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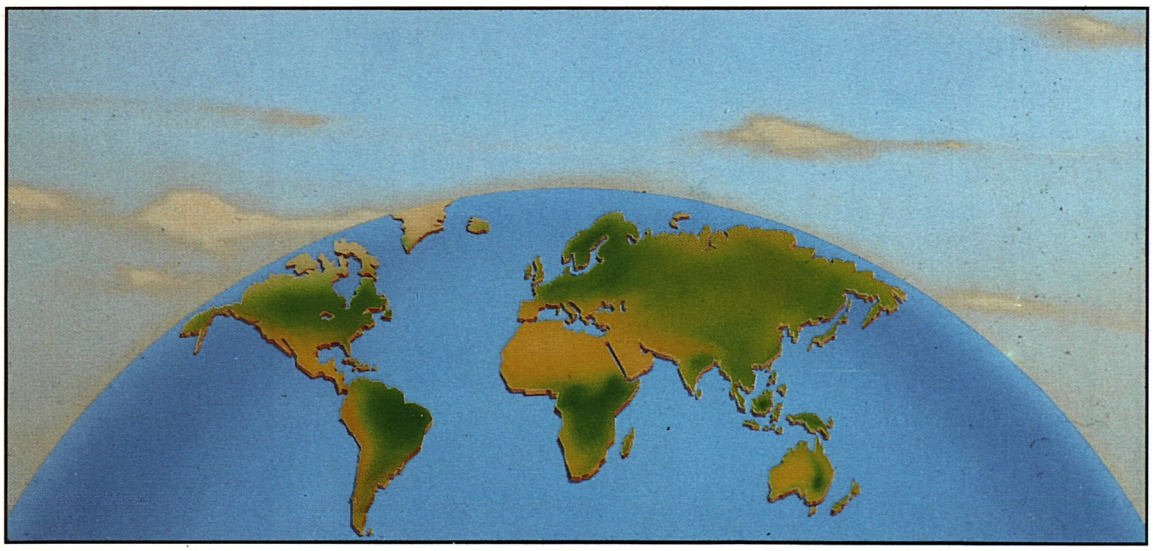
INTERNATIONAL SCIENTIFIC COOPERATION

Consolidated report of activities 1988-90

EC - INDIA



Report

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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.

Commission of the European Communities

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Consolidated report of activities 1988-90

EC - INDIA

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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

India has a long and distinguished record in science and there is a widespread recognition in the country that science has an important contribution to make to the resolution of the dramatic problems of Indian society. This volume presents a summary of International Scientific Cooperation activities with India for which a financial commitment was made during the period 1988 to 1990. It covers 10 joint research projects, 33 postdoctoral fellowships and 3 workshops which conform to the patterns described in the foreword except for some of the early research projects.

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. In addition, it should be noted that there is a bias in the distribution of activities towards fellowships and workshops which are essentially preparatory activities. It is thus to be expected that there will shortly be an expansion in joint research projects as research proposals are generated from these preparatory activities.

The objective of compiling this volume is to show what has been achieved in the framework of International Scientific Cooperation with India. 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 Indian and European, 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 India and the European Community.

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 Science, 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 several of the chapters. Within each chapter, research projects (if any) 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 postdoctoral 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 India has been the contribution of the Indian Government's Department of Science and Technology 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,
Director, Science and Technology Cooperation with non-Member Countries, Commission of the European Communities (1987-91)

Further information on International Scientific Cooperation with India is available from:

International Scientific Cooperation,
Directorate General for Science,
Research and Development,
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1049 Brussels, Belgium.

Department of Science and Technology,
Technology Bhavan,
New Mehrauli Road,
New Delhi 110016, India.

Bibliometric study of Indian and EC research output

In order to see the work supported by the International Scientific Cooperation (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 India and scientists of the EC during the years 1986-90.

The source of data was the Science Citation Index (SCI), published by the Institute of 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 12 Indian journals included. Although the language biases are known to give a distorted impression of the amount of scientific production in some countries, they will have little effect on India where the main language for scientific communication is English. It may also be presumed that articles co-authored between Indian and EC scientists, with which this analysis is concerned, will be published primarily in non-Indian English-language journals. Thus the SCI provides 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 where the authors are from India and an EC Member State.

In 1986-90, Indian scientists authored or co-authored an average of some 10,300 papers per year in SCI journals, a figure that has remained rather constant during the 1980s. During the same period EC production averaged 164,000 papers, and nearly 400 papers per year were co-authored by Indian and EC scientists; neither figure showed any tendency to rise or fall. The co-authored papers represented about 4% of Indian output but only 0.24% of EC output in SCI journals during these years.

The leading EC Member States in terms of co-operation with India in scientific production were the UK and Germany, with France and Italy forming a second group, and Netherlands, Belgium and Spain, a third, see Table 1.

Member State	Papers per year	Percent of output
UK	155	0.28%
Germany (FR)	128	0.36%
France	53.3	0.18%
Italy	48	0.30%
Netherlands	22	0.18%
Belgium	15.7	0.26%
Spain	14.7	0.20%

Table 1: Mean numbers of papers in SCI journals co-authored by Indian and EC scientists, and percent of EC Member State total, 1986-90.

It is interesting to see that scientists from all seven countries showed a rather similar propensity to co-author pages with Indians - the variation in percentage being only by a factor of two from France and Netherlands (0.18%) to Germany (0.36%). Both Germans and Italians are more likely than British scientists to seek an Indian partner.

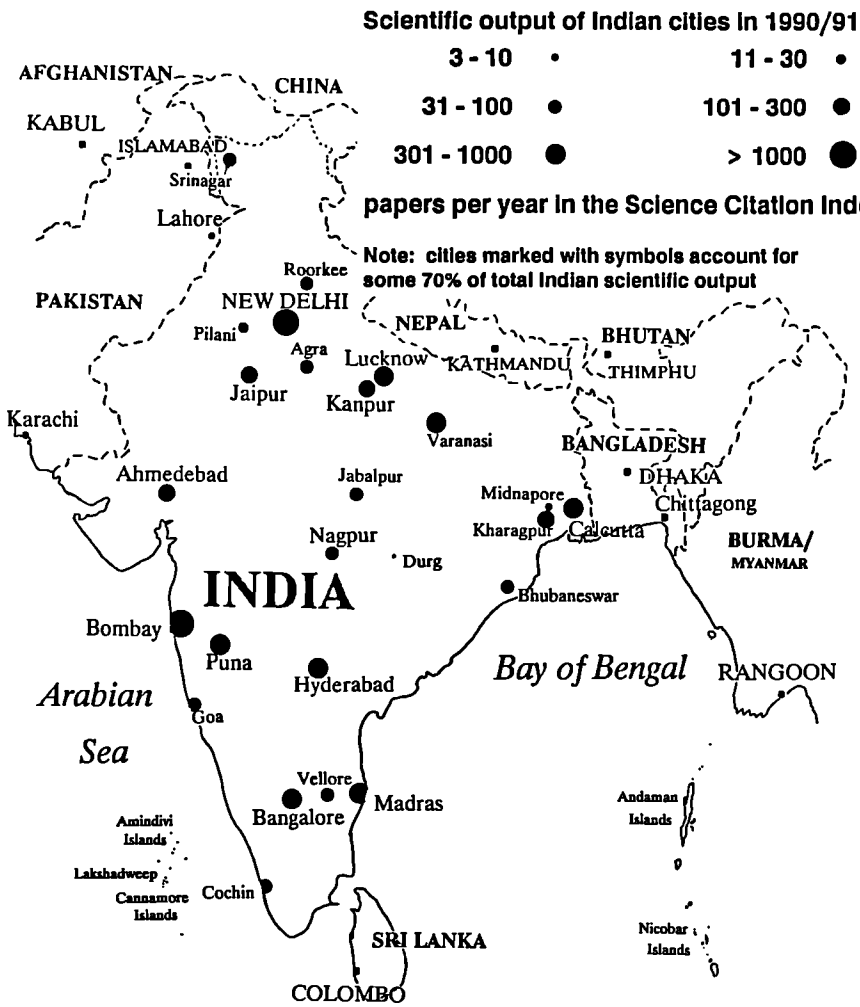
An analysis was made of the papers in the eight subject areas (using the classification scheme adopted for this report, which is standard for the ISC programme) in which Indian-EC scientific co-authorship occurred. Because of the large numbers of papers and the steady change over time expected as a result of the similar analysis effected previously for Mexican-EC cooperation, the analysis was conducted only for the end years of 1986 and 1990. The percentages of papers in each of the eight areas were similar to those found in the earlier study, see Table 2 (opposite). Physical, mathematical and engineering sciences dominated with 39% of the Indian-EC co-authored papers. They were followed by health and biomedical sciences and chemical sciences with 16 and 15%. The life sciences as a group (including agricultural and biological sciences) accounted for 32% of the total (cf. Mexico 30%). However this percentage only rose slightly over the period (from 29 to 34%) whereas for Mexico it nearly doubled.

From the publications listed in the present report that are attributable to the research projects and post-doctoral fellowships supported under the ISC programme an analysis was made of the numbers appearing in SCI journals. So far, only 46 have appeared or are expected: 3 in 1989, 29 in 1990 (mostly in health and biomedical sciences), and 14 in 1991.

Science Area	Number of papers/year co-authored with EC			
	India	%	Mexico	%
Agricultural	18	5	5	4
Biological	40	11	19	15
Chemical	56	15	20	16
Earth	16	4	8	6
Environmental	9	2	1	1
Health and Biomedical	62	16	13	10
Materials	29	8	8	6
Physical, Math. and Eng.	149	39	50	40
Total	**379**		**124**	

Table 2: Distribution of papers in SCI co-authored with EC scientists between scientific areas for India (1986, 90) and Mexico (1986-90).

Clearly, results are only just starting to emerge from the programme and as the projects come to fruition many more papers will appear in 1991 than have been listed here. Indeed, 29 of these 46 publications stem from just two projects (nos 5 and 8). It is perhaps significant that the areas of health and materials sciences, in which they are located, both show an increasing amount of India-EC co-publication, whereas several other fields (biological, chemical and earth sciences) all show a decline in co-authored output in 1990 compared with 1986.



Map of India showing principal cities and locations of joint research projects and fellows' home institutions. The boundaries shown on the map are purely illustrative and have no political significance.

Scale 1 cm = 290 km

Names of cities with populations greater than one million are shown in larger type

1 AGRICULTURAL SCIENCES

Summary

The three research projects described here along with some of the postdoctoral fellowship reports deal with the biotechnological approach. The first two reports concern *Brassica*, a genus of major agricultural importance both in Europe and India for oil and fodder. The first of these projects (page 10) has the principal objective of developing cytoplasmic male sterile and fertility restorer lines in oilseed mustard (*Brassica juncea*) and rapeseed (*B. napus*) with a view to hybrid seed production in these crops, and to increased disease resistance, oil quality and yield. The second project (page 15) consists of a molecular characterisation of the nuclear genome of *Brassica* species with a view to identify, isolate and characterise species-specific repeated DNA fragments that will be useful for the screening of hybrid material currently being produced in the researchers' laboratories.

The third research project (page 21) concerns woody, leguminous *Acacia* and *Leucaena* species that offer potential for firewood, fodder and pole production from degraded and marginal land. The studies reported here focus on tissue culture techniques in order to overcome propagation difficulties encountered especially in hybrid material, and on *Rhizobium* and mycorrhizal inoculation in order to improve plant growth particularly on infertile soils without the use of scarce or costly fertilizers.

Postdoctoral fellowship studies include a phytochemical examination of hops (page 26), basic photosynthesis studies (pages 25, 29, 32), the metabolism of organophosphate insecticides (page 30), the dynamics of nitrogen turnover in soils into which crop residues have been incorporated (page 28) and an ergonomic study of a small-scale agricultural machine (page 31).

Joint research projects

1 Transfer of cytoplasmic male sterility and nuclear genes from wild species to oilseed mustard (*Brassica juncea*) by sexual and somatic hybridisation.

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D. Pental
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7 Jor Bagh, New Delhi 110 003, India.

Contract number and duration: CI1*/0193, April 1988 to March 1992.

Background and objectives

The main objective of the project is to develop cytoplasmic male sterile (CMS) lines and fertility restorer lines in oilseed mustard *(B. juncea)* and rapeseed *(B. napus)* so as to develop the capability of hybrid seed production in these crops. Such systems would be useful for increasing the production of oilseeds in India and other countries of the world, including Europe, where rapeseed is a major oilseed crop.

The technical objectives of the research programme were:

1 To identify varietal crosses that will give high increase in yield from the F1 seed.

2 To identify different cytoplasmic male sterility (CMS) systems and to manipulate these by both conventional and molecular methods to develop stable CMS lines and fertility restorer lines.

3 To develop suitable protoplast culture techniques and to use the techniques of somatic cell hybridisation for transfer of alien nuclear and organelle genes.

4 To develop the techniques of genetic transformation of *Brassica* species.

Materials and methods

Conventional plant breeding methods like diallel crosses have been used for studying combining ability and extent of heterosis in crosses of different varieties of *B. juncea* and *B. napus*. CMS systems - Polima, Ogura, *B. tournefortii* and *B. oxyrrhina* (the first two are of spontaneous origin and the latter two are alloplasmic in nature) were procured from France (M. Renard, INRA, Rennes) and from India (S. Prakash, IARI, New Delhi). Hybrids between alien species and crop species have been made either by conventional methods or by embryo rescue. Genetic transformation has been done by both *Agrobacterium*-explant interaction and by plasmid-protoplast interaction. Protoplast regeneration systems have been developed for different Indian varieties of *Brassica* species by modification of established procedures. Selectable marker genes have been introduced by modifying the existing protocols for genetic transformation of model systems e.g. tobacco. Plasmic vectors were constructed in collaboration with the lab of J. Schell at the Max Planck Institut, Kln. *Agrobacterium* constructs were received from J. Schell's group. A restriction map of *B. oxyrrhina* mitochondrial genome has been made in collaboration with the lab of G. Pelletier at CNRS, Versailles.

Results and discussion

Extent of heterosis in *B. juncea* and *B. napus*. Diallel crosses of different *B. juncea* varieties of Indian and Russian origin and synthetics were made in the growing season of 1988-89 and 1989-90. A few combinations gave very high heterosis for yield (64-179%) over the better parent. The three best combinations of *B. juncea* varieties identified in diallel crosses - RH30 x Skorospieka, Varuna x Donskaya and Pusa bold x Pusa Barani were crossed and F1 plants were grown in a replicated plot level trial to ascertain the extent of hererosis. The hybrid plants show high vegetative vigour and substantial increase in yield (actual data will be available after April 1991). Similar experiments have been set up with rapeseed and a combination of ISN 706 x HSN 8 and ISN 706 x GSL 1 have been found to be more productive than the better parent. These varieties have been identified as targets for the introduction of cytoplasmic male sterility and fertility restorer genes.

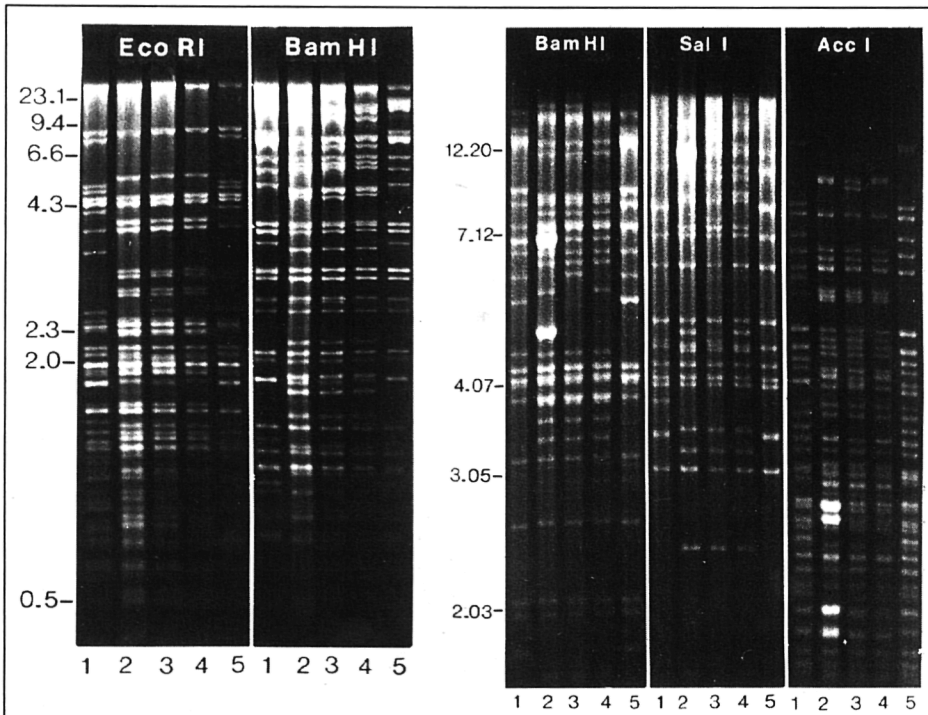
CMS systems. Four different male sterile systems - Polima, Ogura, *tour* and *oxy* have been studied and are at various stages of development for hybrid seed production.

