includes that on hyperthermia and insulin in fed, starved and diabetic mice. Work in the UK includes that on virus infection in mice and on stress, starvation, feeding and diabetes in man.

It is noted that the project is part of a larger, international, collaborative programme aimed at characterising the glucose transporter protein, from its molecular biology to its function in man. It has links to a project in the USA, on the mechanism of translocation (*in vitro* studies), and to another joint UK - India project on the molecular biology of the glucose transporter.

22 Community-based control of malaria and leishmaniasis in Ecuador and Peru

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Contract number and duration: C11*/0766, January 1991 to June 1992.

Objective

The achievement or improvement of community-based control of malaria and leishmaniasis in Ecuador and Peru.

Materials and methods

The project will commence with an exchange of current knowledge on malaria and leishmaniasis control in an International Workshop, paying particular attention to the results of another EC supported project in Ecuador (Community based malaria control in Ecuador: an intervention study (TS2/62). Thereafter, household interview surveys will be carried out in a number of different selected areas of Peru and in Ecuador, with a total sample of around 5,000 households. An "in-depth longitudinal" study will be made in a number of communities (30 to 60 households each), in which team members will perform a strict surveillance of the activities of all the community members during two months.

There will be a campaign in different communities of each study area, with similar communities serving as controls, to provide health education (using new teaching methods developed by the Heidelberg team, to be presented through a workshop on educational techniques); impregnation of mosquito-nets by community health workers; application of floating *Bacillus thuringiensis israelensis* preparations, produced locally in coconuts; use of insecticide-impregnated soap to avoid extradomiliary transmission; and environmental engineering (water management techniques) and the introduction of larvivorous fish.

The campaign against leishmaniasis will include the identification of transmission foci near the communities; health education using the new didactic methods directed to early detection, an early search for appropriate treatment and the avoidance of unnecessary exposure to infection. Consideration will be given to the use of pain-relieving ointment in areas where only cutaneous leishmaniasis exists.

The Ecuadorian team will extend its activities to the entire coast of Ecuador and work through the National Service for Malaria Eradication (SNEM), non-governmental organisations and the general health services, for the introduction of impregnated mosquito-nets and the development of educational methods that can easily be applied at the community level.

To measure the impact of the campaigns, a second household survey will be applied (on a similar sample to that used for the first) in the same study areas, in control and campaign communities.

Entomological studies will be carried out from the beginning and will last for 12 months. They will include the identification of *Anopheles* species present in the study areas, and determination of man-biting activity, the human blood index, the sporozoite index (determined through ELISA), the oogonic cycle and determination of mosquito survival. Data from the latter will be used for the application of the malaria mathematical model.

With respect to leishmaniasis transmission, the species of sandflies present in the study area and their behaviour will be studied.

Computer analysis will be made of the data and their interpretation. A second International Workshop will be held at which the analysis of the project results will be presented and discussed.

Report

Staffing. The first step was to hire and to train the main field staff in the different project areas. Whereas in Ecuador the project could rely on experienced field workers from a former EC-supported project, in Peru new personnel had to be selected and trained. These preparatory activities took place from January to June 1991.

Study areas. These have been defined, as follows:

18 communities in the canton (district) of San Lorenzo, northeast Ecuador (Esmeraldas province);

28 communities in the canton (district) of Muisne, northeast Ecuador (Esmeraldas province);

10 communities in the district of Chulucanas ("Alto Piura"), north coast of Peru (Department of Piura);

63 communities in the district of Tambopata, Upper Amazon region of east Peru (Department of Madre Dios);

4 communities in the catchment area of Pilcopata, Upper Amazon region of east Peru (Department of Madre Dios).

In the first two areas, both leishmaniasis and malaria research activities were carried out; in the last noted only leishmaniasis and in the rest, only malaria.

Household surveys. After initial collection of data on the demographic, geographic, ecological and socioeconomic characteristics of each study area, household interview surveys were carried out in each area. Activities included training of supervisors and local interviewers and, after the interview process, blood sampling (taken from the people who reported having had malaria attacks during the 6-

month period preceding the interview; negative controls were also taken). The blood smears were examined by three independent microscopists and the serology (immunofluorescence of *P. falciparum*) by two highly experienced professionals.

Some numerical details on the household surveys can be found in Table 1.

	San Lorenzo	Muisne	Alto Piura	Madre de Dios
Communities	18	28	10	63
Housholds interviewed	744	693	1372	1733
Persons included	4166	3880	7030	8844
Coverage of households	90%	93%	99%	90%
Non-responses	14	16	2	8
Interviewers	63	55	81	72
Supervisors	8	6	8	9

Table 1. Parameters of interviews in four study areas

Each geographical area included a fixed number of communities intended to be covered totally. However, due to the absence of some families on the day of interview, it was not possible to attain 100% coverage. However, the number of non-responses was low.

In-depth studies. In addition to the formal interview survey, in-depth studies (longitudinal studies) were carried out in each study area. Each field worker remained for two months in a particular community and did a close follow-up of the daily activities of each community member (using the technique developed by W. Ruiz), including the documentation of illness episodes, particularly of malaria. Futhermore the correct use of mosquito nets was monitored.

Entomological studies. Parallel to these activities, three teams of entomologists were doing night-bite collection of mosquitoes in order to determine, ultimately, the vectorial capacity of the study area.

Workshop. In July 1991, a three-day workshop was organised in Catacaos (north Peru) where the field workers and project supervisors of all study areas discussed their work and presented a rough design of the campaign activities for the future. The Ecuadorian team presented their experience of community health education gained during the previous two years. Peruvian health authorities attended the workshop and gave assurance of their deep interest in the project.

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**Immunochemical characterisation of antigenic enzymes and other
Schistosoma mansoni immunogens of interest for immunodiagnosis
and/or immunoprotection**

Fellowship period: January 1990 - December 1990

Summary of results

Alkaline phosphatase (AKP). Integral membrane-bound glycoprotein of the *S. mansoni* (Sm) surface, native m.w. = 260 kD; Sm AKP was obtained from adult worms by partial purification by n-butanol extraction and purification by passage of butanolic extract (BE) through a specific anti-AKP monoclonal antibody (McAb) affinity column and elution with 2 M MgCl₂. This salt concentration induced a conformational change that decreased AKP affinity for the McAb and eluted it from the support; it was, however, deleterious to the activity.

Alternative protocols were tested. KCl or CsCl were stimulatory but did not elute AKP from the column even at 4 M or in combinations with MgCl₂ concentrations <2M. KCl or NaCl at 0.15 M in 50 mM Tris/HCl pH 8.0 favoured AKP. ZnSO₄ was inhibitory at >1μM. Glycerol 5% acted as an effective stabiliser and triton X-100 at 0.1% (w/v) kept the enzyme in solution.

BEs obtained under the above conditions contained a soluble AKP whose activity lasted for many months at 4°C. The enzyme exhibited also some degree of thermo-resistance (30 mins at 75°C).

0.1-1% β-octylglucoside was partially inhibitory on AKP; on the other hand, 0.5-1% SDS was stimulatory, a result leading to good dissociating conditions from the affinity column. Gel filtration through Sephacryl S300 in 0.5% SDS allowed a homogenous, very active dimeric enzyme preparation to be obtained for use in structural analysis.