Polima system. This system has been found to confer stable male sterility on *B. napus* variety ISN 706. Flowers of CMS plants, as shown in Figure 1, have rudimentary anthers which do not produce any functional pollen. This CMS system has been found to be stable during the normal winter growing season in Delhi and during the summer season in the off-season nursery at Leh. It is unstable in other *B. napus* varieties and does not confer male sterility on *B. juncea*. Restorer gene for this cytoplasm has been transferred from *B. napus* variety Italy to Indian varieties - ISN 706, HNS 8 and GSL 1. This system will be made available to plant breeders for further development in the year 91-92.

Figure 1: Flower of *Brassica napus* ISN706 that has Polima Cytoplasm.

Ogura system. Ogura system available in *B. napus* variety Audor has been transferred to two Indian varieties - ISN 706 and GSL 1. Surprisingly, it has been found that these two varieties restore the sterility conferred by Ogura cytoplasm. There are no previous reports of any *B. napus* variety that can restore this cytoplasm.

Restorer genes for this cytoplasm have been reported only in radish. The genetics of restoration in the Indian varieties will be studied in 1991-92.



Restriction profiles of chloroplast DNA from normal *B. juncea*, Pusa bold (1), CMS *B. juncea*, Pusa bold (2), *B. tournefortii* (3), CMS *B. napus*, BO 15 (4), normal *B. napus* BO 15 (5). The chloroplast DNA profiles of CMS lines are exactly similar to the restriction profiles of *B. tournefortii* and are dissimilar to those of normal *B. juncea* and *B. napus*

Restriction profiles of mitochondrial DNA from normal *B. juncea* Pusa bold (1), CMS *B. juncea*, Pusa bold (2), *B. tournefortii* (3), CMS *B. napus* BO 15 (4), normal *B. napus*, BO 15 (5). The picture shows digests with three different restriction enzymes. Highly stained bands in the digest of CMS *B. juncea* are due to a linear plasmid. The profiles of CMS lines are more or less similar to the profiles of *B. tournefortii* and differ significantly from the profiles of normal *B. juncea* and *B. napus*

Figure 2: Chloroplast and mitochondrial DNA analysis of CMS systems earlier reported to be of spontaneous origin. Analysis has shown that this CMS is alloplasmic and that the cytoplasm has come from *B. tournefortii*

Tour system. An analysis of 58 different species of *Brassica* and allied genera for chloroplast DNA restriction profiles has shown that a CMS system which was earlier recorded as being of spontaneous origin has come from *B. tournefortii*. The chloroplast DNA profiles of CMS *B. juncea* and *B. napus* were markedly different from DNA profiles of normal *B. juncea* and *B. napus* and similar to the profiles of *B. tournefortii*. However, some slight differences were observed in the profiles of mitochondrial DNA of CMS lines and *B. tournefortii*. As no restorer system is available for this cytoplasm, we intend to transfer restorer genes from *B. tournefortii* to *B. juncea* for developing restorer lines for *tour* cytoplasm. The transfer of genes from *B. tournefortii* to *B. juncea* and *B. napus* is being done by both sexual hybridisation and somatic cell hybridisation.

Oxy system. Alloplasmic lines of *B. juncea* with *B. oxyrrhina* cytoplasm are male sterile. Such lines suffer from chlorosis, probably due to incompatibility between the nuclear genome of *B. juncea* and the chloroplast genome of *B. oxyrrhina*. No restorer system is available for this cytoplasm. The organelle and nuclear genes are being transfered from *B. oxyrrhina* to *B. juncea* by somatic cell hybridisation.

Protoplast culture and somatic cell hybridisation. In the original proposal a scheme was suggested for the genetic manipulation of oilseed *Brassica* species using the techniques of somatic cell hybridisation. Oilseed *Brassica* species - *B. napus*, *B. juncea* and *B. carinata* (Ethiopian mustard) are allopolyploids with genomic constitution of AACC, AABB and BBCC respectively with AA genome coming from *B. campestris*, BB genome from *B. nigra* and CC genome from *B. oleracea*. According to the scheme, a wild species e.g. *B. oxyrrhina* (OO) is first sexually crossed with one of the monogenomic species generating genomic configuration of OA, OB and OC. Protoplasts isolated from such plants can be fused with protoplasts from any of the monogenomics e.g. *B. nigra* (BB) to make hybrids with genomic constitution OABB. When such hybrids will come to meiosis, recombination may be possible between the nuclear genomes of *B. oxyrrhina* and *B. campestris*. Additionally, there will be interactions between the organelle genomes of *B. oxyrrhina* and *B. nigra*. Thus, hybrid plants could be selected that have chloroplast genome of *B. nigra* and recombinant mitochondrial genomes of *B. oxyrrhina* and *B. nigra*. This model is being applied for the transfer of *B. oxyrrhina* and *B. tournefortii* nuclear and organelle genes to *B. napus* and *B. juncea*. Selection of somatic hybrid plants will be based on the presence of marker genes in the monogenomic species. Therefore, the three monogenomics - *B. campestris*, *B. nigra* and *B. oleracea* - have been transformed with different drug or herbicide resistance-conferring genes. As a prelude to somatic cell genetics protoplast culture and regeneration systems have been developed for the three monogenomic species.

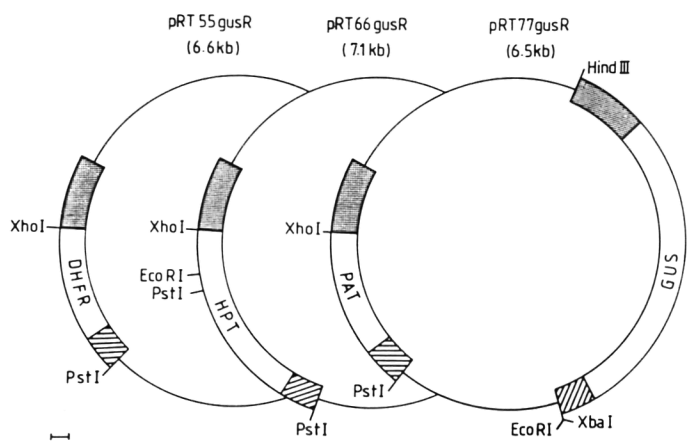


Figure 3: Map of the three plasmid vectors used for the genetic transformation of *Brassica oleracea* protoplasts.

Genetic transformation of *Brassica* species. Most extensive transformation work has been done on *B. oleracea*. This species has been transformed both by plasmid-protoplast interaction and by *Agrobacterium*-explant interaction. Three different plasmid vectors carrying three different marker genes - *hpt* for resistance to hygromycin, *dhfr* for resistance to methotrexate and *bar* for resistance to phosphinothricin - were made in collaboration with the group of J. Schell at the Max Planck Institut, Köln (Figure 3). The vectors also carried *gus* gene for efficient histochemical identification

of transformed plants. Plasmid uptake into protoplasts using polyethylene glycol gives absolute transformation frequencies of 10-30%. Molecular analysis shows that plants transformed with this procedure carry 1-5 copies of the marker gene. Transformation frequencies with *Agrobacterium*-mediated vectors were lower than those for protoplast-plasmid interaction experiments. *Agrobacterium* vectors were also received from the laboratory of J. Schell. Regenerating hypocotyl explants were used for transformation experiments. Only single copy insertions were observed in this method of transformation. Using similar methods *B. nigra* has been transformed with the *neo* gene conferring resistance to kanamycin. Transformation of *B. campestris* with *bar* gene is under way. Progeny analysis of transformed plants of *B. oleracea* and *B. nigra* has shown that the alien genes are inherited in a stable manner. *B. tournefortii* and *B. oxyrrhina* have been crossed with transformed *B. oleracea* and *B. nigra* and F1 plants with marker gene are being grown for fusion work.

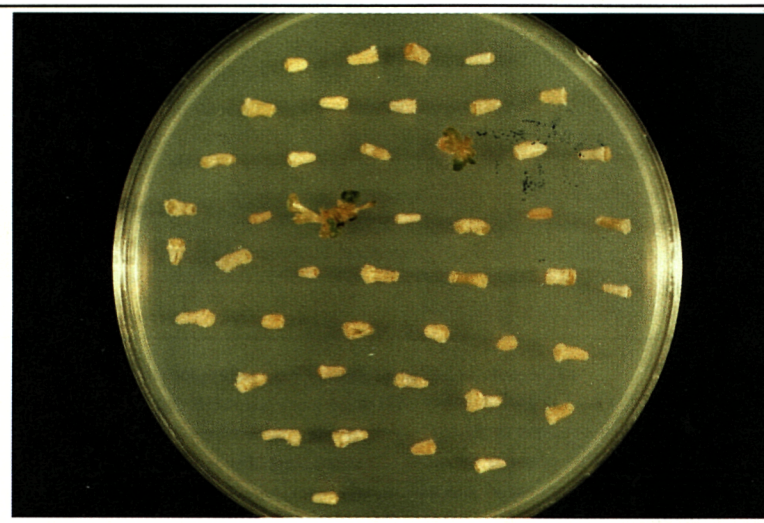


Figure 4: Origin of transformed plants from hypocotyl explants treated with *Agrobacterium* strain carrying a binary vector conferring resistance to hygromycin.

Conclusions

As conceived in the original project we have been able to combine the techniques of classical and molecular genetics to manipulate oilseed *Brassica* species for developing the capability of hybrid seed production in these crops. However, systems other than the Polima system will take more time to develop for hybrid seed production. The results achieved so far are promising. The general strategy adopted for somatic cell hybridisation could be used for transferring genes, both organelle and nuclear, from different alien species to oilseed *Brassica* species. Efficient transformation systems developed for *Brassica* species could be used in future for introducing genes of agronomic interest into these plants.

Publication

Pradhan, A.; Mukhopadhyay, A. and Pental, D. (1991). Identification of the putative cytoplasmic donor of a CMS system in *Brassica juncea*. *Plant Breeding*, in press.

2 Molecular analysis of nuclear genomes of *Brassica* species

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Contract number and duration: CI1*/0194, April 1988 to March 1992

Objectives

Brassica species are of major agricultural importance both in Europe and India for oil and fodder. Knowledge of the genome organisation of *Brassica* species is scanty, so the objective of this research is to undertake detailed molecular characterisation of the nuclear genome of *Brassica* species. Our immediate aim is to identify, isolate and characterise species-specific repeated DNA fragments of *Brassica*. This will be useful for the screening of interspecific and synthetic hybrids being produced in this laboratory.

Results

Nuclear DNA of *B. campestris* (YID1) was restricted with different restriction enzymes. A distinct band was obtained on restriction with the enzyme HindIII. The smallest of the band was 177 bp and the next one was at 354 bp. These distinct bands were eluted from the agarose gel and cloned into pUC plasmid. A number of clones were picked up at random and screened for repeated DNA sequences. Clones which lighted up with total DNA as probe were further characterised by sequencing. Eight different clones were sequenced (Figure 1). The consensus sequence was obtained from these difference clones and was found to contain many direct sub-repeats as well as indirect repeats. The average GC content of the sequences is 40%. The *B.campestris* consensus sequence exhibited greater than 98% homology to that of *B. oleracea*. The homology with *Arabidopsis thaliana* was about 40%. This sequence also showed 75% homology to the 177 bp repeat of the *Raphanus sativus* and 60% homology to that of *Sinapis alba*. These sequences also fold into a tRNA-like structure. The sequence shows homologies to the tRNA intergenic promoters and tRNA reverse transcription primer binding site. The observed homology indicates that these sequences may be originated from a tRNA.

Distribution of *B. campestris* tandem repeat in other *Brassica* and plant species. The tandem repeat of *Brassica campestris* on hybridisation with different *Brassica* species and its wild relatives were carried out. The tandem repeat hybridises at lower stringency conditions to nearly all the *Brassica* except *B. nigra* (IC 257), *Eruca sativa* and *B. tournefortii*. However, in high stringency conditions the tandem repeat hybridises to only one wild ally *Diplotaxis erucoides*.

	10	20	30	40	50
1	AAGCTTCTTA	CAAAGAG*AT	TCATCCTGGT	TTCATTGGAA	CGACGAAGAA
2	----------	-------*--	----------	----------	----------
3	----------	-----T-*--	----------	-CG-------	-A--------
4	----------	-------*--	----------	----------	----------
5	--------C-	-----T-*--	----------	--G-------	-A---T----
6	----------	-----T-*--	----------	--G---T---	------T---
7	--------C-	-----T-C--	----------	--G-------	-*---T----
8	--------C-	-----T-*--	----------	--G-------	-----T----
Con	AAGCTTCTTA	CAAAGTG*AT	TCATCCTGGT	TTGATTGGAA	CGACGAAGAA

	60	70	80	90	100
1	GCTGTGCTAT	TCCCAAACTG	GGAAACTGGA	ATCACCTGAT	TTGAAAGTGG
2	----------	----------	----------	----------	----------
3	-T-A-TT---	----------	----------	----AT----	----------
4	----------	----------	----------	----------	----------
5	----------	----------	----------	----------	----------
6	----------	----------	-------A--	----------	----------
7	---------A	----------	----------	----------	----------
8	-----CA---	----------	-A--------	----------	----------
Con	GCTGTGCTAT	TCCCAAACTG	GGAAACTGGA	ATCACCTGAT	TTGAAAGTGG

	110	120	130	140	150
1	GATAACTTCT	TCATCC*AAC	TCCTAT*GAG	ATTTATTCAA	C*TTCCTGGT
2	----------	------*---	------*---	----------	-*--------
3	-------C--	-***--C---	------*A--	----------	-*---A----
4	----------	------C---	------*---	----------	-*--------
5	----------	------C---	------*---	--------TT	AC--------
6	----------	------C---	------*---	----------	-*--------
7	----------	------C---	------*---	--------TT	AC--------
8	----------	--TA--C---	------AAG-	----------	-*--------
Con	GATAACTTCT	TCATCCCAAC	TCCTAT*GAG	ATTTATTCAA	C*TTCCTGGT

	160	170	180	190
1	GATTCTCC*A	CC**ACTTTA	TAGTATCCAA	ATCAAT
2	--------*-	--**------	-*--------	------
3	C-------*-	--**------	-*-------T	CAAGC-T
4	--------*-	--**------	----------	-----GCTT
5	--------*-	--**-G----	-*--------	-----GCTT
6	--------*-	--**------	----------	--A--GCTT
7	--------*-	--**-G----	-*--------	-----GCTT
8	--------G-	--GG------	-*--------	--A-GCTT
Con	GATTCTCC*A	CC**ACTTTA	T*GTATCCAA	ATCAAGCTT

Figure 1: Nucleotide sequence of eight cloned tandem repeats from *B. campestris* and their consensus (Con) sequence.

```
aAGCTTCTTACAAAGAGATTCGTCCCGGTTTGATTGGAAACACAAGAAGTTGTCCCATTC        1
---------------------A---T-----------------------------*----        2
---------------T----AT------------------------T-------A-----        3
aAGCTTCTTACAAAGAGATTCATCCTGGTTTGATTGGAAACACAAGAAGTTGTCCCATTC        CON
 ^HindIII            ^FokI
   ^AluI          ^HinfI
                       ^EcoRII
                         ^ScrFI

CACAAACTGATAAACTGGAATCAACC*GATTTCAAAGTGGGATAACTTCTTCATCCCAAC        1
--------AGGTG-T-A-----*---T-----G---C------------*G---------        2
---------GG------------TAT--------------------------------T-        3
CACAAACTGGGAAACTGGAATCAACC*GATTTGAAAGTGGGATAACTTCTTCATCCCAAC        CON
                  ^HinfI                        ^MboII
                                                    ^FokI

TCCGATGAGATTTATTCAACTTTCTGGTGATTCTCCACCACTTTATGTATCCAAATCAagctt     1
---T-----------------------------------------------------------     2
---------------------------------------------A-----------------     3
TCCTATGAGATTTATTCAACTTCCTGGTGATTCTCCACCACTTTATGTATCCAAATCAagctt     CON
                      ^EcoRII          ^HphI               ^HindIII
                         ^ScrFI                              ^AluI
                                 ^HinfI
```

Sequences of three cloned tandem repeats of *D. erucoides* namely m13D-E, m13D-Q, m13D-K are shown marked as 1, 2 and 3 respectively. The consensus sequence derived from these three sequences is shown below them. Sequence of clone E (sequence number 1) and the consensus (con) are given with the nucleotides that differ from sequence number 1 are given while the identical nucleotides are represented by dashes. Nucleotides not present in a sequence are indicated by asterisks.