Similar to mammalian intestinal AKP, L-Phe was inhibitory for SmAKP (50% at 10 mM). L-Cys at 0.1 mM was 100% inhibitory; this inhibition was aminoacid-specific and not due to the -SH since β-mercaptoethanol (β-ME) at similar concentration did not inhibit AKP; however, 5% β-ME (as in SDS-PAGE) converted the active AKP tetramer (260 kD) and dimer (130 kD) into inactive monomers (65 kD). The expression of the SmAKP activity would thus depend on a S-sensitive monomer-monomer interaction.

Phosphodiesterase (PDE) type I. Integral membrane-bound glycoprotein of the parasite surface showed a native m.w. similar to AKP; it was also present in BEs. The Sm PDE is not retained by the anti-AKP affinity column; it is sensitive to SDS. Like ADP, PDE is also recognised as an antigen by antibodies present in *S. mansoni* - infected patients.

Schistopapain. Soluble S-dependent papain-like Sm protease was partially purified from adult worm homogenates by dialysis vs. HgCl₂/acetate pH 4. Active peaks at pH 5.5 and 6.5 were found with chromogenic or fluorogenic CBZ-PHe-Arg- or CBZ-Arg-Arg- synthetic peptides. Kinetic studies indicated high affinity for the CBZ-Phe-Arg- substrate at pH 5.5. Studies done with analogue synthetic peptides confirmed the Phe-Arg substrate specificity.

Reductive SDS-PAGE (12.5%) and AgNO₃ staining revealed polypeptides of m.w. 65, 40, 27 and 14 kD in the acid/Hg extracts; 27 kD was predominant. Blotting with a rabbit anti-Sm 31 kD (cathepsin B) or anti-Sm 32 kD (S-dependent haemoglobinase) showed weak reactivity of anti-Sm 31 and no reactivity of anti-Sm 32 with the 27 kD component suggesting that cathepsin B-like (papain) activity may be associated with this polypeptide.

The enzyme has been now purified through a kininogen affinity column and HPLC and it is ready for sequence analyses.

Enzyme immunoassays. The immunological sensitivity and specificity of the IgG-dependent AKP immunoassay (APIA) was reported previously to be about 93% and 100%, respectively; it is, so far, an excellent diagnostic assay to be used in the field. Due to the above results on AKP, the biochemical sensitivity of APIA was improved by using preparations of better antigenic quality and by introducing a washing step with 0.1 M MgCl₂ before Sm AKP detection which resulted in a significant decrease of the overall time of reaction.

APIA done with the Sm AKP from a South American strain and sera from patients infected with *S. japonicum*, *S. haematobium* or *S. intercalatum* was negative or exhibited little reactivity suggesting little or no cross-reaction of Sm AKP with that from other schistosome species. Sm AKP is then a specific marker for Sm infections. This might be relevant for diagnosis in areas where infections by more than one schistosome species are present; preparation of immunologically-based maps of endemic areas; and decisions on medical treatment and control measures.

A PDE immunoassay (PDEIA) was developed. Anti-Sm PDE antibodies could be detected sequentially in the same well after anti-AKP detection, using the same individual antibodies and captured antigenic material. PDEIA discriminated between infected and non-infected patients in a manner similar to APIA but with a slightly different pattern of response: a few APIA (+) individuals were PDEIA (-) and *vice versa*. The possibility of detecting the response of the same individual to two (or more) different defined enzyme antigens in a combined APIA/PDEIA test increased the sensitivity of diagnosis.

An immunocapture assay similar to APIA was also developed to detect the anti-papain IgG antibody response. Using fluorogenic substrates at pH 5.5 and 6.5, anti-schistopapain antibodies were found in sera of Venezuelan Sm-infected patients. The pattern of response was different from that with APIA and/or PDEIA. Additional studies are necessary to assess the diagnostic value of this assay (infected vs. non-infected, immune vs. non-immune patients) in relation to the possible inhibition of activity caused by antibodies in some patients.

Publication

Cesari, I.M.; Pujol, F.H.; Alarcon de Noya, B.; Noya, O.; Hoebeke, J. and Bout, D. (1991). Immunodiagnosis based on enzyme markers. *In: Application of Modern Technology in the Development of Techniques and Immunodiagnostic Reagents in Schistosomiasis*; eds. Bergquist, N.R. and Tzotzos, G.T. Intercept Publications, Ferndown, UK. In press.

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Glucose-6-phosphate dehydrogenase and haemoglobin variability in Ecuadorian populations.

Fellowship period: March 1991 - February 1994

Background and objectives

Glucose-6-phosphate dehydrogenase is known to be highly polymorphic in many human populations and more than 300 variants have so far been described. Many of these variants are associated with more or less severe haemolytic episodes, particularly those common in populations exposed to a malarial environment. Equadorian coastal lowlands as well as the Amazonian districts are exposed to an endemic malaria by *P. falciparum* and *P. vivax*. In spite of this, only few and occasional data are so far available on the distribution of G6PD and haemoglobin polymorphisms with reference with the incidence of malaria in Ecuador.

The objectives of this project are:

1. To search for a genetic polymorphism of G6PD and haemoglobins in Ecuadorian populations exposed to a malarial environment.
2. To identify and characterise G6PD variants associated with haemolytic diseases and haemoglobin hereditary disorders (structural and quantitative).
3. To isolate the genes responsible and identify their molecular variations.
4. To acquire a technical background in the manipulation of DNA and the technology of sequencing to be applied to a number of genetic diseases in Ecuadorian populations.

Materials and methods

The research is part of studies already in progress on the epidemiology of genetic diseases and genetic variability in the Ecuadorian population.

Population studies. The G6PD and haemoglobin genetic variation will be studied by the usual electrophoretic techniques on representative samples of Ecuadorian populations especially those belonging to the malarial districts. The G6PD variants identified by electrophoresis other than polymorphic ones will be submitted to further analysis as described below.

Biochemical characterisation. The G6PD variants identified on the basis of their electrophoretic mobility patterns in standard buffer systems will be studied according to the characterisation protocol suggested by WHO, i.e:

- 1 separation by electrophoresis on different substrates;
- 2 activity expressed in I.U./g Hb or % of the average activity;

- 3 activity at different pH;
- 4 Michaelis-Menten constant determination (K_m);
- 5 thermodenaturation rate;
- 6 activity against different substrates.

Xq 28 gene manipulation. If new variant genes are found they will be studied at the DNA level in order to identify the structural basis of the variations.

Mitochondrial DNA. The mitochondrial DNA polymorphisms will be studied by the polymerase chain reaction in a PCR machine with a protocol provided by A. Stoneking and T. Kocher of the Department of Biochemistry, University of California - Berkeley. The DNA sequencing of P.C.R. amplified fragments will be based on the method of Sanger, with modifications by Wrishnik and Higuchi. After autoradiography sequencing, gels will be read and DNA sequences will be stored in a computing system for further comparisons.

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Use of monoclonal antibodies and oligonucleotides (cold probes) in the detection of papilloma virus infection

Fellowship period: October 1990 - March 1991

Background

At the beginning of the fellowship, it was decided to take as a model the application of molecular biology, and in particular non-radioactive probes (cold probes), to the study of a tuberculosis gene. The study of the papilloma virus was developed in parallel.

Tuberculosis study

This was chosen as a model because, at Bogota, work had already been done on a project to describe a gene, *mt40*, and the corresponding protein, which seemed to be specific to *Mycobacterium tuberculosis*. This gene and its product are thus potentially interesting from the diagnostic and epidemiological point of view. These results had yet to be validated and strategies had to be developed to obtain the necessary biological reagents needed for the study of clinical samples. These methods are the same as those that could be employed for the study of the human papilloma virus (VPH) involved in cancer of the uterus. Moreover, at the cold probe laboratory of the Pasteur Institute, research on mycobacteria had already been undertaken and had resulted in the selection of several specific sequences of different types or complexes of the *Mycobacterium* family. The strategy developed for this work has enabled the use of these sequences for clinical diagnosis and epidemiological study of mycobacteria.