Figure 2: Nucleotide sequence of three cloned tandem repeats (1,2,3) from *D. erucoides* and their consensus (CON) showing the available restriction sites.

Isolation and characterisation of *D. erucoides* 177 bp tandem repeat DNA. The nuclear DNA of *D. erucoides* was restricted with HindIII and separated on 1% agarose gel. DNA bands around 180 bp were eluted from the gel and cloned into pUC plasmid. A number of recombinant clones were picked up at random and screened for the presence of 177 bp repeat DNA sequences. The sequences of three clones were derived and shown in Figure 2. The consensus sequence was also derived from these sequences. Homology searches with other known tandem repeats were carried out using the algorithm of Wilbur and Lipman. The *D. erucoides* tandem repeat showed 90% homology to that of *B. campestris* and 94% homology to *B. oleracea* repeat DNA sequences which showed homology of 72% with *S. alba*, 70% with *R. sativus* and 43% with *A. thaliana*. These sequences do not show homology with any other known sequence.

Identification and characterisation of different repeat DNA families from *B. nigra*. Nuclear *B. nigra* DNA was restricted by BamHI and fractionated on 0.6% low melting agrose gel. DNA fragments in the range of 0.3 to 1 kbp were electroeluted from the gel. These fragments were ligated to BamHI linearised pUC13 plasmid and transformed. A number of recombinant clones were picked up at

random. These clones were screened for repeated DNA sequences using labelled *B. nigra* DNA as a probe. Three different families of repeated DNA sequences were identified and characterised. One clone pBNBH34 showed a ladder pattern clearly indicating a tandem repeat. This clone shows a ladder with BamHI as well as HindIII. To isolate more units belonging to this family, HindIII library in pUC plasmid was also constructed and clones hybridizing to pBNBH34 were selected. In all, five clones, one belonging to BamHI family and the other four from the HindIII library, were further characterised by sequencing. The sequences are shown in Figure 3.

```
AAGCTTCAATATATATAGCAATTAACGAAAAACTCAATATTTTCGTAGGGGTTAA-CCCA          IC21
AAGCTTCAATATATATAGCAATTAACGAAAAACTCAATATTTTCGTAGGGGTTAA-CCCA          IC18
AAGCTTCAATATATATAGCAATTAACGAAAAACTCAATATTTTCGTAGGGGTTAAACCCA          IC26C
AAGCTTCAGTATATAGAAGATTTAACGAAAAACTCAATATTTTCGTAGGGGTTAA-CCTA          IC34
AAGCTTCAATATATATAGCAATTAACGAAAAACTCAATATTTTCGTAGGGGTTAA*CCCA          Con


CAAATTTTGGGTGATATTAAAAAAA-ATGACAATATTGTTTTATTGA-AAATTTGGATCC          IC21
CAAATTTTGGGTGATATTAAAAAAA-ATGACAATATTGTTTTATTGA-AAATTTGGATCC          IC18
CAAATTTTGAGTGAAATTAAAAAAACATGAGAATATTTTTTTATTGGCAAAGTCGCATCC          IC26C
CAAATTTTGAGTGAAATTAAAAAAAGATGACAATATTTTTTTATTGCATAGTTGGGATCC          IC34
CAAATTTTGRGTGAWATTAAAAAAASATGACAATATTKTTTTATTGA*AAATTTGGATCC          CON
                                                  [   ]
                                                  BamHI


TCCAC-TTACATATTTTGAAGCTCAAAGTGGCTAGGAACCTTTGTCGTCTCTGTTTCTCT          IC21
TCCAC-TTACATATTTTGAAGCTCAAAGTGGCTAGGAACCTTTGTCGTCTCTGTTTCTCT          IC18
TCCACATT-CATATTATGATGGTCAAAGAGGTTGGGAACCTTTGTTGTCTCTGTTTCTCT          IC26C
TCC-CCTTACATATTATGATGTTCAAAGAGGTTAGAAAGTTTTGTTGTCTCCGTTTCACT          IC34
TCCACMTTACATATTWTGAWGBTCAAAGWGGYTAGGAACCTTTGTYGTCTCTGTTTCTCT          Con


AGATAATAGTGATTTT-ATCGTTGTTAGTCCACTTATTTAAGTAGTCTATGAATTTAGAC          IC21
AGATAATAGTGATTTT-ATCGTTGTTAGTCCACTTATTTAAGTAGTCTATGAATTTAGAC          IC18
AGATAATA-----TTT-ATAGTTGTTAGTCCACTTATTTAAGCAGTATATGAATTTAGAC          IC26C
AGATAATAGTGA-TTTCATAGTGGTTAGTCCACTTATTTATATAGTATTTAAATTTACAA          IC34
AGATAATAGTGATTTT*ATMGTTGTTAGTCCACTTATTTAAGTAGTMTATGAATTTAGAC          Con


TAAATTAATTGTTA-AACATTAGAATGATGTACATGTACCTTGAAAGGGTGTTTATAATA          IC21
TAAATTAATTGTTA-AACATTAGAATGATGTACATGTACCTTGAAAGGGTGTTTATAATA          IC18
TAAATTAATTGTTACAA-ATTAGAATGATGTACATGTACCTTGAAATGTGGTTTAGAATA          IC26C
TAAATTAATTGT-AAAA-ATTAGAATAATGTCCATATATCTTGAAAGATGGTTTAAAATA          IC34
TAAATTAATTGTTA*AACATTAGAATGATGTACATGTACCTTGAAAGGKKGTTTADAATA          CON


CATTAATAAGAATATAGGATATGGTTAAATATATTTA-AA-CAACACTAAAATTATAAGCTT        IC21
CATTAATAAGAATATAGGATATGGTTAAATATATTTA-AA-CAACACTAAAATTATAAGCTT        IC18
CATTAATAAGAATATAGAATATGGTTAAATA--TTTATAAACAACACTAAAAGTATAAGCTT        IC26C
CATTAATTCAAATGTAGAATGTTGTTAAATATTTTT--AAACAACAGTAAAAGTACAA-CTT        IC34
CATTAATAAGAATATAGRATATGGTTAAATATATTTA*AAACAACACTAAAAKTATAAGCTT        Con
```

Figure 3: The sequence of clones 21, 18 and 26, which are the tandem repeat from HindIII library, and clone no. 34 the tandem repeat from BamHI library. The sequence starts the HindIII sites and the BamHI site is internal. Consensus is given as Con.

Characterisation of pBNBH35 and pBNMB05. Two other clones from BamHI library pBNH35 and pBNMB05 were characterised by sequencing. Both these clones were identified as repeated DNA sequences. These sequences are dispersed in nature. This was confirmed by time course digestion of *B. nigra* with BamHI. Both these clones were sequenced on both strands. Plasmid pBNBH35 is 498 bp in length with an average GC content of 46%. Both of the dispersed repeated DNA sequences contained a number of direct and inverted sub-repeats.

Species specificity of the different *B. nigra* repeated DNA sequences. The tandem repeat (pBNBH34) of *B. nigra* (B genome) does not hybridise to *B. campestris* (A genome) under high and low stringency conditions. It hybridises to *B. oleracea* (C genome) but the pattern is very different under high stringency conditions.

The dispersed repeat pBNBH35 is species-specific DNA and does not hybridise to *B. oleracea* and *B. campestris* even under low stringency conditions. Plasmid pBNBH35 can be used as a strict B genome marker. This repeat DNA shows hybridisation signals as with *B. juncea* and *B. carinata*, which is to be expected as both amphidioploids contain *B. nigra* genome. *B. napus*, which does not contain *B. nigra* genomes, does not show any hybridisation signal. The other dispersed repeat pBNMB05 hybridises to *B. oleracea* but not to *B. campestris* under high stringency conditions.

Discussion

Tandem repeated DNA sequences have been detected in a number of species belonging to the *Cruciferae* family. In plants such as radish, mustard, cauliflower, *Sysimbrium irio* and *B. campestris* the satellite DNA has been cloned and sequenced. The basic monomeric unit of the satellite is about 180 bp long in all these plants except in *S. irio* in which it is 220 bp in length.

The hybridisation experiments clearly indicate that there is greater sequence homology between *D. erucoides* and *B. campestris* tandem repeats than with other wild allies of *B. campestris*. This is of significance since *D. erucoides* also gave stronger hybridisation signals than some *Brassica* species. The 177 bp tandem repeated DNA of *D. erucoides* has been cloned and characterised. This repeat accounts for nearly 15% of the genome and consists of many mostly identical copies of the monomer units that have been arranged in tandem. The nucleotide sequence of *D. erucoides* tandem repeat was sequenced with the aim of establishing its exact homology with the *B. campestris* tandem repeat. The 96% homology between *B. campestris* and *D. erucoides* tandem repeats confirms the hybridisation results.

These results support recent studies by Yangino, based on chloroplast DNA restriction pattern and fraction I protein large subunit types, which indicate closer relationships between *B. campestris* and *D. erucoides* than with some species of *Brassica* genera, such as *B. nigra* and *B. tournefortii*. Mizushima, also, has carried out genome interrelationship studies in *Brassica* and allied genera, based on cytogenetic studies in which chromosome pairing in hybrids is a measure of the chromosomal relationships between parental species. The chromosomal relationship found between *D. erucoides* and *B. campestris* - four bivalents were retained in this interspecific hybrid - was closer than for hybrids between species of the *Brassica* genera itself. This also supports our data that *D. erucoides, B. tournefortii, B. adpressa, S. arvensis* belong to another group based on the presence and sequence divergence for the 176 bp tandem repeat. This sequence data gives definite support to the nuclear, cytogenetic and chloroplast studies indicating similarity between *B. campestris, B. oleracea* and *D. erucoides*, it is possible that *D. erucoides* is a progenitor of *B. campestris* and *B. oleracea*.

The three different repeated DNA sequences of *B. nigra* reported belong to different families. One is a tandemly repeated DNA sequence (pBNBH34) with a unit length of 348 bp. This family of repeated DNA sequences is highly AT-rich (74%). These sequences do not show homology with any of the plant repeated DNA sequences reported so far.

The other two repeated DNA sequences namely pBNBH35 and pBNMB05 are of dispersed nature as confirmed by partial digests of genomic *B. nigra* DNA. The average GC contents of both sequences is 45%. Sequence analysis of these repeated DNA sequences reveals a number of inverted and direct subrepeats suggesting sequence duplication and rearrangements. These sequences do not show homology with any other reported plant or eukaryotic repeated DNA sequences.

Genome-specific repeated DNA sequences are useful as molecular markers in simple hybridisation experiments. The specific DNA can distinguish a particular genome type from others. Molecular markers are simpler and more accurate for identification of hybrids than conventional ones such as morphological traits, isozyme analysis and determination of fertility or cytological analysis.

None of the cloned repeated DNA sequences namely pBNBH34, pBNBH35 and pBNMbo5 hybridise to *B. campestris* (A genome) under high stringency conditions. These repeated DNA sequences can be used to demonstrate the presence of *B. nigra* in synthetic and somatic hybrids. pBNBH35 does not hybridise to *B. oleracea* (C genome) *or to B. campestris* (A genome) and can be used as a strict *B. nigra* (B genome) marker. The characteristic banding pattern observed in *B. genome* was in combination with other genomes such as A or C. This is shown by the similar hybridisation patterns observed with synthetic and natural *B. juncea* and *B. carinata* using probe pBNBH35.

Conclusion

As well as being an important crop for European countries *Brassica* is an important oilseed crop in India where *B. juncea* accounts for nearly 80% of that grown there. *B. nigra* and *B. campestris* are the parents of the amphidiploid *B. juncea*, so work was initiated on these two species. The main objective was to isolate and characterise species-specific probes from these species for *B. campestris*. The 177 bp tandem repeat of *B. campestris* which does not hybridise to *B. nigra*, *Eruca sativa* and *B. tournefourtii* is used for screening wild hybrids containing *B. campestris* as one male parent. This tandem repeat is used to identify synthetic *B. juncea* as well as wild hybrids such as *Eruca-Brassica*.

Three repeated DNA families of *B. nigra* have been identified and characterised. Of these, two are species-specific as they do not hybridise to *B. campestris or to B. oleracea*. They can be used as species-specific probes for the characterisation of hybrids.

Our interest is to isolate more species-specific probes from *B. nigra* to use them for the characterisation of additional lines of *B. nigra* on a *B. napus* background. Already two clones pBNH34 and pBNBH35 have been used for such characterisation in collaboration with M. Delseny of University of Perpignan, France.

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3 Tissue culture and nitrogen fixation of tree legumes for wasteland development

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Contract number and duration: CI1*/0195, April 1988 to March 1992

Background and objectives

The main objectives of the project were to select suitable tree legumes for afforesting the vast wastelands, their mass multiplication by tissue culture and the selection of suitable microbes.

The technical objectives were:

1 Selection of suitable *Acacia* species and *Leucaena* hybrids for semi-arid regions.

2 Development of tissue culture protocols for *Acacia* species and psyllid-resistant *Leucaena* hybrids.

3 Nodulation of the tissue culture propagated plants with efficient strains of *Rhizobium* and also their inoculation with mycorrhizae.

4 Transfer of tissue culture propagated plants to the field and their evaluation for (a) clonal uniformity and (b) increase in biomass.

5 Selection of microbes for increasing biomass production.

Materials and methods

Seeds of ten *Acacia* species were obtained from CSIRO, Australia. The species are as follows: *Acacia ampliceps, A. auriculiformis, A. bivenosa, A. holosericea, A. ligulata, A. maconochieana, A. salicina, A. sclerosperma, A. stenophylla, A. victoriae.*

The seeds of *A. nilotica* were obtained from the Pakistan Forest Research Institute, Peshawar. Seeds of *Leucaena leucocephala* and their hybrids were obtained from Nitrogen Fixing Tree Association (NFTA), Hawaii. The hybrids are as follows:

(1) *Leucaena retusa* x *L. shannoni*

(2) *L. leucocephala* x *L. diversifolia*

(3) *L. diversifolia* x *L. leucocephala*

(4) *L. diversifolia* x *(L. pallida x L. leucocephala)*

(5) *L. leucocephala* x *L. esculenta*

(6) *L. leucocephala* x *L. pallida*

(7) *L. pulverulenta* x *L. leucocephala*

The *Rhizobium* and mycorrhizae were isolated from the soils of Gwal Pahari. Some *Rhizobium* strains were also obtained from USDA and NFTA.

For selection of suitable species for Gwal Pahari soils the plants were first raised in polythene bags and later transferred to the field. No organic input was given. Plants were irrigated only in the first year.

Results and discussion

Figure 1: Tissue culture propagation of *Acacia* species. Phyllode forming *Acacia* sp. shows phyllode formation in culture

For tissue culture propagation seedling explants were used. This was due to the non-availability of adult trees in the near vicinity. The seeds were raised under aseptic conditions on Murashige and Skoog basal medium and explants were excised from 2-3 week old seedlings. All explants possessed preformed axillary buds (cotyledonary node, single node segments and terminal portion). The medium was always supplemented with cytokinins and sometimes a combination of cytokinin and auxin. Depending on the species, the axillary bud grew into a solitary shoot or it gave rise to a cluster of shoots. From the solitary shoot, single node segments were excised and sub-cultured. In species where a cluster of shoots was formed, shoots were separated and recultured on fresh medium of the same composition. For *Acacia*, the initial growth was slow and it took almost eight weeks to get a reculturable size shoot/shoot cluster. However, the multiplication rate was in the range of 8-9 fold every eight weeks.