Results

Within the above framework and benefitting from the experience, the infrastructure and the assistance of the laboratory, the following results have been achieved.

Detection of *M. tuberculosis* with the help of an oligonucleotide marked with biotin and corresponding to a part of the sequence of the gene *mt40*. After amplification of the gene by the PCR method, it was confirmed that the gene was not amplified unless it came from the ADN of *M. tuberculosis* and not from any other source of mycobacteria. The presence of the amplified product was revealed by hybridisation with the oligonucleotide probe marked with biotin and having a sensitivity and a specificity usable for diagnostic and epidemiological work. Most of the biological reagents had been prepared in the same laboratory.

Detection of *M. tuberculosis* with the help of a plasmid recombinant of the gene *mt40* and marked with acetyl-amino-fluorine (AAF). Having produced the gene *mt40* using the PCR technique, it was then cloned in a plasmid (pUC) and marked with AAF in accordance with the technique used in the laboratory. As a consequence a greater sensitivity was obtained with the AAF probe than with the oligonucleotide probe marked with biotin; these results are in accord with those described by J.L.

Guesdon and his group. Further, the Southern blot technique could be used more specifically to reveal the presence of *M. tuberculosis*. The capability of the plasmid marked with AAF to detect *M. tuberculosis* was equally verified on 18 strains coming from isolates derived from Greek tuberculosis sufferers.

Other *Mycobacteria* studies. In order to apply the methodology and to use the reagents produced, a study has started on biological samples coming from gazelles suspected of being infected with *M. bovis*. It has not been possible to identify the type of mycobacteria which caused the infection but the presence of inhibitors of the PCR reaction was noticed in the samples. Attempts were made to isolate ADN by different techniques in order to eliminate these inhibitors because they constitute what is one of the most important limitations on the application of PCR to the direct detection of pathogenic agents in biological samples. The methodology followed in this study could thus be applied to the VPH model.

Study of the human papilloma virus (VPH)

This study, which was the principal aim of the fellowship, was carried out as follows:

Amplification and purification of plasmids. Plasmids containing the entire genomes of the viruses VPH6, VPH11, VPH16 and VPH18 were obtained from Lucott of the College de France. They were amplified and purified in sufficient quantity to carry out all the work. At the same time the plasmids pUR290, pUR291 and pUR292 which are used in expressing proteins were amplified and purified.

Obtaining oligonucleotide probes marked with biotin. Oligonucleotides corresponding to VPH16 and VPH18 (associated with cancer of the uterus) were built up and marked with biotin and purified in order to test their capacity to detect viral ARN for use in the succeeding stages.

Isolation and purification of fragments of the virus coding for the proteins E6 and E7 of VPH16 and VPH18. The strategy of fractioning and isolation of genes coding for the proteins E6 and E7 (oncogenic) was discussed with F. Thierry of the Oncogenic Virus laboratory at the Pasteur Institute, a specialist in VPH.

Genes corresponding to the proteins E6VPH16, E7VPH16, E6VPH18 and E7VPH18 were purified and cloned in the expression plasmids. For E7VPH16 the gene isolated by PCR had to be used, employing the corresponding oligonucleotides which were synthesised by Igolen in the organic chemistry laboratory of the Pasteur Institute like all the other oligonucleotides used during the fellowship.

Expression of the proteins E6 and E7 of VPH16 and VPH18. After having cloned in the plasmids pUR recombined with their respective genes, the expression of proteins was induced. Through the electrophoresis technique, SDS-PAGE, it was established that the proteins were present in the bacterial extract transfected with the corresponding plasmid. For E6VPH18 the protein was successfully purified, starting from a massive culture (on the advice of O. Puijalon). The other proteins will be purified back in Colombia, assuming that all the original material prepared during the fellowship will be available.

Study of synthetic anti-peptide monoclonal antibodies. This study showed that synthetic anti-peptide monoclonal antibodies derived from the proteins E6 and E7 of VPH16 and VPH18 produced in Colombia were of type IgM and of weak affinity. Having available now complete proteins resulting from the present work, these antibodies could be studied more closely in Colombia. In order to continue this study advice was received from S. Muller (University of Strasbourg) and from F. Traincard (Hybridolab, Pasteur Institute).

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Oligosaccharide assembly and the infectivity of *Leishmania major*

Fellowship period: June 1990 - May 1991

Summary

Recent studies have demonstrated that the life cycle of *Leishmania* spp. includes sequential development from a dividing non-infective stage to a resting infective stage within both the sandfly midgut and axenic culture. At present, the molecular basis of this development (metacyclogenesis) is not understood. Several studies have shown that cell surface carbohydrates of *Leishmania* spp. undergo modification during metacyclogenesis, concerning mainly the Gp63 glycoprotein and the LPG lipophosphoglycan, which are known to play a key role in the process of infection. Therefore, the study of the assembly of oligosaccharides of glycoproteins and glycoproteins are of special interest as it might provide molecular clues to the adaptative mechanisms of promastigotes for survival within the vertebrate host.

Characterisation of the *L. major* strain. The Schneider (Sch) line of *L. major* promastigotes was isolated from BALB/c mice infected with a cloned strain of *L. major* (LV 39), in Schneider's medium + 10% of FBS. It was found that Sch promastigotes were non-infective during the log phase; in stationary phase an increase in infectivity occurred from early to late phase. Metacyclogenesis of Sch promastigotes was accompanied by a change in the affinity of the surface carbohydrates towards the peanut agglutinin (PNA). The highest proportion of PNA-minus forms was detected within the late stationary phase. Thus, the virulence properties exhibited by stationary phase Sch promastigotes could be attributable to the proportion of PNA-minus forms present within the cell population.

N-glycan assembly. Sch promastigotes of *L. major* were harvested from cultures at different phases of growth and labelled with D-[2-³H]-mannose for 1 h. Sequential lipid extractions were allowed to obtain oligosaccharide-P-P-dols (oligo-P-P-dols), a glycoprotein pellet and an aqueous fraction containing soluble oligosaccharide material.

HPLC analysis of the oligosaccharide moieties from oligo-P-P-dols revealed the presence of two major populations of molecules: one (I) behaving as $\text{Man}_5\text{GlcNac}_2$ and the second (II) behaving as $\text{Man}_6\text{GlcNac}_2$, and representing 10-15% and 75-90%, respectively. Structural analysis is further required to establish whether they are similar or not to those reported for *L. mexicana*.

HLPC analysis of the oligosaccharides released from mature glycoproteins indicated the presence of two major oligosaccharide populations, referred to as II and III, and behaving as $\text{Man}_6\text{GlcNac}_2$ (42%) and as $\text{Man}_7\text{GlcNac}_2$ (54%), respectively. It is of interest to determine whether the structure of N-glycans of mature glycoproteins of *L. major* are similar or not to those reported for *L. mexicana*.

Anionic exchange chromatography of oligosaccharides obtained from the aqueous fractions has shown the presence of a population of neutral oligosaccharides (45%) and a population of phospho-oligosaccharides (54%). Mild acid hydrolysis of the latter fraction and subsequent incubation with alkaline phosphatase produced 100% of phosphate-free oligosaccharides, which indicated the presence of phosphodiester bonds. HPLC analysis of both neutral oligosaccharides and phosphate-free oligosaccharides revealed the presence of molecules migrating from 3 to 8 monosaccharides. The origin and polymeric and anionic nature of the material isolated awaits clarification: it might originate from nascent LPG molecules.