Acacia victoriae. A. maconochieana, A. schlerosperma and *A. bivenosa* shoots, multiplied under *in vitro* conditions, could be rooted with 90-100% success. The cultures are maintained for over 75 cycles without any loss of vigour.

Leucaena leucocephala, once reported to be a wonder tree, is now questioned even for roadside plantation because of its susceptibility to attack by jumping plant lice (psyllids). Since it is a fast growing tree with versatile uses, attempts have been made to obtain trees resistant to jumping plant lice. By applying conventional techniques of plant breeding (hybridisation of *L. leucocephala* with psyllid-resistant *Leucaena* species), many valuable hybrids were obtained at NFTA, Hawaii.

The commercial exploitation of these hybrids is still awaited due to their semi-sterile nature and segregation in F_2 generation. The conventional methods of rooting of cuttings have failed for these hybrids. Seeds of seven of these hybrids were obtained and tissue culture methods have been developed through an enhanced axillary branching method. The multiplication rate is 4-6 fold every four weeks depending on the species and 90-100% of them could be rooted under *in vitro* conditions. The plantlets at the hardening stage were nodulated with *Rhizobium* strain NGR 8 and CB 81.

Large scale multiplication and transplantation to the field of *Acacia* species and *Leucaena* hybrids is scheduled for the monsoon of 1991.

Rhizobium and mycorrhizae were isolated from local soil for *Leucaena* and *Prosopis* respectively. Rhizobium isolates were tested for their efficiency of nodulation of nitrogen fixation while the mycorrhizae was tested for colonisation.

In *Leucaena* rhizobium isolates A1, A3 and NGR-8 were selected for field trial along with *G. mosseae, G. caledonius* and indigenous mycorrhizae. After 12 months, the observations indicate that the inoculation of the plants with both rhizobium isolate NGR-8 and *G. caledonius* and isolates A-3 and *G. caledonius* increases the biomass by 62% and 72% respectively. Indigenous mycorrhizae stimulated nitrogenase activity of isolate A-1 while *G. mosseae* inhibited the activity. Maximum colonisation of *Leucaena* was observed with indigenous mycorrhizae.

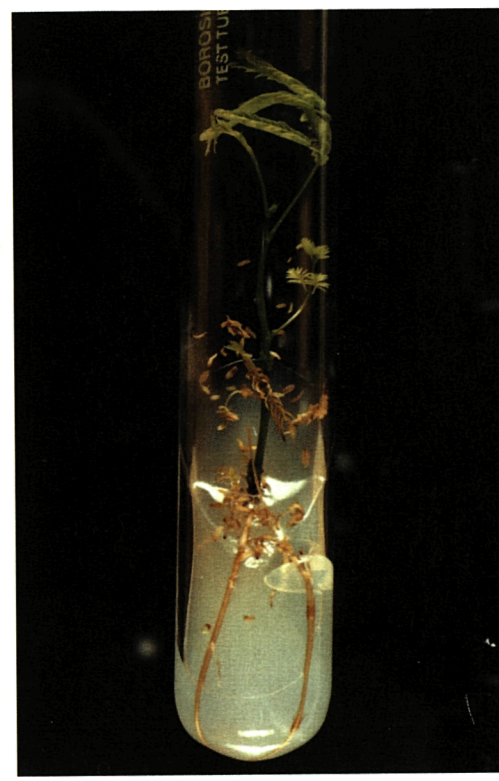

Figure 2: Tissue culture propagation of *Leucaena* hybrids. All *Leucaena* hybrids produced 3-4 shoots and were multiplied by culturing single node explants or terminal portion. Roots were produced from basal cut end without any callusing

In *Prosopis juliflora* nitrogenase activity declined with the onset of winter. The nodulation and nitrogenase activity was greatest with Tal-600 and was not observed during spring. Among mycorrhizae, maximum colonisation was observed with *G. calospora*. Total biomass was maximal in plants inoculated with indigenous mycorrhizae or *Rhizobium* isolate Tal-600 alone. Dual inoculation, however, led to decreased biomass compared with single inoculations.

Conclusions

As conceived in the original project it has been possible to select a few *Acacia* species from Australia which are suitable for wastelands in the Indian sub-continent. Tissue culture propagation protocols have been developed for four *Acacia* species and seven *Leucaena* hybrids. Microbial interaction studies observed over 12 months indicate that, in *Leucaena*, dual nodulation with rhizobium and mycorrhizae leads to increased biomass production. However, in *Prosopis juliflora* dual inoculation was not successful.

Postdoctoral fellowships

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Phytochemistry and plant biotechnology of the bitter and volatile components of Humulus lupulus L. cultivars.

Fellowship period: February 1989 - January 1991

Summary

Various cultivars of hop (*Humulus lupulus* L.; fam. Cannabidaceae) are used in the brewing industry to impart a bitter flavour and hop aroma to the well known beverage 'beer'. The glandular hairs (lupulin glands) of the hop cones (hops) contain a resinous fraction of α- and β- acids, which is responsible for the bitter taste of the beer and a volatile fraction (the essential oil) which contributes to its aroma. Various investigations concerning the intact plants as well as cell suspension cultures of hop were undertaken as there is still an incomplete understanding of the subtle chemical quality factors of hops and their impact on beer quality.

As supercritical fluids are known to penetrate more efficiently into plant materials and can extract the soluble components to a greater extent, experiments were carried out on extraction from the cones and leaves of hops by supercritical CO_2 at different combinations of temperature and pressure. The best parameters were thereby established for the complete extraction of the bitter and volatile constituents from the hops.

A biotechnological approach to induce the hop cells to produce their flavour and aromatic principals resulted in the isolation of 1-phenyl-(4'-hydroxy)-n-nonanone as a new compound from the cell suspension cultures of *Humulus lupulus* cv 'Wye Northdown'.

In order to know about the enzyme systems present in these cells and their potential to transform exogenously added substrates, the cell lines were inoculated with geraniol and a mixture of four isomers of farnesol. The cells were able to oxidise, isomerise and metabolise the added components, the corresponding aldehydes of farnesols and geraniol being the main biotransformation products. This conversion was found to be enhanced when the cells were propagated in a two-phase culture medium.

Besides the use of hops in breweries, they have also been used in medical treatment since the middle ages, owing to their supposed antimicrobial activities. This fact prompted us to determine the possible activities of extract and the essential oils of various available hop cultivars against Gram-positive and Gram-negative bacteria, a yeast and a fungus. The experiments gave a comparative idea of the activities of the essential oils and the extracts of the same plant, and further on of the differences between the various cultivars.

The possible occurrence of the glycosidically-bound volatiles in aqueous extracts of cones of *Humulus lupulus* L. cv 'Brewer's Gold' and aerial parts of *Cannabis sativa* L. was also studied. The extracts treated with β-glucosidase failed to yield any volatile compounds. However, we detected some

monoterpene alcohols in the acid hydrolysed hydrodistilled fraction of both the plant materials. *Cis*-3-hexen-ol-1, hexanol-1, 7-methyl-octan-1-ol, pinocarveol and eugenol from *C. sativa* L., and *cis*-3-hexen-ol-1, and *cis*-3-octen-ol-1, benzyl alcohol and *trans*-sabinene hydrate from *H. lupulus* L., cv 'Brewer's Gold', were a few of the components identified.

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Nitrogen mineralisation and immobilisation transformations in soil

Fellowship period: June 1990 - July 1991

Summary

The soil microbial biomass is the agent of transformation of organic C and N back to simple inorganic forms. It is also a source and sink of plant nutrients in soil. Straw incorporation at harvest is believed to decrease nitrate leaching losses because the biomass that develops on the straw has a high N demand. However, the dynamics are poorly understood. Residue incorporations at harvest may have several advantages such as the economical management of farm by-products, a decrease in the input of fertiliser nitrogen to the soil and decreases in the leaching of nitrate and the output of greenhouse gases to the atmosphere. It may permit the development of suitable management strategies for the efficient use by crops of organic and inorganic soil nitrogen sources. The aim of the work is to evaluate the effects of straw incorporation on soil microbial biomass and soil N dynamics to help towards a better understanding of the nitrogen cycle. The following experiments have been set up and have achieved, to date, the main results noted. The experiments are still in progress so that their results have not yet been published.

Effects of straw incorporation on soil microbial biomass C, N and ninhydrin-N. Soil microbial biomass dynamics was studied in a clay soil containing recently added and actively decomposing wheat straw. Biomass C, N and ninhydrin-N increased several fold due to straw incorporation in soil.

Estimates of soil nitrogen balance using ^{15}N-labelled sugar beet tops. Soil nitrogen dynamics studies using ^{15}N-labelled sugar beet tops showed that microbial biomass derived nitrogen preferentially from sugar beet tops rather than from inorganic fertiliser nitrogen. The amounts of microbial biomass C and N were greater in clay soil than in sandy soil following the addition of sugar beet tops. After 40 days of incubation, the mineralisation of total inorganic N was at a faster rate in the sandy soil than in the clay soil. Over short incubation periods (40 days), there was an excellent "nitrogen balance" in soils amended with ^{15}N-labelled sugar beet tops and ^{15}N-labelled inorganic fertiliser nitrogen, suggesting that N losses, e.g. due to denitrification, were very small.

Standardisation of the diffusion method for nitrogen-15 analysis. This was amongst the various analytical techniques required in the experimental work.

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Characterisation of the nature and location of proteases responsible for chloroplast degradation during senescence.

Fellowship period: August 1990 - August 1991

Summary

The turnover of thylakoid membrane proteins was studied. The objective was to characterise the degradation products of a D1/D2/Cyt 6559 complex of PSII reaction centre during senescence and photoinhibition. The degradation products were studied by gel electrophoresis and immunoblotting.

Polyclonal antibodies (immunoglobulins, IgG) against electroeluted D1 and D2 proteins were synthesised in rabbits following hypodermal or lymph node treatments. The lymph node treatment was quicker and yielded high titre of antibodies against both D1 and D2 polypeptides. These polypeptides will be used for studies on the turnover of D1 and D2 proteins.

The unsolved problem of whether the turnover of proteins in D1/D2/Cyt 6559 complex of PSII reaction centre is an enzymatic or a radical-damage process is answered by characterising the enzyme associated with this complex. Current studies at the Welsh Plant Breeding Station, Aberystwyth are on the molecular mechanism of turnover of LHCP II protein during senescence of *Lolium temulentum*.

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In vitro/in vivo metabolism of a novel organophosphate insecticide

Fellowship period: August - September 1990

Summary

The basic aim of the present study was to investigate the metabolism in mammals and birds of a novel organophosphate insecticide developed at IICT, Hyderabad, India using unlabelled and ^{14}C-labelled chemicals.

ß-esterases are known targets of oxons generated by oxidation of thiophosphates, and therefore their inhibition can provide a very sensitive biochemical measurement of NADPH-dependent oxon formation by microsomal mixed function oxidases (mfo). Our studies on the interaction of this novel analogue with hepatic microsomal preparation of rat with and without-NADPH supplementation have revealed progressive NADPH-dependent inhibition of microsomal carboxylesterase (α-naphthyl acetate esterase) suggesting oxidative desulphuration of the analogue by mfo to form an active oxon that subsequently phosphorylates the enzyme. Similar results were obtained with rat hepatic microsomal butyryl cholinestrerases (BuCh). However, carboylesterase appeared to be more sensitive to inhibition that BuChE, and was used as a marker of oxon formation in subsequent studies with warfarin-resistant rats and Japanese quail. Resistant strains of rats showed less inhibition of α-NaE activity than susceptible ones under similar experimental conditions. Following the time course of enzyme inhibition, warfarin-resistant rats seemed to activate the analogue more slowly than susceptible strains did and/or hydrolysed the activated product faster than their counterparts. Japanese quail also showed NADPH-dependent activation of the analogue by hepatic mixed function oxidases as assessed by inhibition of microsomal α-NaE activity.

Interestingly, the inhibition of α-NaE activity in the bird was much faster than in rats. This may be due to a difference in oxidation-hydrolysis balance; birds have an inherent deficiency of α-esterases.

The analyses of hepatic microsomal metabolic extracts of unlabelled chemical by TLC, HPLC and those of ^{14}C-labelled radio TLC scanner established that the analogue undergoes detoxification without involving mfo as well, presumably by hydrolysis of carboxylester bond. Separation, purification and identification of metabolites are in progress.

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Ergonomic evaluation of multifunctional engine-operated knapsack-type brush cutter.

Fellowship period: February - July 1991

Summary

The noise levels of the multifunctional brush cutter were determined with the help of sound level meters, together with their effect on the operators' hearing. The amplitude of vibration and the frequency of its main components were evaluated by means of accelerometers. The effect of noise and vibration on the performance of the operators were investigated. Data on sweat loss, skin temperature, core temperature and metabolic rate were determined to assess the thermal load on them. The measurements of energy expenditure while operating the brush cutter were made in the field with different cutter blades for mowing grasses. Measurements of energy expenditure were repeated with a cultivator attachment for field interculture on different types of weeds. Ergonomic measurement included the force required by the operator and the cultivator's angle of working, the work-rest schedule and records of muscular activity by electromyography, as well as safety aspects.

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Expression and modification of genes for the photosynthetic (thylakoid) membrane in higher plants.

Fellowship period: August 1990 - January 1991

Summary

Investigations have been carried out to identify light-responsive *cis*-elements from the promoters of nuclear-encoded genes psaF and petH, coding for subunit III of photosystem I and ferredoxin-$NADP^+$ -oxidoreductase, respectively. For this, suitable deletions of the promoters were hooked to the reported gene for ß-glucuronidase (GUS) and the fusion constructs were introduced into tobacco cells via *Agrobacterium*. Preliminary assays in the transient expression system and with transgenic plants show redundancy and multiplicity of the regulatory elements. In addition, certain *trans*-acting factors interacting with photoregulated (psaF, petH and rbcS) and a constitutive (CaMV35S) promoter have been characterised.

Success has also been achieved in expressing plastocyanin in *E. coli* by employing fusion gene constructs containing coding regions for plastocyanin with coding regions for signal peptide of bacterial protein azurin or for hydrophobic domains of transit peptides responsible for targeting proteins into thylakoid lumen. Products from both types of constructs are found in the periplasmic space of bacteria thereby establishing the functional equivalence of two domains. Since the mutant plastocyanin gene - generated after site-directed mutagenesis - is also expressed in bacteria, it should be possible to understand the structure-functional aspects of the protein by bio-physical methods.

Publication

Herrmann, R.G.; Westhoff, P.; Tyagi, A.K. and Link, G. (1991). Biogenesis of plastids in higher plants. *In:* Herrmann R.G. (ed.), Plant Gene Research Vol 6. Cell Organelles - Plastids, Mitochondria, Glyoxysomes, Peroxysomes, Springer, Wien - New York, in press.

2 BIOLOGICAL SCIENCES

Postdoctoral fellowships

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NMR spectroscopy in living tissues

Fellowship period : September 1990 - August 1991

Summary

Vital information on a metabolic process *in vivo* can be obtained by proton NMR spectroscopy, complementary to anatomical and functional studies by magnetic resonance imaging.

To carry out NMR spectroscopy in living tissues, solvent suppression and localisation of volume of interest are prerequisite conditions. In proton NMR *in vivo* a strong water signal masks weaker signals from metabolites which have very low concentrations (1 mM to 10 mM) compared with water (50 M/1). Hence, to obtain the metabolite spectrum *in vivo*, the water signal has to be suppressed as much as possible. A promising technique in solvent suppression is the selective excitation of the water signal by a narrow band width, frequency selective, pulse at water resonance and the application of a strong field gradient (homospoil gradient) to spoil the coherence of water. Experiments to achieve optimum solvent suppression by this technique are now under way. It is then planned to incorporate the technique in a spin-echo localisation method and in a spectroscopic imaging method for investigation of metabolic processes by proton NMR spectroscopy.

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Effect of Bacillus megaterium on removal of copper by activated carbon from aqueous solutions

Fellowship period : October 1990 - September 1991

Summary

Bacterial exopolymers are known to be efficient at binding metals and could influence the adsorption of copper by activated carbon. A Gram-positive bacterium *Bacillus megaterium* is found to be responsible for removing a high quantity of Cu from aqueous solutions. Resting bacterial cells of *B. megaterium* can remove up to 29.1 mg of Cu per g of cell dry weight. Almost total removal of Cu was achieved with the bacteria in combination with activated carbon up to a Cu concentration of 100 mg/l. Growing bacterial cells are found to have a synergistic effect on the adsorption of Cu by activated carbon.

3 CHEMICAL SCIENCES

Joint research project

4 Charge-transfer processes in ions, molecules and biopolymers induced by ionizing radiation

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Contract number and duration: CI1*/0518, December 1990 to November 1993.

Summary

Using ionizing radiation (u.v., X- and g*-rays) electrons and holes will be generated in a range of systems at low temperatures and their behaviour will be studied using temperature resolved e.s.r. spectroscopy. The systems will contain at least two different electron (or hole) traps which are either separate or part of the same molecule (or ion). Factors which govern initial electron (hole) capture will be investigated, and if, as expected, subsequent transfer of electrons (holes) between the centres occurs, kinetic studies will be used over a range of temperatures in order to probe in greater depth the factors controlling such transfers.

Examples of simple (intermolecular) systems will include glasses containing electron-capture agents (such as Ag^+, NO_3^-, alkyl halides, etc.) and others containing hole-capture agents (such as ethers, aldehydes, toluene, alkyl halides, phosphines, etc.). Examples of intramolecular competition and transfer will include frozen media containing bi-functional or poly-functional molecules (ions). Examples will include organic molecules with S-S, C≡N, C=O and other e^--capture centres having known, fixed separations, and others having hole capture centres similarly disposed within the molecules.

Biological examples will include proteins having multiple e^--capture sites (such as xanthine oxidase) and/or multiple e^--loss sites. Also DNA and chromatin will be studied. In all cases, factors which control specific electron- or hole-capture will be sought.

Workshop

Polymeric membranes

Joint coordinators:

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Summary

The new field of membrane technology was reviewed by scientists from seven European Community countries and from a range of Indian institutions at this workshop held at New Delhi from 30 November to 2 December 1989.

The subject was considered to hold considerable potential for joint European Community-India cooperation in both basic and applied research and in the application of existing technology. Specific recommendations were made for research and development related to the use of membranes in water treatment, including making brackish water potable, in the fertiliser industry, in the sugar, dairy, edible oil and other food and beverage industries, in cell culture and in the pharmaceutical industry. The application of inorganic and ceramic membranes and membranes for gas separation and pervaporation were also covered by these recommendations.

Postdoctoral fellowships

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New routes to pharmaceutically important heterocycles

Fellowship period : August 1990 - July 1991

Summary

Aminoquinoline derivatives are widely used as active antimalarials and strongly inhibit the enzymes acetyl cholinesterase and monoamine oxidase. They are also intrinsically valuable due to their potential anthelmintic and amoebicidal activity. Despite the importance of the 4-amino functionality in particular in such compounds, scant attention has been given to methods for its direct introduction. Moreover, the presence of a 3-carboxylic acid or ester functionality in 4-aminoquinolines further enhances the pharmacological activity of these compounds. Therefore it was planned to develop efficient synthesis of quinoline derivatives containing variants of these two functionalities.

Two different approaches were considered. In the first a systematic study of the reactions of o-aminonitrites with carbon nucleophiles was undertaken. It was found that anthranilonitrile (1) reacts with carbanions derived from substituted acetic acid esters (2) to give 3-substituted-4-amino-2(1H)-quinolones (3) directly (Figure 1).

So far, five different nucleophiles (2) viz. ethyl cyanoacetate, ethyl acetoacetate, diethlyl malonate, ethyl octyloxycarbonylacetate and ethyl phenylacetate have been utilised for this type of synthesis.

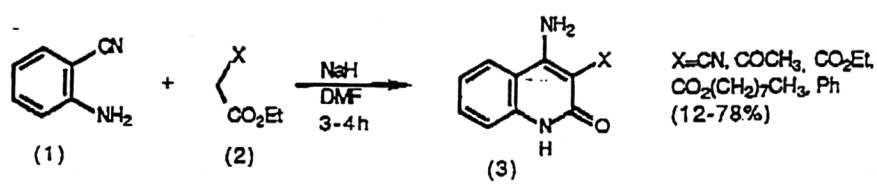

Figure 1 Reaction of anthranilonitrile with carbanions derived from substituted acetic acid esters.

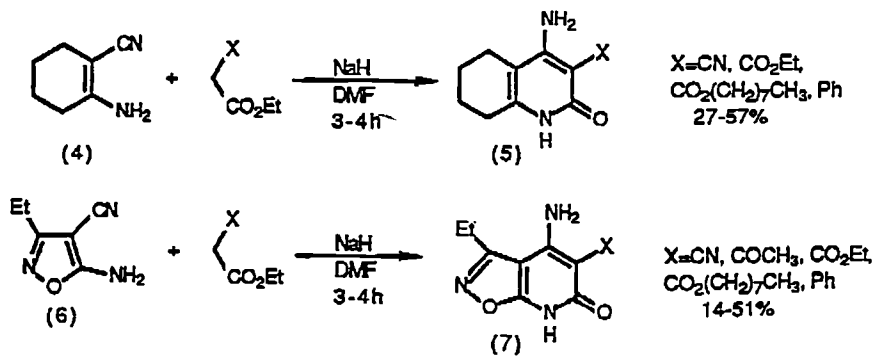

Figure 2 Generality test of the reactions of o-aminonitrites with carbon nucleophiles

To test the generality of the approach both cyclic enaminonitriles e.g. (4) and heterocyclic enaminonitriles e.g. (6) were reacted similarly and gave the desired heterocycles (5&7) respectively in moderate to good yield (Figure 2).

In an alternative approach, shown in Figure 3, a study of the reactions of carbon nucleophiles with cyano-substituted ethoxymethyleneamino derivatives (9) has been initiated which has necessitated the development of a reliable procedure for the preparation of the latter intermediates. This has now been accomplished, and a very satisfactory synthesis of (9) from (8) has been developed.

Work is now in progress to establish the reactivity of (9) towards carbon nucleophiles under both base and metal catalyzed conditions.

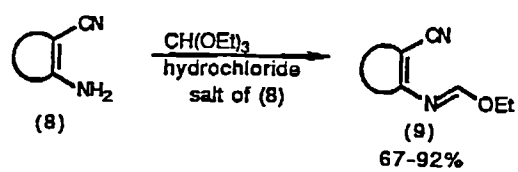

Figure 3 Reactions of carbon nucleophiles with cyano-substituted ethoxymethylene-amino derivatives

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Photoionisation of electron-sacrificial agents in presence and absence of semiconductor colloidal particulate systems

Fellowship period : October 1990 - March 1991

Summary

Three separate investigations were carried out; these were:

Reactions involving electron-sacrificial agents: 2,2'-azinobis-(3-ethylbenzothiazole-6-sulphonate), ABTS, which is a very good electron-sacrificial agent, is found to be photoionised by 308 nm and 248 nm laser pulses, resulting in the formation of $ABTS^{\cdot +}$. Further photoionisation of $ABTS^{\cdot +}$ by 248nm laser always results in the formation of $ABTS^{++}$ and ABTS. $ABTS^{\cdot +}$ reacts with many reducing radicals and the corresponding second order rate constants are determined.

Formation of $ABTS^{++}$ was also observed (λ_{max} = 515 nm) by reactions of many oxidizing intermediates such as, $CO_3^{\cdot -}$, Ag(II), Tl(II), $N_3^{\cdot}$, etc. with $ABTS^{\cdot +}$. The respective rate constants are determined. In short the reactions,

$$ABTS \xrightarrow{-e^-} ABTS^{\cdot +} \xrightarrow{-e^-} ABTS^{++}$$

(reducing agent) (reducing agent)

are thoroughly investigated by laser photolysis and pulse radiolysis techniques.

Photochemical production of phenylcations and their reactions with aromatics. Phenyldiazoniumtetrafluoroborate and its 4-methyl and 4-methoxy derivative were photolysed in 1,1,1,3,3,3-hexafluoro-isopropyl alcohol (HFIP) with 308 nm laser pulses. This leads to a pronounced and permanent depletion of the parent compound, but a signal from a transient is not seen. However, on addition of aromatics such as mesitylene, strong signals with λ_{max} ~ 380 nm are seen. In the absence of nucleophiles other than the aromatics, these species, which do not react with oxygen, have a life time in the ~ μs to ms range. On the basis of their absorption spectra and their reactivity with typical nucleophiles such as halides and alcohols, the transients are identified as cyclohexadienyl cations (CHD) formed from the photoproduced phenyl cations by addition to the ring of the added aromatics. The reactivities of the CHD cations with alcohols, ethers and Br^- are characterised by the bimolecular rate constants for these processes.

ESR investigations of sulphate, phosphate and hydroxyl radicals with furancarboxylic acids. In-situ-photolysis and in-situ-radiolysis investigations of reactions of sulphate, phosphate and hydroxyl radicals with furancarboxylic acids were carried out and the ESR spectra of the resulting adduct radicals were identified and characterised. The effect of pH on the structure and stability of the adduct radicals was also investigated.

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Indirect electrochemical detection in high-performance liquid chromatography

Fellowship period : December 1990 - November 1991

4 EARTH SCIENCES

Postdoctoral fellowships

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Quaternary upwelling and climate in the Arabian sea and Bay of Bengal (Northern Indian Ocean) related to monsoonal events

Fellowship period : September 1990 - February 1991

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Response of middle atmospheric structure and dynamics to changes in solar activity and phases of quasi-biennial oscillation

Fellowship period : August 1990 - July 1991

Summary

Solar activity, QBO and Indian rainfall. Studies were made on the influence on rainfall distribution over the Indian sub-continent of solar activity and of changes in the phase of equatorial quasi-biennial oscillation (QBO). The sunspot number and the phase of the QBO at the 15-20 hPa level during January and February are considered as the source parameters and the annual rainfall, averaged over the entire Indian region, is taken as the dependant variable. It has been found that during the period of low solar activity, excess/normal rainfall (frequent floods) is associated with the westerly phase of the QBO and deficient/normal rainfall (frequent droughts) generally corresponds to the easterly phase. During the period of high solar activity only normal rainfall is observed when the QBO is in its westerly phase, but no clear performance is then found to be associated with the easterly phase of the QBO. It is interesting to note that heavy rainfall is always associated with the westerly phase and no droughts occurred.

The important application of the present study is that if the direction of the QBO at the 15-20 hPa and the level of solar activity during the earlier part of the year are known, it may be possible to give a forecast of the rainfall of the year. It may also be possible to forecast the possibility of occurrence of severe weather conditions, like floods or droughts, which have disastrous effects on agriculture, industry and power generation and which may cause severe strain to the national economy of the country. A detailed study on this topic, including regional distribution of the results, is in progress.

Solar activity and middle atmospheric responses. Investigations are being made into the influence of solar activity, in the 11-year cycle, on changes in the atmosphere parameters (pressure temperature and wind) in the 20-80 km middle atmospheric region. These are being carried out for different latitude zones and different seasons using rocket data which is available over a period of 22 years (1969-1990). The temperature changes in the mesosphere are highly sensitive to solar changes: a warmer mesosphere is observed during the period of solar maximum and a colder mesosphere is noted during solar minimum. The stratosphere, on the other hand, shows a different response in different seasons. The zonal wind in the mesosphere also indicates a strong response to solar activity. The middle atmospheric pressure variations show a direct association with the changes in solar activity, higher near the equator and lower near the poles in summer. The linkage will be studied between changes in the thermal structure and circulation pattern of the middle atmosphere associated with changes in solar activity.

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Complex terrain air quality modelling

Fellowship period : August 1990 - January 1991

Summary

An extensive study of the methods of stability classification in vogue in the UK concluded that the ratio of boundary layer height to Monin-Obukhov length is a superior indicator of stability. A method has been devised to derive this parameter from conventional meteorological data. This is being validated under Indian conditions.

An in-depth examination of different ways of deriving diffusion coefficients led to the identification of a new method which is applicable over all terrains and which incorporates mixing layer velocity scale and turbulence in convective and neutral conditions. These equations have implicit travel time and hence can be used for long distances also. The unconventional parameters needed for the above equations have been derived from conventional meteorological parameters.

Further studies showed that, instead of using conversion factors to obtain air quality standards for one time domain from another, it is more appropriate either to interpolate between two time domains or to evaluate the moving average for the required time domain.

A critical examination of plume rise formulae, supported by experiment, indicated that Moore's formula is more appropriate than others. This is being validated under Indian conditions.

Two models - one for Coastal Diffusion (CDM) and other for Complex Terrain (CTDM) - have been formulated. The parameters required for these models are derived from conventional meteorological data. The computer programmes are being finalised. Based on Indian data the results show that the complex terrain model yields higher concentrations than flat terrain computations.

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Ore formation on rapidly diverging plate boundaries: geothermal metallogenesis on East Pacific rise (geometep)

Fellowship period : February - July 1991

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Mathematical modelling of ocean and coastal waters

Fellowship period : January - June 1991

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Fundamental research for developing methods for seismic refraction data and reflection data

Fellowship period : October 1990 - March 1991

Summary

The Indian subcontinent preserves a diversity in tectonic and geological elements spanning, in age, from the Archean to the present. It also showed super mobility in traversing a distance of about 9000 km, with an anti-clockwise rotation of around 60 degrees, since its separation from the main Gondwana landmass. Such anomalous features require a thorough investigation to ascertain the probable cause, in terms of underlying geological structures, and thereby deduce the geodynamical aspects of this region. P-wave Seismic Tomography has proven to be a powerful tool to get a three-dimensional view of complex geological and tectonic regimes.

For the present investigation an area bounded by latitudes 20 degrees south to 80 degrees north and longitudes 60 to 100 degrees east was selected. Seismic delay time data were extracted from CD-ROM provided by NEIC/USGS for the period January 1964 to August 1987. The selection criteria were to have regional events under the above prescribed limits and recorded by stations within epicentral distance of 90 degrees. Teleseismic events all over the globe and recorded by stations within the regional limits were also selected for the present investigation. The data were corrected for the ellipticity of the earth. The present selection criteria provided 924,000 delay times. Two dimensional histograms for delay times (0.1 s interval) and epicentral distance (0.5 degrees) were prepared. Such a density plot shows large dependence of the data on the Jeffreys-Bullen (J-B) reference model. The J-B model, relative to which these delay times are estimated, is modified in such a way as to remove the epicentral dependence in the data, which in turn leads to a better reference model. After correcting the ISC delay times for the travel time difference between the J-B and the new model quite an improvement was found in the distribution of delay times. This new reference model will be used for future tomographic work. Standard deviations of the delay times for individual stations are estimated and stations with large scatter and standard deviation above 3 will not be considered for the investigations in the future.

Preliminary study of station delay times indicates that the stations over the southern Indian tip and over the entire eastern coast of India and even the Burmese Arc have positive average delay time. This shows that the region is underlaid by low velocity structures and probably the sediments in the Bay of Bengal may also influence such anomaly pattern. Negative delay times are observed over the Aravallis. Localised positive delay times are recorded in the vicinity of Punjab Himalayas and over the Tien Shan regions, negative delay times appear above the Hindu Kush.

A tectonic map of the region, incorporating all available information, has been prepared.

5 ENVIRONMENTAL SCIENCES

Postdoctoral fellowships

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Evaluation of the analysis of PCBs, DDT and related compounds in the aquatic environment.

Fellowship period: February - July 1991

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Environmental impact assessment and management of wastewaters.

Fellowship period: October 1990 - September 1991

Background

The area of operation of this project is the Greater Manchester region (UK) through which flows a configuration of the rivers Irwell, Medlock and Irk. Further, the river Irwell has been shaped into the Manchester Ship Canal. According to the prevailing environmental rules and regulations, sewage (domestic or industrial) can not be discharged directly into the water bodies. The responsibility for treating the sewage lies with North West Water plc., while that for monitoring the water quality of flowing water systems lies with the National Rivers Authority.

Summary

The present research work is being pursued on the following lines:

Wastewater characteristics: Samples of sewage are being analysed for various parameters. Comparison with the observations of the preceding five years is likely to establish a trend which will help in planning a better strategy.

Health of the riverine system: The water samples of the river Irwell system have been analysed for various physico-chemical and biological parameters to assess the health of this aquatic eco-system.

The data based findings of the preceding five years are likely to help in developing a water quality model for future predictive purposes.

Riverine fish and fisheries: The population of fishes like rainbow trout, brown trout, Scottish gobio and loach etc. is to be ascertained in relation to water quality.