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Study of the interaction between infantile malnutrition and Chagas' disease

Fellowship period: February 1989 - January 1990

Background

Protein-energy malnutrition (PEM) is extremely common. 500 million children in the world suffer from PEM and 40,000 of them die each day. Research over the past ten years has shown that one of the most serious consequences of PEM, in terms of life expectancy, is a malfunction of the immune system, concerning essentially the cellular mediated immunity (acquisition of immunological memory, production of interleukin 2-IL2, ...)

The protozoa *Trypanosoma cruzi*, the agent responsible for Chagas' disease, infects 24 million people in Latin America and 65 million are exposed to the risk. At the start of the infection (the acute parasitemic phase), the immune response system of the host infected by *T. cruzi* is characterised by a polyclonal activation, T and B together and an important deficit of IL2, associated with an immunodepression with regard to all antigenic stimulation.

It is thus legitimate to think that PEM in a child, characterised by thymic atrophy and the deficit in the cellular immune response which it brings on, could considerably aggravate the infection. Bolivia, where 35% of the population is estimated to be infected by *T. cruzi*, has also the highest infant (0-4 years) mortality rate for the American continent (200/1000). However, the interactions of infantile PEM and Chagas' disease, both tied to poverty, have never been studied.

Research to be undertaken

The programme aims to reproduce at an experimental level the immunological consequences of infantile PEM (characterised by an attack on the cellular responses and a severe thymic involution) in order to model and to analyse the repercussions on the evolution of the infection *T. cruzi*. The questions posed are, does PEM aggravate infection in young mice? and, does this PEM modify the immune responses to infection with *T. cruzi*? The work will have the following stages.

Establishment of an experimental model of PEM and study of its immune status. After weaning, three groups of mice will be fed, respectively, 100% (control group), 75% and 50% of normal intake. The mortality and weights of all the mice will be monitored regularly. Several mice from each group will be regularly sacrificed from the start of the deficiency test. The thymus will be removed and weighed in order to determine the moment when an involution becomes clearly apparent. The remaining mice will then all be sacrificed and an immunological balance sheet will be drawn up (sub-population of

lymphocytes, tests for proliferation of mitogens, proportions of IL2). Comparison of the results between the groups of mice will enable the impact of PEM on the cellular immune system to be evaluated more precisely.

Influence of PEM on infection by *T. cruzi*. The preceding experiment will be repeated with sufficient mice to assure an adequate survival, allowing for their infection. The mice will be in two groups, one infected with *T. cruzi*, the other not infected. The weight, parasitemy and mortality of these mice will be monitored regularly, up to the 60th day of infection. Comparison of the parameters obtained for the two groups of deprived mice and the control group will allow a judgement to be made of the role of PEM in the eventual aggravation of the acute phase of infection and also of the effect of the association of infection and deprivation on the growth of the mice.

Influence of PEM on the immune response of mice infected by *T. cruzi*. In line with the preceding observations, the experiment will be re-run and the mice killed either at the acute phase or in the chronic stage of the disease, so as to study the non-specific immunological parameters and the specific anti-*T. cruzi* responses (antibodies, proliferative response to parasitic antigens). Comparison of these parameters will allow determination of the effect of PEM on the specific and non-specific immune response.

Expected benefits

The study envisaged will allow a refined analysis of the repercussions of PEM on the immune response and on infection with *T. cruzi*, in a manner that is impossible on human subjects.

Amongst other things, a clear demonstration of an aggravating link between PEM and Chagas' disease *via* the immune response could enable a strategy to be envisaged in which nutritional rehabilitation would restore the immune response and thus limit the infection.

The results of the experimental studies proposed here will allow future research on human subjects to be better oriented on the ground, in response to the request and to the needs of the Bolivian authorities.

7 MATERIALS SCIENCE

Summary

Materials science is a rapidly evolving field and one which is a priority in the Andean Pact countries. In order to promote cooperation in the field, an EC-Andean Pact workshop was held in Mérida, Venezuela in 1990 (page 153).

Topics covered in the reports of activities already initiated include an investigation of the properties of superconducting materials and devices (page 143), the structural properties of ternary semiconductors (page 147) and an application of modern techniques to the dating of ceramics and the identification of archaeological raw material source areas (page 149).



At Centro de Fisica, IVIC, Caracas, M. Octavio (on left) and A. Juan unpack data acquisition equipment for use in experimental studies of superconducting materials and devices (January 1991).

Joint Research Projects**23 Properties of superconducting materials and devices****J. Bindslev Hansen**

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Contract number and duration: Cl1*/0506, January 1990 to December 1991.

Objectives

The objective of the project is to fabricate good quality films of high- T_c superconductors and to fabricate Josephson devices made of Nb-NbO_x-Pb and characterise their microwave response. High- T_c thin films are fabricated using sputtering, electron-beam evaporation and spin-on methods, exploring the various kinds of substrates that yield high-quality thin films. The long range objective of the project is to master the fabrication, patterning and barrier growth techniques that would be required to fabricate Josephson devices based on high- T_c materials and to make useful devices out of them.

Work carried out at IVIC, Caracas**Superconducting materials**

Thick films of high- T_c superconductors. Thick films of Y-Ba-Cu-O have been prepared on various substrate materials, SrTiO₃, BeO, Zr(Ca)O₂, alumina and sapphire. The films were made by mixing powder of the YBa₂Cu₃O_x compound with an organic solvent and painting the solution on the substrates, which were subsequently dried and fired. The effect of the substrate material and the firing temperature on the superconducting properties was studied. The optimum firing temperature was found to be 990°C. In terms of critical temperature, the best substrate material was SrTiO₃; in terms of sharpness of the transition from the normal state to the superconducting state, BeO was found to be superior.

Divergence of the resistance noise of normal-superconductor composites of the critical current. Measurements were carried out of the resistance noise in thick films of Y-Ba-Cu-O and of sputtered thin films of NbN (made at TUD, Denmark) in the proximity of the superconducting/normal transition. The frequency range was from 1 Hz to 100 Hz. At the transition point (around 82°K) it was found that the magnitude of the normalised voltage noise power, S_v/V^2 , rose above its level at room temperature. For a fixed temperature it was observed that S_v/V^2 diverged as the critical current was approached from above and that it was zero below the critical current, I_c . In order to interpret these results, a new critical exponent, x , has been defined for the behaviour close to the transition. If p is the fraction of superconducting material, $I-I_c = (P_c-p)^x$. From measurements on the ceramic YBaCuO films and from theoretical predictions it was found that $x = 1/3$.

Fabrication of thin films of YBaCuO and BiSrCaCuO. Thin films have been made of the high-temperature superconductors YBaCuO and BiCaSrCuO. The YBaCuO films have been fabricated by electron-beam evaporation using multilayer techniques. This work is still in progress. The work on BiSrCaCuO films of the 2212 stoichiometry is at a more advanced stage. Films near the desired stoichiometry were fabricated using resistive evaporation and a multilayer technique in which bismuth was first evaporated and followed by evaporation of CaF₂, SrF₂ and Cu, respectively. The best films were found to be those evaporated onto MgO substrates: they had critical temperatures as high as 87°K, the highest achieved for evaporated films of this composition and stoichiometry. Critical current densities as high 2×10^4 A/cm² were obtained in the best films, near liquid nitrogen temperatures (60°K). Due to limitations in the measuring current, the critical current densities at lower temperatures could not be measured in the best films. Other substrates were also used to make films, including calcium-stabilised zirconia (ZrO₂(Ca)) and SrTiO₃. However, neither the critical temperatures nor the critical current densities of these films approached those for films made on MgO substrates. The salient features of the process used in making these films were the multilayer evaporation, the reduction of the amount of Cu used in the boats to achieve stoichiometry and the post annealing process. Work is underway to explore the possibility of making these films *in situ* as well as characterising some of their Hall-effect properties.

Superconducting devices

Tunnelling effect between normal metals and high T_c superconductors in bulk form. An experimental investigation was made of the tunnelling phenomena in normal-superconducting (N-S) contacts, using both La-Sr-Cu-O and Y-Ba-Cu-O as superconductors. The normal metals used were copper, indium and tungsten. The measured current-voltage characteristics (I-V curves) exhibited a tunnelling conductance which was linear in the applied voltage and which was asymmetrical with respect to a reversal of the voltage polarity. The observed I-V curves were explained by a simple model which incorporated the charging effects in the granular contacts. It was shown that the model led to a tunnelling conductance proportional to the applied voltage. The asymmetry was accounted for by introducing an energy dependent gap in the high-T_c material. The measured energy gap varied from contact to contact as expected for charging effects which offset the apparent energy gap by a voltage given by $V = e/2C$ where C is the capacitance of the granular metallic contact (C is of the order of 0.1 femto-farad or smaller).

Work carried out at the University of Twente

Superconducting materials

Sputtered thin films of YBaCuO high T_c superconductor. Thin films of YBaCuO were fabricated using top-down sputtering, off-axis rf sputtering and laser deposition on various substrates like SrTiO₃, yttria-stabilised zirconia (YSZ), Si with YSZ bufferlayer and MgO. By careful adjustment of the laser

deposition parameters and use of high density targets the so-called "droplet problem" has been completely overcome. By the use of off-axis rf magnetron sputtering excellent films have been grown with $T_c = 90^\circ\text{K}$ and $J_c > 10^6 \text{ A/cm}^2$ at 77°K . Furthermore multilayers of YBaCuO and PrBaCuO have been grown. The latter is not a superconductor and is used in superconductor/normal-metal/superconductor (SNS) device fabrication as a barrier material. Multilayer and bufferlayer systems have been extensively investigated by means of a variety of analysis methods: AES, XPS, XRD and RBS. Interface diffusion is usually negligible and layers can be grown epitaxially as can be seen from RBS measurements. From one of the multilayers, high-resolution transmission electron microscope (HRTEM) pictures were made. The layers show intercalation of CuO planes in agreement with the results of other groups. The structures are, however, fully epitaxial and it was also observed that atomic surface steps on the substrate do not pose any growth problem.

Superconducting devices

SNS junctions and dc SQUIDS. By the use of off-axis sputtering systems, edge type SNS-junctions and dc SQUIDS (Superconducting Quantum Interference Devices) were fabricated based on YBaCuO/PrBaCuO multilayers. Individual junctions exhibit a supercurrent up to 80°K . The $I_c R_n$ product of these junctions usually has a lower limit of 8 mV at 4.2°K . Voltage modulation of the first dc SQUIDS can be observed up to 66°K . The critical current of the junctions varies as $(1-T/T_c)^2$ indicating SNS-like behaviour although insulating barrier layers can not be excluded because of the influence of the depression of the order parameter. The total modulation depth $m = \Delta I_0/I_c$ is roughly 0.2. Noise measurements on one of our dc SQUIDS show a $1/f$ frequency dependence of $2 \times 10^{-8} / f \Phi_0^2 / \text{Hz}$ probably being due to telegraph-like switching noise from flux jumps.

Work carried out at the Technical University of Denmark (TUD), Lyngby.

Superconducting devices

Non-linear dynamics of Josephson junctions, half-harmonic generation in long Josephson tunnel junctions. Experiments were performed in which long Josephson junctions were pumped at 10 GHz and the radiation emitted by the junctions at half the pump frequency was studied. Near the regions in the I-V characteristics where this generated half-harmonic was seen, a high noise rise was also observed at low frequencies suggestive of a bifurcation transition to chaos. However, the presence of higher subharmonics could not be observed and thus the possibility could not be ruled out that the observed noise rise was due to thermally-induced switching between two attractors that are not necessarily chaotic.

Coupled Josephson soliton oscillators. A long and narrow Josephson tunnel junction works as a non-linear oscillator coupled to the resonant transmission line formed by the junction geometry. The effective values of the quality factor, Q, are of the order of 10^6 - 10^7 . The oscillator may be modelled by the so-called perturbed sine-Gordon equation, which involves the action of form-stable solitary waveforms, solitons, within the (self-pumped) junction resonator. This type of oscillator is therefore called a Josephson soliton oscillator. The power coupled out from a single soliton oscillator is of the order of a picowatt (10^{-12} watt). To achieve higher, more useful power levels, devices consisting of arrays of coupled soliton oscillators have been fabricated and tested. For simple coherent coupling between N oscillators it could be expected that the radiated power would increase with the square of the number of oscillators, i.e. as N^2 . Experimentally it has been found that the increase in power is higher than N^2 , indicating an amplification process in arrays of non-linearly coupled oscillators.

Theoretical models have been set up to understand these observations. The first simulations indicate reasonable agreement between the coupling models and the experimental data. Work is still in progress in order to explain fully the complicated non-linear behaviour that has been observed.

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24 Growth and study of the temperature dependence of the structural, thermal, electrical, optical and elastic properties of the I-II-VI₂ ternary semiconductors

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Contract number and duration: Cl1*/0556, April 1990 to March 1993.

Background and objectives

Ternary semiconductors of the type (I-II VI₂) are of considerable interest because of their potential applications in the domains of non-linear optics, light-emitting diodes, photovoltaic detectors and solar cells. Accordingly, a considerable amount of theoretical and experimental work has been done on these semiconductors in order to gain a better understanding of their electronic, electrical and optical properties.

However, their structural, thermal and elastic properties have not been studied in detail and, in particular, the behaviour of their coefficients of expansion, structural parameters and elastic constants as a function of temperature has been measured only very partially. Knowledge of these properties is nevertheless fundamental to the successful fabrication of monocrystals of these compounds as well as to their choice for specific potential applications.

The aim of this project is thus to study the optical absorption, the expansion, the structural parameters, ultrasonic attenuation and propagation speeds as a function of temperature in monocrystals of this family of semiconductors (CuInTe₂, CuGaS₂, ...).

Materials and techniques

Research into structural properties as a function of temperature is carried out starting with a two circle X-ray diffractometer (working on polycrystalline samples) for precise determination of parameters and then on an automatic X-ray diffractometer for determination of structures. Separately, the study of the high temperature phase transition of these compounds will be performed *in situ* using a Raman laser spectrometer. These techniques were developed at the Molecular Crystal Dynamics laboratory of the Université de Sciences et Techniques de Lille Flandre-Artois.

Investigations into the absorption and speeds of propagation of ultrasonic waves in these compounds, their crystal growth and determination of their low-temperature optical properties (by transmission) are carried out at the Semiconductor Research Centre at Mérida.

This project thus enables the capabilities of the Lille laboratory in the domains of structural investigation by X-ray diffraction and of lattice dynamics by Raman diffusion to be combined with the capabilities of the Mérida laboratory in crystal growth, ultrasonic measurements and the determination of the optical absorption spectrum.