Wastewater management: The system of wastewater collection, recycling, treatment and disposal is altogether different in UK from that in India. Research will include the identification of sources, the canalisation of sewage, its treatment and its disposal/utilisation. In the treatment part, emphasis will be on assessment of the efficacy of non-conventional means of sewage treatment, such as biological.

Environmental impact assessment (EIA) of sewage: Selection is in progress of a site for the EIA of sewage from amongst the newly planned Human Settlement Schemes.

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Development of automated field monitors for water pollutants by flow injection analysis.

Fellowship period: December 1990 - May 1991

Summary

The current interest in on-line analytical instrumentation for monitoring water quality parameters can be attributed to the need for prevention and/or control of transient pollution incidents, the gathering of detailed trend data for water management and to ensure that industrial discharges and abstracted, potable and treated waters conform to the required standards.

In addition to the standard laboratory requirements of accuracy, precision, sensitivity and selectivity, any on-line water quality monitor must also involve easy-care instrumentation, which includes the need for reagent stability, hardware and software flexibility, internal diagnostics, stay-clean properties, modular construction and easy on-site maintenance and calibration. The technique of flow injection analysis (FIA) would seem to be ideally suited for this purpose and offers the additional attractions of rapid response, low purchase, maintenance and operational costs and an extensive collection of established laboratory methods.

On-line determination of low residuals of free chlorine and other chlorine species. Since the inception of disinfection, chlorine has been by far the most widely used disinfectant. One aspect of water quality monitoring is the determination of residual free chlorine, and its reaction by-products, especially combined chlorine residuals. A major problem associated with the analysis of chlorine is the need to be able to differentiate between free and combined chlorine.

A new reaction chemistry is evaluated in the present study that does not involve the use of toxic or unstable reagents, and is based on the oxidation of 4-nitrophenylhydrazine by chlorine to yield 4-nitrophenyl diazo cation and its subsequent coupling with N-(1-naphthyl)ethylenediamine to form an azo dye.

Two variations of FIA configurations were optimised: one in which the analyte chlorinated water forms one of the streams in the manifold and the reagent is injected, and others in which chlorine is injected and merged with reagents.

This reversed-FIA method has been utilised for the precise (%RSD in the range 0.5-2%) and sensitive (limit of detection 0.03 p.p.m.) determination of 0-12 p.p.m. of chlorine. The throughput was 60 samples per hour.

A thorough investigation was done to optimise the flow injection parameters such as flow rate, reaction coil lengths, injection volume, concentrations of reagents and carrier.

It is proposed to employ the investigated system for the analysis of chlorine in the presence of other chlorine residuals such as chlorate and chlorite, and their speciation by using gas-diffusion membrane or other on-line separation techniques. A special stress is on the development of present system as an automated field monitor.

A proposal has been put forward for an extension of the fellowship to permit investigation of FIA-based monitors for the on-line determination of trade quantities of nitrate, of mercury and of hydrogen sulphide.

6 HEALTH AND BIOMEDICAL SCIENCES

Summary

The principal topics covered in this chapter are leprosy (page 52) and polio (page 58), two diseases of major public health importance. Both projects involve detailed immunological studies of responses in patients to vaccination with a view, ultimately, to cheap and effective control of these diseases.

Research on leprosy as well as other mycobacterial diseases was stimulated considerably by a joint EC-India workshop (page 60) which allowed a review of the subject to be undertaken and contacts among scientists to be developed.

Finally, a postdoctoral fellowship has enabled a study of environmental risk factors in oesophageal cancer to be carried out (page 61).

Joint research projects

5 Immunology and immunoprophylaxis of leprosy

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Contract number of duration: CI1*/0264/0265, December 1988 to December 1991.

Background

Leprosy is a spectral disease, in which clinicopathological features reflect the status of host cell mediated immunity (CMI). The highly bacilliferous lepromatous (LL) patient, who represents the end of the spectrum, exhibits *M. leprae* specific anergy. At the tuberculoid (TT) pole patients mount a good CMI, with improvement in tissue bacillary load and containment of lesions (Kaufmann and Deo, 1990).

Vaccination is perhaps the cheapest and most effective means of controlling infectious diseases. ICRC vaccine is undergoing clinical trials as an anti-leprosy vaccine in leprosy endemic areas in India. A single dose of the vaccine brings about lepromin conversion in 55% of the patients, associated in some patients with "up grading" and tissue bacillary clearance. High conversion rates (about 95%) were observed in lepromin negative healthy residents of endemic areas (Deo *et al.* 1990).

Objective

The main objective of this project is to investigate the immuno-reactivity of leprosy patients to antigens of ICRC and *M. leprae*, before and after vaccination with gamma-irradiated ICRC bacilli.

Work primarily carried out in Ulm

Characterisation of T cell antigens. The method which has been developed combines the high resolution power of two-dimensional gel electrophoresis with the advantage of direct probing with viable cells (Gulle *et al.* 1990). This device allows the transfer by electroelution of 480 distinct fractions from the gel into soluble phase. Transferred fractions are virtually nontoxic, thus allowing direct probing with viable cells. Using this procedure it was found that T cells from normal healthy individuals recognise a multitude of mycobacterial antigens and that the fine antigen recognition pattern of T cells changes after short-term culture *in vitro*. The procedure has also been applied to the verification of antigen purity of the heat shock protein 65 (hsp 65) on the T cell level and to the identification of antigens within crude bacterial lysates which are recognised by cloned T cells. Currently, this approach is employed for the identification of antigens that are characteristic for ICRC-vaccinated individuals, leprosy patients or contacts, respectively.

T cells directed against shared regions of heat shock proteins. Studies using recombinant proteins of *M. tuberculosis* and *M. leprae* revealed that hsp represent prominent T cell antigens. Hsp are highly conserved and it has been shown that the mycobacterial and human hsp cognates share more than 60% sequence homology (Kaufmann, 1990). Therefore, hsp could be involved in immunopathology of leprosy. To address this possibility, T cells from healthy individuals were activated in vitro with mycobacterial lysates and afterwards tested for cytolytic activity on autologous target cells primed with (1) killed mycobacteria, (2) intact recombinant hsp 65 of mycobacteria or (3) tryptic fragments of recombinant hsp 65 (Munk *et al.* 1989). Strong cytolytic activity was observed on targets primed with killed mycobacteria or with tryptic fragments of hsp 65 but not those primed with the intact hsp 65. To assess whether T cell responses were directed against self epitopes shared by the mycobacterial and human hsp 65, four peptides of at least 10 amino acids length were synthesised, corresponding to identical or almost identical regions of this molecule. Peripheral blood T cells from most individuals, tested after activation with killed mycobacteria, expressed strong cytolytic activities towards autologous targets primed with one or more of these synthetic peptides. By using HLA-DR transfected murine L cells we found that the epitopes were recognised in the context of major histocompatibility HLA-DR (class II) molecules. It is concluded from these experiments that T cells with specificity to self epitopes exist and that such T cells might be activated during mycobacterial infections. Due to their cross-reactivity, such T cells could be involved in pathogenesis of mycobacterial diseases.

Gamma/delta T cells. During the last year we have also started with the phenotypic characterisation of mycobacteria-reactive T cells. This approach was initiated at our department using T cells from peripheral blood of normal individuals and in the future will be applied to the analysis of T lymphocyte sets from leprosy patients. It used to be assumed that all peripheral T cells express a receptor composed of an alpha and a beta chain. More recently it was found that, in addition, a second T cell set exists which expresses a T cell receptor composed of a gamma and a delta chain. It appears that this T cell set has a particular predilection for mycobacterial antigens. We have stimulated peripheral blood T lymphocytes from healthy donors with mycobacterial lysates in vitro and afterwards analysed them phenotypically (Munk *et al.* 1990). Marked expansion of the gamma/delta T cell population was observed in the majority of donors, 7 to 10 days after stimulation. Mycobacteria-activated gamma/delta T lymphocytes expressed receptors for interleukin-2 and secreted interleukin-2 upon restimulation with mycobacterial antigens. Furthermore, stimulation with mycobacterial lysates evoked specific cytolytic activity in the gamma/delta T lymphocytes as demonstrated in the following ways:

1 gamma/delta T cells lysed mycobacteria-primed but not unprimed targets;

2 high concentrations of antibodies against the gamma/delta T cell receptor facilitated killing of unprimed target cells;

3 low doses of this antibody blocked killing of primed targets;

4 gamma/delta T cells from two donors after activation with mycobacterial lysates or with irrelevant bacteria, respectively, only lysed targets primed with homologous agents, whereas in other donors some cross-reactivity was observed.

It is concluded that, upon contact with mycobacteria, gamma/delta T cells are activated which contribute to immunity against infection *via* interleukin secretion and specific target cell lysis. In order to analyse whether a direct correlation exists between gamma/delta T cells and leprosy, gamma/delta T cells will be enriched and isolated from peripheral blood lymphocytes of leprosy patients, contacts and ICRC-vaccinated individuals.

Influence of *M. leprae* on lysis of Schwann cells and mononuclear phagocytes by killer cells. It has been proposed that, in addition to specific T cells, non-specific killer cells contribute to immunity against *M. leprae*. The question was analysed whether *M. leprae* influences lysis of mononuclear phagocytes and Schwann cells by killer cells (Steinhoff *et al.* 1991). Peripheral blood mononuclear cells from healthy individuals were stimulated by mycobacteria plus interleukin-2. Activated cells expressed potent

nonspecific killer activity and were able to lyse Schwann cells and mononuclear phagocytes which had been pulsed with dead *M. leprae*. In contrast, infection of target cells with *M. leprae* did not lead to significant lysis by killer cells. Importantly, infection with viable *M. leprae* as well as IFN-g* stimulation or heat shock induced resistance in otherwise susceptible targets that had been pulsed with dead *M. leprae*. It is concluded that *M. leprae* markedly influences susceptibility of Schwann cells and mononuclear phagocytes by killer cells and it is assumed that the induction of heat shock protein in *M. leprae*-infected cells contributes to resistance.

Work primarily carried out in Bombay

T cell responses to ICRC and *M. leprae* antigens. CMI responses to ICRC and *M. leprae* antigens were studied in patients across the clinical spectrum of leprosy and normal healthy individuals. The ability of the lymphocytes to secrete cytokines (IL-2, IFN-g*) after antigen stimulation was also monitored. Frequency analysis of antigen-reactive T cells in tuberculoid leprosy patients showed comparable frequencies to ICRC and *M. leprae* antigens. Fractionated ICRC and *M. leprae* antigens were blotted on nitrocellulose and solubilised antigens were presented to lymphocytes from leprosy patients, contacts and healthy individuals. A distinct pattern of antigen recognition was obtained. Responses in LL patients were directed to high molecular weight antigens (174-165 kD) of ICRC/*M. leprea* while TT patients responded to lower molecular weight antigenic fractions 11-15 kD.

As an extension of this part of work we plan to study responses of lymphocytes to antigens of ICRC/*M. leprae* fractionated by methods developed by Kaufmann's group. An attempt was already made in this direction. Sonicates of ICRC were taken to the University of Ulm, fractionated and electroeluted. These fractions were tested on T cells from tuberculoid leprosy patients. Preliminary data obtained have shown that TT patients recognise several ICRC antigens but responses are higher to low molecular weight fractions. A large batch of ICRC antigens was fractionated at Kaufmann's lab and it is planned to test these fractions at the Cancer Research Institute in India. Similar studies would be conducted on *M. leprae* antigens.

Immunoprecipitation of ICRC and *M. leprae* antigens with sera from leprosy patients. Reactivity of sera from leprosy patients (BL, BT, TT, LL), contacts, LL in ENL, tuberculosis patients and healthy individuals to ^{125}J-labelled ICRC and *M. leprae* antigens was analysed. Our data indicate that, using *M. leprae* antigens, it is not possible to distinguish seroreactivities of leprosy patients and healthy individuals (Chiplunkar *et al.* 1990b).

Sera from lepromatous leprosy patients identified major antigens of 47, 36, 21 and 14 kD from the labelled *M. leprae* sonicate. Sera obtained from BT, BL, LL in ENL, TB patients and healthy donors also showed a similar pattern of reactivity. Immunoprecipitation of ^{125}J-labelled ICRC antigens with sera from LL patients showed reactivity to 134, 94, 81, 48, 36, 29, 21 and 14 kD antigens. The 21 kD protein of ICRC was consistently immuno-precipitated by sera of LL patients, and LL in ENL. The 14 kD protein of ICRC was precipitated by sera of contacts. Sera from few LL patients also precipitate a 14 kD protein of ICRC. This band is not seen when the ICRC antigens are immunoprecipitated with sera of normal individuals or TT patients, but consistently appears in the sera of healthy contacts of multibacillary patients. This observation may suggest that development of antibodies to 14 kD protein of ICRC indicates exposure to *M. leprae*. This study will be expanded.

Cytotoxic effectors mechanisms in leprosy. The role of natural killer cells and antibody-dependent cellular cytotoxic mechanisms were assessed in leprosy patients and healthy idividuals (Chiplunkar *et al.* 1990a). Impaired NK cytotoxicity observed in LL patients could be significantly modulated using cytokines (IL-2, IFN-g*). We are now studying the lymphokine-activated killer cells (LAK) with respect to their ability to distinguish mycobacterium-pulsed and nonpulsed targets.

Immunotherapeutic trial of the ICRC vaccine. ICRC vaccine was administered to LL patients who were clinically classified as nonresponders to MDT (MDT-NR). These patients showed no clinical improvement or a drop in their BI despite 2 to 3 years of supervised MDT. The patients have been followed up before and 6 to 12 months after vaccination. In another trial the vaccine was given to newly-diagnosed LL patients and the patients were followed up as mentioned earlier.

Immunological studies conducted on MDT-NR LL and new LL patients have shown that increased proliferation of lymphocytes and cytokine production in response to *M. leprae* and ICRC antigens was observed in 50-60% of the patients 12 months after vaccination. An interesting observation was the increase in proliferation of lymphocytes in response to ICRC/*M. leprae* antigens with a concomitant drop in tissue BI. An increase in the frequency of *M. leprae* and ICRC reactive T cells was also observed after vaccination (Gangal *et al.* 1990).

T cell lines were established from vaccinated MDT-NR LL patients. The T cell lines were tested for their reactivity to fractionated ICRC and *M. leprae* antigens. It was observed that long-term cultured T cell lines showed reactivity to lower molecular weight antigens of ICRC and *M. leprae* (11-15 kD) and a similar reactivity pattern was also observed in TT patients.

Using the technique developed by Kaufmann, it is proposed to screen the antigen reactivity patterns of ICRC-vaccinated LL patients.

Collaborative activities

Heinz Gulle (a PhD student from the University of Ulm) worked at the Cancer Research Institute (February 1990). *M. leprae* antigens fractionated on 2D gels and electroeluted were brought to India. These antigens were tested on lymphocytes obtained from leprosy patients and contacts. The Cancer Research Institute provided all research facilities needed for this project and the experiments were completed successfully.

Gangal and Chiplunkar visited Kaufmann's lab in August/September 1990. Chiplunkar was able to fractionate ICRC antigens using the method developed by Kaufmann's group. Preliminary experiments were conducted on blood samples of *M. leprae* patients examined by B. Schoel in Kaufmann's lab. This work will be extended further as a collaborative project.

A joint Indo-EC symposium on leprosy and mycobacterial diseases was held at Lonavla from 6-9 November, 1989. See page 60.

A group meeting, attended by Kaufmann, Schoel, Deo, Gangal and Chiplunkar, was held at the University of Ulm on 13 August 1990 to take stock of the work done and to plan future activities.

Future projects

ICRC and *M. leprae* antigens fractionated by Kaufmann's method will be tested on ICRC vaccinated patients.

Skin biopsies (paraffin and frozen) of TT and LL patients and ICRC-vaccinated LL patients will be screened for lymphocyte infiltration. Several monoclonal antibodies brought from Kaufmann's laboratory are being tested on skin biopsies of TT patients. The results obtained from these preliminary experiments will help in designing future experiments.

The analysis of T cells from skin biopsies and lesions will be attempted.

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6 North Arcot district polio control programme

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Contract numbers and duration: CI1*/0316/0318, October 1989 to September 1994.

Background

The scientific objective of this project is of vital importance for India: the goal is to assess whether the injectable poliovaccine produced by the Institut Mérieux is better suited than the commonly used oral vaccine, supplied by the Soviet Union, in eradicating poliomyelitis in India. The enhanced potency of the Mérieux vaccine has already been demonstrated in other countries.

The wide-scale Indian test covers an entire rural region (North Arcot, Tamil Nadu) and is applied to a cohort of 500,000 children. The medical and political choice to move the Indian vaccination policies from one vaccine type to another rests upon the results of this joint research. The downstream industrial and political implications of this project are paramount.

Within the region, the area in which the oral polio vaccine is being tested is designated OPV and the area in which the injectable vaccine will be tested is designated KPV.

At present, polio viruses (wild poliovirus in the KPV area and wild and vaccine virus in the OPV area) circulate in both KPV and OPV since cases continue to occur in each area. While in the KPV area it may be assumed that only wild poliovirus circulates, in the OPV area, wild and vaccine viruses could be expected to circulate. Faecal samples of children in the OPV and KPV areas will be collected in order to look for circulating wild and vaccine viruses in order to undertake further virological studies. These studies are being undertaken in collaboration with Institut Pasteur, Paris.

One of the possible problems consequent upon the global eradication of poliomyelitis will be the inability to pinpoint the origin, nature, transmission and the reasons for endemic and epidemic polio in unimmunised and immunised populations. The standard epidemiological investigations may not reveal the origin and nature of outbreaks of paralytic poliomyelitis because a high proportion of poliovirus infections are subclinical.

Usually, methods of identifying strains of poliovirus are serological using either polyclonal or a panel of monoclonal neutralising antibodies. Advances in molecular biology have helped us to gain insight into a variety of viral genomic structures. These techniques have been developed and applied to the poliovirus identification not only to define whether sporadic or epidemic cases of polio are caused by wild/vaccine/recombinant strains of poliovirus but also for defining whether the causative wild strain of poliovirus is already in circulation in that region or imported from elsewhere.

Objectives

The aim of the project is to define the characteristics of the strains of polioviruses of epidemic and non-epidemic years in North Arcot District and to define the variations if any in the immune responses of the population to these strains. In order to achieve the above aim the following objectives have been defined.

1 To characterise poliovirus strains causing paralytic poliomyelitis in the pre- and post-epidemic periods in North Arcot District.

2 To determine the relationship of epidemic and non-epidemic strains with prototype poliovirus strains used in vaccine preparation.

3 To establish the origin of the prototype epidemic strains in India: their evolution and relationship with co-circulating poliovirus in the country and in the world.

4 To determine gaps in protection to currently circulating poliovirus strains among the local population.

5 To determine the quality of protection conferred by vaccines OPV and KPV against prototype wild circulating poliovirus strains.

Materials and methods

Antigenic analysis of monoclonal antibody characterisation. Studies on poliovirus strain characterisation have been initiated with collaboration from Institut Pasteur, Paris. Sixty three poliovirus strains isolated in the Department of Virology, CMC Hospital, Vellore between January 1987 and December 1989 have been taken up for strain characterisation. All these isolates are from children with paralytic poliomyelitis and include strains from the epidemic year, which was 1987, and from post-epidemic years 1988 and 1989. These isolates were first tested with a panel of monoclonal antibodies at Institut Pasteur. Subsequently the monoclonal antibodies were obtained from Institut Pasteur and further testing is currently under way at Vellore. Also, to further characterise these Indian strains, genomic analysis of selected poliovirus isolates (of all three serotypes) has been initiated. Results are expected to be available in mid-1991.

The Institut Mérieux in Lyon is supplying the Indian researchers in Vellore with a total of 1,400,000 doses of the injectable vaccine (IMOVAX DPT Polio) of which 350,000 will be a free gift.

Workshop

Leprosy and other mycobacterial diseases

Joint coordinators:

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Leprosy and tuberculosis were the main themes of the workshop which was held at Lonavla (Bombay) from 6 - 9 November, 1989. Both basic and applied issues were covered in the fields of immunology, molecular biology, chemotherapy and pathology along with the use of modern biotechnological tools for diagnosis and treatment.

As well providing a forum for a discussion of the state of the art in this important research area, the workshop has promoted further European Community - India scientific cooperation and several joint research proposals have been made.

A full report of the workshop has been published in the editorial section of the *International Journal of Leprosy* **58**, (3), 566-70.

In addition, the full proceedings of the workshop were published as 32 separate papers in the journal *Tropical Medicine and Parasitology* **41**, (3), 291-370 (Supplement II).

Postdoctoral fellowships

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Study of alkylated DNA adducts as possible indicators of human exposure to N-nitroso compounds in a high risk population for oesophageal cancer in Kashmir.

Fellowship period: April - September 1991

7 MATERIALS SCIENCE

Summary

Materials science is a fast-developing field, particularly because of the generation of new materials, often with industrial applications, and because of the availability of new analytical techniques. Joint research projects described in this chapter concern conducting polymers (page 64), superconducting materials (page 67 and page 72) and ionically-conducting glasses (page 74).

EC-India superconductivity research received a further boost through the organisation of a workshop (page 78) which built on the joint research projects already in hand and allowed other results to be presented, contacts to be generated and ideas discussed.

The postdoctoral fellowships have also included studies on superconductivity, with one linked to a research project (page 79), as well as semiconductors (page 90), and in studies of more traditional materials, advanced techniques have been applied to rock engineering problems (page 81) and to the fine structure of cotton fibres (page 82).

Joint research projects

7 Synthesis, characterisation and applications of some conducting polymers

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Contract numbers and duration: CI1*0324/0325/0325(01), February 1989 to January 1992.

Background and objectives

Interest in conducting polymers has been fuelled in recent years by immediate applications in lightweight batteries and displays and by possible applications in the longer term in the area of molecular electronics. However, long-term chemical stability and mechanical properties continue to be a major concern. Hence intense effort is now being devoted to the search for conducting polymers with improved physical and chemical properties.

Materials and Methods

Successful utilisation of conducting polymers in the future will depend to a considerable degree on detailed understanding of conducting mechanisms. The structure of doped polymers is complex and no consensus has yet emerged despite many detailed studies of materials such as transpolyacetylene, polypyrrole and polyanaline. Much of the effort in the present project has been devoted to the synthesis of processable conducting polymers and copolymers that are likely to be suitable for use in polymer batteries and in polymer electronic devices. A wide range of experimental techniques is being applied, including dielectric relaxation, optical spectroscopy and muon spin relaxation. In addition, theoretical techniques are being used to unravel fundamental characteristics of well known and also of new conducting polymers.

Results

Materials production and characterisation. Many conducting polymers have been synthesised at NPL, Delhi, under the contract. These include polyparaphenylene, useful as an n-type electrode in lightweight batteries. In addition, copolymers of benzene and pyrrole, and also of benzene and aniline, have been produced by electrochemical methods, giving conductivities in the doped form as high as 0.45 S cm^{-1} at room temperature.

A particular effort has been made to synthesise polypernigraniline, which has a topological degeneracy similar to that of transpolyacetylene, and might therefore be expected to exhibit exotic solitonic behaviour. The preparation of fully oxidised (polypernigraniline) and fully reduced polyaniline (leucoemeraldine) was carried out by oxidation and reduction of half-oxidised polyaniline base (emeraldine). The formation of polypernigraniline has been confirmed by infrared measurements which show no absorption association with hydrogen in the region 3000-3400 cm^{-1}.

Substituted polyanile has also been synthesised and the copolymers poly-o-toluidine-o-anisidine and polyaniline-o-toluidine have been prepared. Detailed spectroscopic studies of these conducting copolymers have been completed and measurements are in progress of electrical conductivity as a function of temperature. In addition, polyaniline-anisidine has been prepared and spectroscopic and conductivity measurements have been completed.

An ongoing study of the nature of the charge distribution in doped polymers is being carried out at the Rutherford Laboratory near Oxford, using muon spin relaxation techniques. So far experiments have been confined to polypyrrole but it is planned to extend the measurements, in the near future, to deuterated polyanaline now being prepared at NPL, Delhi.

Theoretical Studies. Theoretical studies of conducting polymers have been carried out at both Oxford and NPL, Delhi. At Oxford, computational methods for determining equilibrium geometries and dynamics of molecules using CNDO and INDO semi-empirical methods have been developed. A self-consistent geometry optimisation process has been worked out in detail and can be applied to any system where electron-lattice coupling is important; it makes possible the study of a wide range of classical defects and examination of the behaviour of molecular systems in ground, excited or charged states. This computational technique lends itself to the study of chemical reaction pathways and to the effects of external perturbation, e.g. an electric field, on molecular systems. This new approch has been applied to the study of the energetics and dynamics of excitations in conducting polymers, particularly polyacetylene.

At NPL, Delhi, an attempt has been made to extend to organic conductors some theories developed for inorganic semiconductors. Estimates of the mobility of charge carriers in polymers show good agreement with experiment. Calculations of the electronic structure of poly-a-naphthalene oxide-pyrrole have been carried out using the valence-effective-Hamiltonian (VEH) technique. A similar study of the band structure of polythianaphthene has also been completed.

Studies relevant to device applications. It has been discovered at NPL, Delhi, that the copolymers poly-o-toluidine-o-anisidine and polyaniline-o-toluidine are soluble in common organic solvents and hence are likely to be processable; their thermal stability is at present under investigation. Flexibility is also an important parameter for device applications and it has been found that highly flexible films can be produced by electro-chemical synthesis of polypyrrole films in an aqueous medium containing the sodium salt of p-toluene-sulphuric acid.

Studies of semiconducting polypyrrole/metal and polyaniline/metal junctions have been carried our and it has been shown that excellent rectification can be obtained with an indium/polyanaline junction.

The use of conducting polymers for biomolecular electronic devices has also been investigated. A large number of experiments have been carried out on the immobilisation of enzymes (glucose oxidase and urease) in conducting polypyrrole and polyaniline matrices.

Future Emphasis. Fundamental studies of transport mechanisms will continue. The study of semiconducting polymer/metal junctions will expand and the development of transistors and lightweight batteries will be pursued. The study of optoelectronic properties (electrochromism and nonlinear optical effects) will intensify with applications to photonic devices in mind. The development of a glucose biosensor based on conducting polypyrrole will be a priority. There has been close and fruitful collaboration between researchers at NPL, Delhi, and at the Clarendon Laboratory, Oxford; this close collaboration will continue.

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8 Studies on polycrystalline, bulk, thin films and single crystals of high temperature superconductors

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Background

High temperature superconductivity (HTSC), a zero electrical resistance state at temperatures above that of liquid nitrogen (77°K), was discovered in 1987 in copper-based mixed oxide materials. The discovery has three important implications: the need for a better understanding of the phenomenon of superconductivity, the possibility of achieving higher transition temperatures (and eventually to synthesise materials which superconduct at room temperature); and the tremendous potential for the exploitation of these materials for practical applications. Because of this there is an unprecedented worldwide interest in the study and exploitation of these materials.

Objectives

Broadly, the following objectives are being pursued in this joint collaboration programme of two French and two Indian laboratories:

1. Exploration of new superconducting materials for achieving higher transition temperature;
2. Investigation of fundamental properties of relevance to basic scientific understanding as well as technological applications.

Materials and Methods

Polycrystalline bulk materials. Y(RE)$Ba_2Cu_3O_7$ (denoted as Y(123) systems). The Y(123) system has been studied extensively by many laboratories in the world. This research will concentrate on only four types of materials based on Y(123) - these are: Zn dopes, Ba completely replaced by Sr, Y replaced by La and Ca; metal oxide (PbO) additives to Y(123).

Zn doped Y(123). Zn is divalent and non-magnetic and has been shown to substitute for Cu in the Cu-O_2 plane. This results in a T_c depression of around 13-15°K/atomic % which is the largest known for any cationic substitution in these systems. The Hall constant of Zn substituted samples were systematically measured as a function of T and the carrier concentration (p) was evaluated using a single band model. It was shown that p hardly varies as f(Zn) whereas 6% of Zn completely quenches superconductivity. The normal state resistivity increases and the Hall mobility decreases as a f(Zn). This poses challenging problems to the existing theoretical models which we reviewed. It is possible that our results can be qualitatively understood in terms of carrier localization.

Ba replaced by Sr. Controversial results are reported as to the existence of superconductivity in Y-Sr-Cu-O. We found superconductivity in this material at 72°K confirmed by electrical and magnetic measurements. However, electron diffraction and EDX analyses revealed the presence of other insulating phases. It is quite possible that the Y-rich phase (16% according to EDX analyses) is superconducting. Further investigations are in progress.

Metal oxide additives to Y(123). The aim is to study the influence of these additives on the normal and superconducting properties of pure YBaCuO. Preliminary ac susceptibility measurements show that the samples containing 2 mole % of PbO have a T_c=91°K and the width of the transition (of the imaginary part of the susceptibility) is around 1°K. Magnetisation measurements are being carried out to estimate the critical current density.

$LaCaBaCu_3O$ system. This system is interesting from several points of view. It is a tetragonal superconductor whereas the well known YBaCuO has an orthorhombic structure. The (110) mirror twins do not occur in $LaBaCaCu_3O_y$. Consequently comparison of flux creep in YBaCuO and $LaBaCaCu_3O_y$ can give information on the influence of the (110) mirror planes in flux pinning. Though this material was earlier studied by half a dozen laboratories in the world, contradictory results were reported on the structure, room temperature resistivity and T_c. We have shown that this system has a tetragonal structure and, depending on the heat treatment and the atmosphere, T_c can vary between 60 and 83°K. However, chemical analyses indicate only 60 to 70% phase purity. Further work on magnetic measurements is in progress.

Bi(2212) and (223) system. We have shown that in these two systems, T_c can be varied by heat treatments and by certain substitutions. Detailed Hall measurements were made to establish a relation between T_c and the carrier concentration. Some relevant models such as bi-polaron and Hirsch's model were discussed for analysis of data.

The effect of Zn and Ni substitution in the Bi(2212) system was investigated. Zn was found to depress T_c faster than Ni though both of them are divalent and go to the Cu sites (note there is only one Cu site in this system whereas there are two Cu sites in Y(123) compound).

Tl compounds. Contarary to the Bi cuprates there are a variety of Tl cuprates. The (1212) and the (2223) systems have been studied with several substitutions. In particular, when Th and Ce are added to the (2223) system, multiphase samples are obtained with a predominant (2201) phase with T_c going up to 75°K. Preliminary Hall measurements indicate p-type conductivity. Again, Zn substitution at the Cu sites depresses T_c at a rate of 12-15°K/atomic %.

Observation of $T_c > 200°K$. There have been frequent reports from different parts of the world on the observation of unstable superconductivity at $T_c > 200°K$ in Y(123) compounds. In some of the samples (made by NPL), a resistivity drop was observed at 210°K and zero resistivity at 200°K. A small diamagnetic signal in the ac susceptibility measurements was observed at 213°K which disappeared after thermal cycling. The relative volume fraction of this extra high T_c phase was estimated to be 1% of the stable 90°K phase. Further work is necessary to consolidate this observation.

Thick and thin films. There are high hopes of applications of high T_c materials in magnetic shielding, microwave cavities, bolometers etc. These require large area coatings or shapes. With these in mind a programme of plasma sprayed coatings has been started. A systematic study of heat treated YBaCuO coatings (5 cm^2 area, 100 to 200 microns thick) show that one can obtain a $T_c = 92°K$ on these coatings. It is interesting to note that other laboratories have reported $T_c < 90°K$ by using similar techniques. First measurements of the transport critical current density by pulsed current technique give a value of 70 A/cm^2 at 77°K. The intragranular critical current at 10 K and at low fields is of the order of 10^5 A/cm^2. Thin films of superconductors have also been obtained by DC sputtering. For the first time, it has been shown that polycrystalline CuO can be used as a substrate to grow BiPbSrCaCuO films with a $T_c = 75°K$. The results are expected to improve once the quality of the substrate surface and the heat treatment schedules are optimised. Further, it is quite possible that CuO film can be used as a buffer layer on a cheap substrate such as alumina.

Methods

Several physico-chemical and physical methods have been used in this project. The physico-chemical methods included X-ray diffraction, electron diffraction and microscopy, oxygen determination by iodometry, electron microprobe analysis for composition determination, scanning electron microscope for morphology, etc. The physical methods used were: ac and dc resistivity, ac and dc susceptibility, dc magnetisation and microwave absorption. The first two methods are routine analyses to determine the effect of heat treatment and substitution on T_c and the quality of the sample whereas the last two methods were used to study the critical current (J_c), pinning mechanism and intergranular Josephson weak-links. In addition, Hall effect was measured (see above) to determine the nature of the carriers and to establish a relation between T_c and the number of carriers. A short discussion on magnetisation and microwave absorption studies follows.