Results

Crystal growth. Monocrystals of CuInSe_2 , CuInTe_2 , CuGaSe_2 , CuGaS_2 , CuInS_2 and AgInTe_2 have been grown; the first three by the directional solidification method, the remaining three by the chemical transport method.

Structural investigation. High temperature ($\sim 400^\circ\text{C}$) X-ray diffraction studies were made on the compounds CuInTe_2 and AgInTe_2 . The results are in the process of being analysed. The determination of the structure of a CuGaS_2 monocrystal was also made so as to investigate the electron density in the crystal; the data are being processed.

Raman diffusion. A study of lattice vibrations in CuInSe_2 and CuGaSe_2 has been made to help resolve a controversy which appeared in the literature on the spectrum of vibration of these two compounds. The results obtained remove all ambiguity and have been submitted for publication.

Low temperature optical properties in CuInTe_2 and CuGaS_2 . Measurements of optical transmission were made between 5°K and 300°K . For CuInTe_2 the variations with temperature in the width of the forbidden band, E_g , were determined and the deformation potential was calculated. For CuGaS_2 the position of the first excitonic level was determined as well as the variation with temperature of the mid-height width of this peak.

Publications

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25 Dating of ceramics by thermoluminescence and geological study of archaeological raw-material source areas

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Contract number and duration: Cl1*/0618/0710, June 1990 to August 1993.

Background

Until the 1970s the main objective in Ecuadorian archaeology had been to establish a cultural sequence of its then little-known archaeological remains. However, beginning with the 1974/75 excavations at Real Alto (Valdivia culture), new perspectives have been emerging on social organisation and on regional and long distance trade. The need became apparent for refined dating techniques and for raw-material source identification to study the genesis, development and relationship of local, regional and long distance trade.

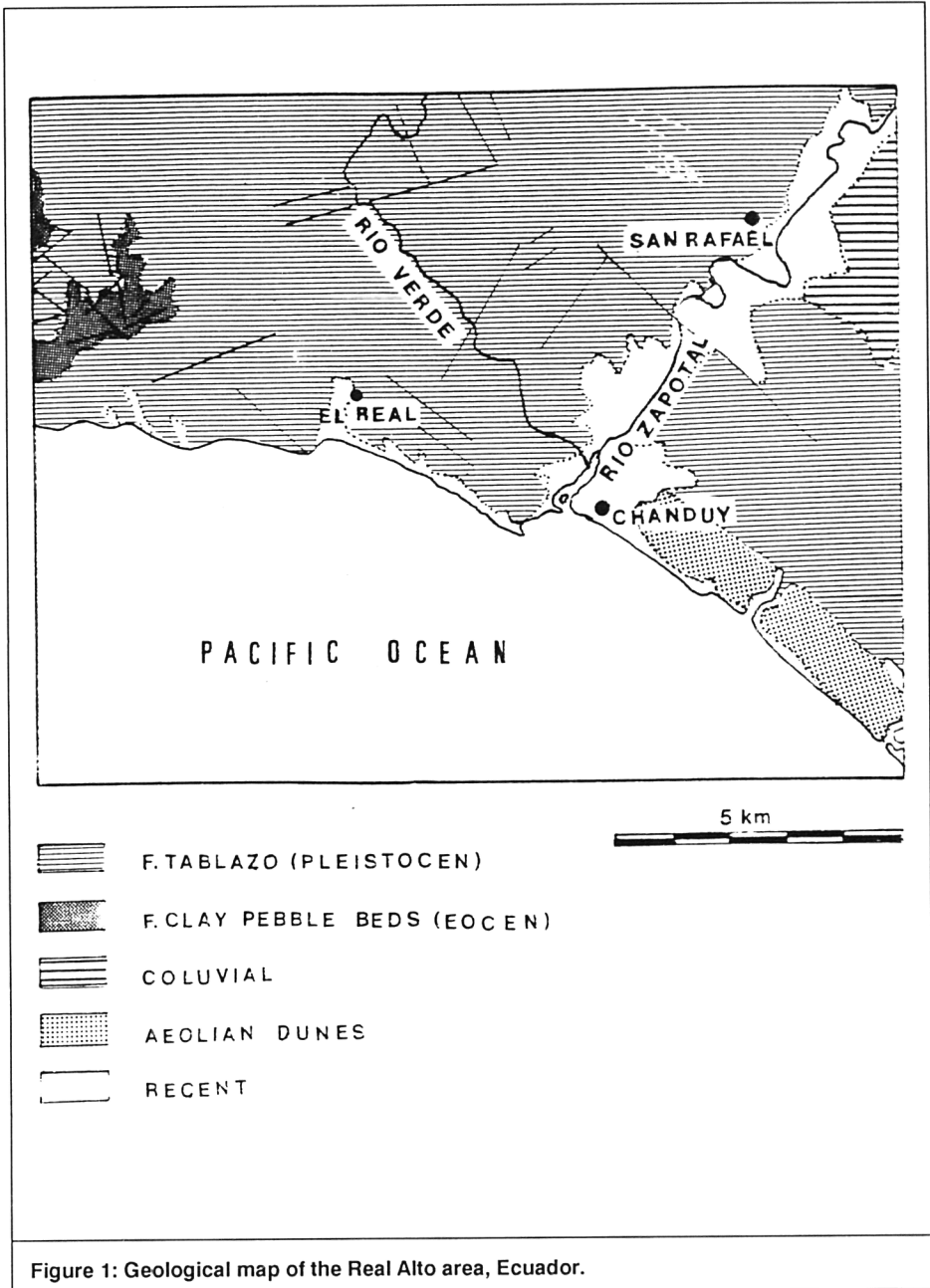
It should also be pointed out that too few systematic and scientific excavations have been carried out recently in areas of interest because of the activities of illegal diggers. There is a serious danger that extremely important information will soon be lost.

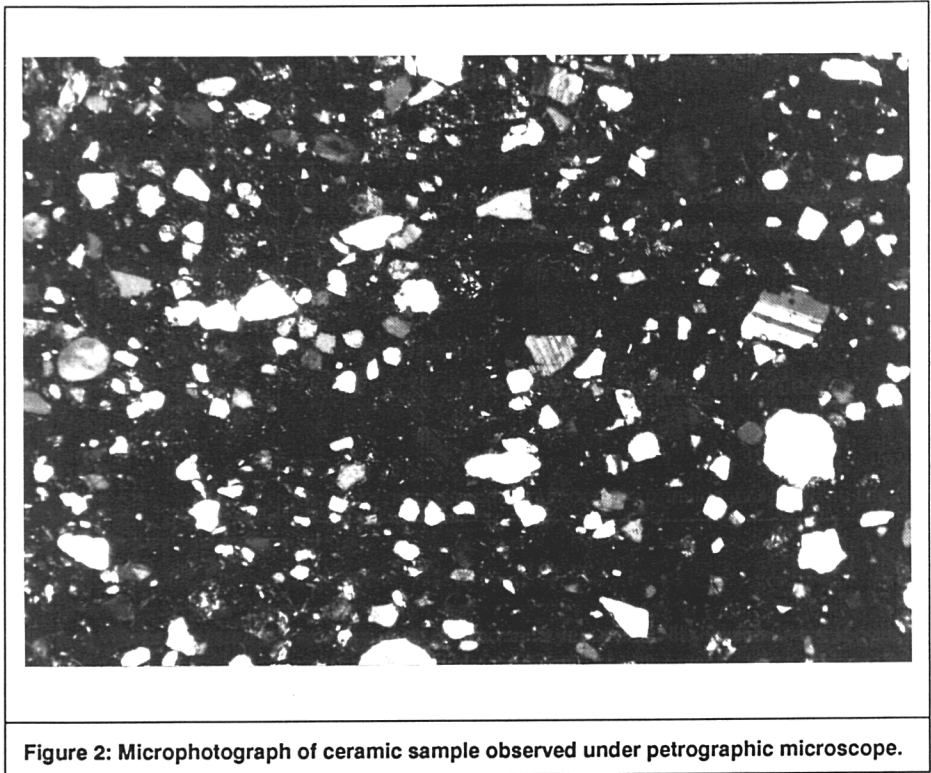
Objectives

For dating of the most ancient periods, a study will be made of ceramic artefacts from Chanduy valley, mainly from the site of Real Alto. This will be useful for calibration of the thermoluminescence (TL) method for accurate use in Ecuadorian archaeology, and for cross-referencing the TL dates obtained with those determined by ^{14}C assays. In the second and third year, the objective will be to make an original contribution by dating strata of archaeological sites related to more recent periods but lacking absolute chronology.

Ceramic technological analysis will be used to determine intrinsic classification criteria, and to establish clay source areas.

Provenance identification of mineral raw materials such as turquoise, lapislazuli, sodalite, obsidian, native copper, will be made of archaeological artefacts.





Materials

In the first field study (August 1990), research was concentrated on the Chanduy valley area, near Real Alto, where 12 important sites from different periods and 300 other related sites are located. Even today, potters apparently still use the same clay sources as their ancestors. So the Chanduy valley is a good laboratory for the initial phase of our project.

A number of ceramic and carbon samples were taken from secure archaeological contexts. Specifically, for ceramics:

1. Real Alto: Unit 1, 30 samples from 5 strata (Valdivia); Unit 2, 15 samples from 3 strata (Valdivia-Proto Machalilla)
2. Punta Tintina: Unit 3, 7 samples (Valdivia temprano)
3. Lama de Villones Sur: Unit 4, 16 samples from 3 strata (Guangala back to Valdivia)
4. Simon Bolivar Est, Pozo de Cateo B: Unit 5, 15 samples from 3 strata (Machalilla temprano).

These samples should cover approximately the period from 4000 B.C. to 1200 B.C. (a small number should belong to the Guangala period, 200 B.C. - 800 A.D.), and will be dated by the thermoluminescence method and also studied by polarising light microscopy, X-ray diffraction, spectroscopic methods, electron microprobe analysis, image processing and statistical analysis.

Results

In Milan

Thermoluminescent dating analysis of samples from Unit 1 is in progress. The dating programme will be performed using the fine-grain technique. The thermoluminescent properties of the samples analysed are generally good enough, the natural TL signal being always very high but not extremely regular. Storage of irradiated samples at temperatures ranging from 50 to 200°C, to check the stability of the high temperature traps, is in progress.

Saturation water has been measured, and is low (8-15%).

Potassium content of shards and soils has been measured by flame photometry: average concentrations have been obtained (1-2% K₂O). Uranium and thorium contents are obtained by total alpha counting; Th/U ratio is evaluated by pairs technique. Unusually low alpha counting-rates (less than 0.5 c.p.m.) were measured in all samples.

Environmental gamma dose has been measured *in situ* with a portable Geiger-Muller counter for external dosimetry. These results will be cross-checked with those obtained in an indirect evaluation of the radioactivity of the soils.

In Barcelona

84 ceramic samples were studied (the same as taken for TL dating). Polarising microscopy studies enabled study of the principal crystalline components and their morphology and granulometry to be determined. X-ray diffraction analysis permitted identification of the primary phases existing in the clay, and the reaction phases (gehlenite, spinel, haematite) of the crystalline fraction; it also allowed calculation of the percentages of the named phases.

Dating analysis by the ¹⁴C method was started. So far, various groups of ceramics of different manufacture have been recognised and it has been possible to verify and discuss them in connection with archaeological dates. Two characteristic groups have been established; one which contains illite (0.2 to 8.1%) and one which does not. The cooking temperature does not exceed 850-950°C, in accordance with the reaction components detected. The clay materials used in the fabrication of ceramics are free of carbonate components, since the products of corresponding reactions do not appear (diopside, wollastonite, oxide). Materials of volcanic origin exist (olivine, amphibole). These have come from the primary clay and have remained during the whole process without transformation. The abundance of plagioclase also confirms a volcanic influence in the production zone.

Workshop**New industrial materials****Joint Coordinators:****C. Miravittles**

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As a result of the priority status given to this subject in International Scientific Cooperation with the Andean Pact countries, this workshop was organised with the objective of bringing together Andean Pact and EC country scientists active in the field in order to review their work, generate contacts and discuss ideas, with a view to establishing the bases of possible joint research activities.

For the purposes of the workshop, materials were defined as substances with physical and chemical properties that make them useful as components of machines, apparatus, etc. It thus is immediately obvious that scientific research on materials cannot be separated from technological development and that in effect, in this field, a continuous gradation exists between science and technology. In recognition of this, participants were invited both from the academic and from the industrial sectors and a total of 41 scientists from 13 countries took part in the event held in Mérida, Venezuela from 26 November to 1 December 1990.

The principal themes developed during the workshop fell within the area of solid state chemistry, and included aspects such as synthesis and/or growth of crystals of the materials of interest, characterisation of these materials by physical and chemical techniques and the study of structure-property relations. The materials covered were principally polymers and ceramics.

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Study of the magnetic and transport properties of heavy fermion systems

Fellowship period: October 1989 - September 1990

Summary

The work of the fellowship included preparation of samples of YbPdCu₄, YbNiSn and YbAgCu₄ and their characterisation by X-ray diffraction; training in and use of the Hall effect and magnetoresistance techniques; training in and use of the dilution refrigerator; measurements of electrical resistivity, magnetoresistance and Hall effect on the YbPdCu₄ and YbNiSn samples; and measurements of magnetic susceptibility and magnetisation on the YbNiSn sample.

Measurements were made of Hall effect (1.3-30°K) and magnetoresistance (1.3-20°K) of the YbNiSn compound. A sharp peak in the Hall constant was observed around the ferromagnetic transition at 5.5°K. Also, a non-linear dependence of the Hall resistivity with the magnetic field was found between 1.3 and 20°K. A high magnetoresistance had maxima between 1.3 and 4.2°K. Further results on magnetic measurements on YbNiSn are being analysed and will be published.

Publication

D'Onofrio, A.; Hamzic, A. and Fert, A. (1991). Magnetotransport properties of YbNiSn. *Physica B*, 171 (1-4) 266-8.

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Theoretical study of the properties of heavy fermions, with special reference to those related to superconductivity and relaxation of magnetic impurities in these systems.

Fellowship period: May 1990 - November 1990

8 PHYSICAL, MATHEMATICAL AND ENGINEERING SCIENCES

Joint research project

26 Phase transitions in disordered magnetic systems

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Contract number and duration: Cl1*/0409, September 1989 to August 1992

Objective

The study of equilibrium and non-equilibrium models of magnetic systems which incorporate some sort of microscopic disorder.

Materials and methods

The study involves the techniques of statistical and solid-state physics and computer simulations of the system behaviour.

Results

Some significant progress can be reported towards each of the two main objectives of the initial proposal. In the study of the properties of disordered magnetic systems, progress has been made along three separate lines and some definite results have been or are about to be published. The planned cooperation between the two groups on that matter has also been initiated.

On the first line of the study, the critical behaviour of the magnetic model system usually identified as a fully frustrated classical XY antiferromagnet in a two dimensional lattice, with domain second nearest neighbour interactions (assuming that $J_2 = J_1$ using standard notation) has been simulated in a computer by the Monte Carlo method. The ground state of that system is degenerate with respect to rotations of all spins on one sublattice in addition to being degenerate with respect to global rotations. Henley's result, that the temperature does indeed introduce an anisotropy, was first checked numerically. There is consequently an Ising-like broken symmetry in the ordered state at $T > 0$. The Monte Carlo results achieved are consistent with only one critical temperature and one correlation length, l , both for magnetisation fluctuations and for fluctuations of an appropriate Ising-like order parameter. Critical fluctuations of the magnetisation seem to cross over from a Kosterlitz-Thouless-type behaviour to $l \sim t^{-\nu}$ ($\nu \sim 1$) as l becomes large enough ($l > 10$ lattice units) for anisotropy effects to become dominant. Values of several critical indexes are obtained. The critical effects produced by small amounts of impurities in that model are currently being analysed.

Work along the second line has produced two series of results. In the first one, the so-called driven diffusive lattice-gas systems are discussed as models for fast ionic conductors, the associated hydrodynamic equations and expressions for transport coefficients are derived, and mean-field theory, Monte Carlo results and experimental observations are compared. The comparison between model and real behaviours helps to understand some properties of those materials which seem to be characterised by the occurrence of nonequilibrium steady states and phase transitions. In particular, the study suggests the existence in nature of a novel (nonequilibrium) universality class (Marro *et al.*, 1991).

The second series of results (González-Miranda and Marro, 1990) concerns the so-called reaction diffusion lattice-gas model, i.e. an interacting particle system out of equilibrium whose microscopic dynamics is a combination of creation-annihilation (reaction) or Glauber processes and diffusion or Kawasaki processes. The Glauber rate $c(s;x)$ at site x when the configuration is s satisfies detailed balance at temperature T , while the Kawasaki rate $\Gamma c(s;x,y)$ between nearest-neighbour sites x and y satisfies detailed balance at a different temperature, say T' . A report is made there on the phase diagram of that system, as obtained from a series of Monte Carlo simulations of steady states in two dimensional lattices with arbitrary values for T' , T and Γ , thus generalising previous analytical and numerical studies for $\Gamma \rightarrow \infty$ and/or $T \rightarrow \infty$. When the rates are implemented by the Metropolis algorithm, the system is observed to undergo various types of first- and second-order (nonequilibrium) phase transitions, e.g. one may identify Onsager (equilibrium) as well as Landau (mean-field) types of continuous phase transitions.

In a sense, the third line of research combines the experience and objectives of the other two. That is, the focus of interest is in the study of nonequilibrium steady states, phase transitions and critical phenomena in disordered systems. A simple, systematic method has been developed to investigate the existence of stationary probability distributions for interacting particle (or spin) lattice systems exhibiting steady nonequilibrium states. The latter originate in a competition between several creation-annihilation (or spin-flip) kinetic mechanisms, where each mechanism acts presuming a different bath temperature, particle (or spin) interaction strengths and/or chemical potential (or magnetic field). It follows the existence of stationary probability distributions for a large class of those systems which may thus be studied by simply applying the techniques of equilibrium theory. The method is illustrated with several examples having practical interest (Lopez-Lacomba *et al.*, 1990). In addition, exact results have been found for one- and two-dimensional lattice, Ising-like model systems in which a competing kinetic process induces the presence of nonequilibrium steady states dominated by a kind of (dynamic) disorder. The model, which may be relevant in relation to the spin-glass problem, serves also to uncover some novel features of nonequilibrium phase transitions and critical phenomena.

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Theoretical study of electronic systems in one dimension with the techniques of the density functional formalism

Fellowship period: November 1989 - October 1990

Summary

The fellowship project concerned the theoretical study of electronic systems in one dimension with the techniques of the density functional formalism. In particular, we proposed to develop an explicit approximation for the kinetic energy of these systems as a functional of the total electronic density. An explicit form for this density functional, $T[n]$, would allow the study of the electronic properties in terms of the total electronic density, without explicit reference to the wave-functions. This is particularly useful in order to include, in a self-consistent way, the Coulomb interactions with the exchange and correlation corrections to the total energy. The usual procedure of solving the Schrödinger equations in the effective one-electron potential and iterate until convergence may become very inefficient, while the explicit functional $T[n]$ reduces it to a simple minimisation problem which may be treated with standard techniques.

There were already explicit functional approximations for the kinetic energy of independent electrons in three dimensions, but the peculiarities of low-dimensional systems required a special treatment, mainly because of the stronger divergence associated with the Fermi surface, which appears in the linear response function of these systems. The simple density functional approximations which may be directly applied to one-dimensional systems, like the Thomas-Fermi of the gradient expansion, completely neglect the non-analytic character of this response function. A one-dimensional version of the averaged density approximation for the kinetic energy of independent electrons has been developed and tested for a few characteristic problems, to evaluate its accuracy. The project has opened possible lines of future work on the application of these density functional techniques to systems like quantum wires, of important technological interest, and also on some fundamental open problems.

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Self-regulated galactic evolution

Fellowship period: January 1990 - December 1990

Summary**Work at the Royal Observatory of Edinburgh (ROE)**

In the ROE, A. Parravano continued his study of a proposed mechanism of the self-regulation of star formation (HRWGC). The main results were recompilation of observational data to test the mechanism; coupling of the HRWGC mechanism with the FIRSF; and application of the HRWGC hypothesis to SO and infrared luminous galaxies.

Work at Osservatorio Astronomico di Roma (OAROMA)

At the OAROMA the work initiated in the ROE was continued and, simultaneously, work was undertaken on a line of research (galaxy formation) which was completely new to the fellow. The main results were the application of the HRWGC to large Virgo cluster galaxies and investigation of the photo-ionising radiation flash as a trigger of efficient H₂ cooling in free-metal gas clouds.

Publication

Parravano, A. and Mantilla, J. (1991). A self-regulated state for the interstellar medium: radial dependence in the galactic plane. *Astronomy and Astrophysics*, 250(1) 70-83.

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EC - Andean pact countries**

C. Kessler

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The first international scientific cooperation activity with a country in the Andean Pact group (Bolivia, Colombia, Ecuador, Peru and Venezuela) was initiated in 1987, and this volume presents a summary of all activities for which a financial commitment had been made before the end of 1990. It thus covers joint research projects, fellowships and workshops, in various stages of completion: some being totally finished, some in progress and for those projects just starting, a work programme only is presented.

The objective of compiling this volume is to show what has been achieved in the framework of international scientific cooperation with the Andean Pact countries. The strength of these achievements lies with their firm scientific foundation and this is reflected in the style of presentation of this volume; however, the contents are orientated towards a wide readership, to allow not only the scientists, both from the Andean Pact region and from Europe, to place their work in a wider perspective, but also to provide a concise account of the activities to other scientists, government officials, diplomatic representatives and all those interested in science in the Andean Pact countries and the European Communities.

This report covers 26 research projects, 28 fellowships and two workshops, and the summaries included here demonstrate the results of these actions in terms of *research findings, productive contacts developed and scientific publications*. These results are impressive, especially considering that many of the activities are still at an early stage of development and further output will be generated before they are completed.

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