Magnetisation. Magnetisation has been studied in detail as a function of H and T of several thin film samples of YBaCuO. The polycrystalline films had a low value of remanent magnetisation (M_r). This may be related to the low values of intergrain critical current either due to parasitic phase trapped at grain boundaries or to a Josephson junctions (weak links) mechanism. J_c is of the order of $10^4 A/cm^2$ at 10°K. Similar studies on well oriented films indicate $J_c = 10^7$ A/cm^2. The temperature dependence of M_r on such films, which is due to thermally activated flux creep phenomena, was analysed using Anderson-Kim theory. The flux pinning energy U_0 was estimated to be of the order of 80 meV. We have also found a correlation between the surface impedance and M_r. This indicates that a granular structure of the films which lowers M_r also increases the surface impedance. M_r has also been studied as f(T) of Zn substituted Er(123) ceramic samples. J_c decreases from 10^6 to 5.10^4 A/cm^2 at 10°K as the concentration of Zn increases from 0 to 5 atomic %. A systematic increase in the pinning energy from 20 to 40 meV was found as a function of Zn. However, the decrease in J_c may be attributed to the increasing disorder produced by Zn in the Cu-O plane as also evidenced by the broadening of resistivity transitions.

Microwave absorption. Field-modulated microwave absorption is a very sensitive technique to investigate the microwave properties of superconductors. The data obtained will be useful in the development of high-performance microwave devices such as high-Q cavities, high-speed transmission lines, antennae etc. With this in view the quality of both polycrystalline and oriented thin films of YBaCuO has been examined. The behaviour of polycrystalline films is distinctly different from the well-oriented ones. The granular nature of the films is also evident in these measurements. The presence of additional odd harmonics close to T_c has been observed when a dc magnetic field was superimposed on the modulation field. Detailed studies are in progress.

Summary of main results

Laboratoire de Physique des Solides de Bellevue, Meudon.

- Set-up of a very sensitive ac susceptometer fabricated in collaboration with a French industrial organisation and the Institut d'Electronique Fondamentale, Orsay to evaluate the screening effect and the critical current on bulk ceramic samples, tiny single crystals, thin films and plasma sprayed coatings of superconductors.
- Preparation of large area superconductors (YBaCuO) by plasma spray with $T_c = 92°K$.
- Establishment of a relation between charge carriers and superconductivity in Bi and Y based superconductors.
- Study of structural, electrical and magnetic properties of $LaCaBaCu_3O_y$ and Y-Sr-Cu-O.

LEPES, Grenoble

- Design of a chamber for physico-chemical characterisation of thin films of YBaCuO and to study the effect of annealing with or without oxygen.
- Magnetisation studies of YBaCuO thin films, estimation of pinning energy, critical current.
- Influence of Zn on the critical current of YBaCuO samples made by NPL, Delhi.
- Microwave absorption properties of thin films.

IIT, Madras

- Optimisation of heat treatment schedules to obtain the 110°K phase in the Bi (2223) system.
- The effect of the addition of PbO, $BaBiO_3$ and $BaPbO_3$ to YBaCuO on its electrical and magnetic properties - samples given to Bellevue.
- Study of the formation of 1212 phases in the TlMSrCaCuO system (M=rare earth, Pb, Bi, Th).
- Phase formation in the Tl-Ba-Cu-O system.

NPL, Delhi

- Superconducting properties of Zn and Ni substitued Bi(2212) and Tl(2223) systems.
- Specific heat measurements on Y1-2-3 and Bi (2212) systems.
- Observation of T_c above 200°K in some cuprates.

Conclusions

The joint project on superconductors between France and India has progressed well to the satisfaction of every partner. Several original results have been obtained; in the areas of material development, fundamental and application aspects and the development and use of sophisticated analytical techniques. Each partner derived benefits from the expertise developed by others. The materials prepared and well characterised in India were studied in France by the French scientists and also by the Indian scientists during their stay in France. In particular, two Indian scientists were trained in magnetic measurements and in the interpretation of the data and one of them was also trained in thin film preparation by a sputtering technique. A detailed report at the end of the project will further amplify these conclusions.

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9 Study of non-stoichiometry and valence states in superconductive thallium and lead cuprates

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Objective

The objective of the project is to establish relationships between non-stoichiometry, valence state of cations and the superconducting properties of the thallium and lead cuprates. Such a study should allow the optimisation of superconductivity in mixed valence copper oxides to be understood and, especially, to be controlled.

Materials and methods

For this purpose, the different known series of layered thallium cuprates and lead cuprates will be synthesized. The partners will study the effect of oxygen non-stoichiometry by varying the experimental conditions of the different as-synthesized samples, i.e. using different oxygen pressures at low temperature (400-600°C). For these different samples the valence state of the different cations will be studied by spectroscopic techniques (X-ray absorption, ECSA, Auger, XPS). Study of the non-stoichiometry and structure will be performed by X-ray and neutron diffraction and electron microscopy and the superconducting measuremènts will be carried out from the magnetic and electrical point of view. A similar study will be performed by varying the cationic ratios, especially the thallium and lead content, in the structure. This latter study is of capital importance since it has been shown recently that there is a homogeneity range which can influence drastically the superconducting properties. Again, for such synthesis, the results depend on the oxygen presence so that synthesis has to be performed at different oxygen pressures and the effect of annealing has to be understood. It should also be pointed out that in such materials there exist problems of homogeneity of the samples; a "statistical" study of the structural and physical properties will therefore be made in various samples in order to understand the distribution of extended defects. In a final step, after having established correlations between superconductivity and chemical bond and structure, an attempt will be made to optimise the superconducting properties of some of those materials,

Summary of work in Caen

Synthesis of various samples; several samples will be prepared twice, by both partners simultaneously, in order to test the reproducibility of the synthesis and of the thermal treatments.

In the same way the diffraction study will be partly made in Caen.

Tasks which are specific to Caen concern the following points:

- Study of the different samples prepared in Bangalore and Caen by high resolution electron microscopy: structure and extended defects.
- Some selected samples will be studied by neutron diffraction in Grenoble.
- Study of the magnetisation versus temperature using a SQUID magnetometer.
- Study of the valence states of the elements of X-ray absorption spectroscopy in LURE.

Work in Bangalore

Synthesis of novel oxide systems including cuprates and characterisation by various methods.

Exchange of samples with Caen.

Study valence states of metals and oxygen.

Raman spectroscopy.

Preparation of reviews in collaboration with scientists in Caen.

10 Structural aspects and ionic conduction in boron sulphide and oxisulphide-based glasses

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Background

Ionically-conducting glasses are currently the subject of extensive study because of their potential use in solid-state devices (e.g. rechargeable lithium batteries or electrochromic displays). In this regard, the Bordeaux group were the pioneers in 1983 in preparing glasses in the B_2S_2-Li_2S-LiI system. These glasses exhibit amongst the highest room temperature Li^+ ionic conductivities known in the solid state (10^{-3} Scm^{-1} at room temperature). Furthermore, their electrochemical properties make them suitable as electrolytes for lithium cells, since they have negligible electronic conductivity and stability with respect to lithium metal. These materials have been studied in the form of thin films or compacted powder in all solid-state batteries.

From a more fundamental point of view, because homologous crystalline compounds are not known to exist, and because these glasses require rather special preparation and handling techniques due to their extreme hygroscopic nature, very little is yet known about their atomic structure and about the mechanisms of ionic conduction which occur.

This is, in fact, the case for all ionically-conducting glasses, for which there is no generally accepted mechanism describing the conduction process. Two main approaches can be distinguished. One considers that the conduction process is governed by the number of mobile ions produced by dissociation equilibria whereas the other assumes that the ionic mobility is the important factor; this is then governed by local ion jump mechanisms, which can be either independent or correlated. Another approach, proposed by the Bangalore group, is to regard the glass structure as being inhomogeneous, with relatively ordered clusters connected together by a more disordered tissue material.

Knowledge of the local atomic structure of these glasses is also necessary in order to validate a given ionic conduction mechanism. A variety of techniques can be used for the purpose, such as Raman scattering, infra-red absorption and nuclear magnetic resonance (NMR) spectroscopies also X-ray or neutron diffraction. Computer modelling of the structure, associated with the experimental determination of the radial distribution function (RDF) by diffraction methods is an important adjunct.

Objectives

The study of amorphous (non-crystalline) solids is a field of increasing importance, from both fundamental and applied points of view. Such materials, unlike many of their crystalline counterparts, have the advantage that they exhibit isotropic properties and can readily be fabricated in different shapes and forms. Furthermore, in general they can be prepared over large compositional domains and therefore can exhibit properties which greatly vary and which can be controlled by changing the composition.

Amorphous materials do not have the long-range structural order (periodicity) characteristic of crystalline solids, and as a consequence their properties are not as well understood as those of crystals where the simplifying presence of periodicity allows their behaviour to be described readily. Nevertheless, non-crystalline solids possess a degree of local (short-range) order, the study of which is accomplished using those experimental techniques (e.g. infrared, Raman, NMR, ESR) which are sensitive to it. More recently, structural modelling using computer-based techniques has acquired increasing significance due to the availability of high-power computers.

Understanding of the conduction mechanisms of ionic transport is currently based on various models; this also has to be correlated with the structural aspects. Elucidation of these transport mechanisms is an important goal from a fundamental point of view, and furthermore is the key to the design of new materials with improved ionic performance.

The aim of the research project is to combine the complementary expertise of the participating groups in order to elucidate the local structure and conduction mechanisms of the superionic glassy system Li_2S-LiI-B_2S_3.

Materials and methods

These hygroscopic materials have been discovered and well characterised by the Bordeaux group in terms of their ionic conductivity, and by using chemical and thermal analysis, Raman scattering and electron spin resonance (ESR) spectroscopies.

Both the Bangalore and Cambridge groups have considerable expertise in terms of complementary theoretical approaches for ionic conduction in amorphous materials: the Bangalore approach emphasises structural aspects (cluster-tissue model and "structural unpinning number"), whilst the Cambridge work emphasises more the microscopic aspects of the process ("diffusion-controlled relaxation"). In addition, these two groups have access to advanced experimental techniques which will be of considerable assistance in the furtherance of the project, namely high-resolution magic-angle spinning (MAS) NMR in both Bangalore and Cambridge and ionic Hall effect in Cambridge.

Tasks of Cambridge group

Structure determination. The structure of glasses in the Li_2S-LiI-B_2S_3 system will be studied using a variety of techniques. Amongst the experimental methods for structure determination are:

Nuclear Magnetic Resonance. Magic angle spinning (MAS) NMR will be used to obtain high resolution spectra using the ^{11}B and ^{7}Li isotopes (natural) in the glasses under study. This will provide information on the local structural environment of both the network-modifying ions (Li+) and network forming cations (B) as a function of total Li content and, separately, as a function of doping-salt (LiI) concentration. The new technique of quadrupolar nutation, which has previously been used for the study of B-containing oxide glasses, will also be used to distinguish between tetrahedrally and triagonally coordinated B sites.

Diffraction. Diffraction experiments will be undertaken on the glasses using X-rays and, if ^{11}B isotopically-substituted glasses can be prepared (to avoid absorption problems with the ^{10}B nucleus), neutron diffraction experiments will also be performed.

The results obtained from the above and other experimental structural probes will be correlated with those obtained by computer simulation techniques.

Ionic transport studies. The mechanism of Li+ ion transport in the Li_2S-LiI-B_2S_3 system will be investigated using both experimental and theoretical approaches. The experimental techniques, detailed below, are both sensitive to the ionic mobility, μ, in the expression for the conductivity $= ne\mu$, where n is the mobile ion concentration and e is the electronic charge.

Ionic hall effect. The (Hall) mobility of ions in a glassy host will be measured as a function of total Li^+ ion concentration and also doping salt concentration. Various theoretical predictions of the compositional dependance of the mobility will be differentiated thereby. In addition, the temperature dependence of the (Hall) mobility will be measured and compared with that obtained from conductivity measurements.

Travelling-wave mobility studies. It is proposed to measure the mobility of ions for the first time using the travelling-wave technique, in which the electric field associated with the surface acoustic wave (SAW) of a piezoelectric transducer causes motion of charge carriers, resulting in a DC voltage being developed across the sample placed on the SAW device. The mobilities obtained using this novel technique will be compared with those obtained from the aforementioned Hall effect method.

NMR relaxation-time studies. Measurements of the NMR spin-lattice relaxation time, T_1, of the ^7Li nucleus in the glasses under study will also shed light on the conduction mechanisms, if ionic diffusion-mediated relaxation is the dominant NMR relaxation mechanism operative in these glasses.

Quasi-elastic neutron scattering. If ^{10}B-free samples can be prepared (see structure determination (diffraction) above), QENS measurements can also be performed which give direct information on the ionic diffusion coefficient. The results of these measurements, as a function of temperature, will be compared with those obtained from the other techniques and ionic conductivity.

Theoretical efforts will be concentrated in two areas.

Diffusion-controlled relaxation model. It has been suggested that the time (frequency) dependent behaviour of ionic transport in glasses can be understood in terms of the CDR model, in which correlation between ions takes place in a pair-wise fashion and transport involving interstitialcies is assumed. Development of the theory to embrace QENS will be pursued, and the results of the proposed experiments will be compared with the predictions of this theoretical approach.

Ionic hall effect. Currently, there is no theory for the ionic Hall effect in solids (crystalline or amorphous). Efforts will be made to develop a theory for this effect in terms of the different microscopic transport mechanisms possible.

Tasks of Bangalore group

Theoretical. Molecular dynamics simulation will be performed to understand the structure of and dynamics in Li_2S-B_2S_3-LiI glass. The now successfully simulated structure of selenium glass will be used as a starting configuration and extended by a decoration technique. 50% of selenium atoms are substituted by sulphur atoms and the rest by BS_2B groups and equilibrated minimum energy structures will be obtained at appropriate glass densities.

The last ion conduction in Li_2S-B_2S_3-LiI glasses will be examined using the model of structural unpinning recently developed and used successfully for AgI-Ag-Oxy salt glasses. The effect of anion and cation substitutions in Li_2S-B_2S_3 glass will be examined in response to the indications from the model.

Experimental. Since Li, B and I are all spin-bearing nuclei, the structure of Li_2S-B_2S_3-LiI glasses will be examined using HR MAS NMR studies.

High resolution electro-microscopy will be used to investigate the ultra-micro structure of Li_2S-B_2S_3-LiI glasses to ascertain the presence of cluster tissue texture in these glasses. The results will be used to develop an appropriate theoretical model for ionic conduction which incorporates the ultra-micro structure features of glasses.

Task of Bordeaux group

Preparation of the glasses. The glasses from the Li_2S-B_2S_3-LiI system are prepared by classical quenching of the melt in an inert atmosphere. This yields a limited vitreous domain, which will be enlarged by the setting up of an hyperquenching system. The availability of the new compositions of glasses is important to extract more information about the system itself as well as to improve further the ionic conductivity.

New glasses will be derived from this system by the incorporation of oxide modifier or glass-former. These oxysulfide glasses are expected to be more resistant to moisture attack, while retaining good performance.

All glasses will of course be chemically analysed.

Characterisation of the glasses. This will involve the following:

Ionic Conductivity. The determination of the pre-exponent and activation energy of the conductivity from the analysis of complex impedance diagrams is a routine technique.

Thermal analysis. The determination of the glass transition temperature is achieved by the DSC technique.

Density. The density of the glass will be determined by the Archimedes method, using an appropriate fluid.

Infrared and Raman spectroscopy. These techniques will be essential for the determination of the local structural entitities present in the glasses, and how they change with composition.

EPR. The detection of paramagnetic defects by this technique will be a confirmation of the structural hypothesis.

An important part of the work will be the correlation of the evolution of all the properties mentioned above with the composition of the glasses in order to determine how each factor influences the conduction process.

Workshop

Superconductivity

Joint Coordinators:

B. Raveau

Laboratoire CRISMAT (CNRS), 6 boulevard du Maréchal Juin, 14050 Caen Cedex, France.

C.N.R. Rao

Indian Institute of Sciences, Sir C.V. Raman Avenue, Bangalore 560 012, India.

The India-EC joint workshop on superconductivity was held in Bangalore from 30 January to 2 February 1991.

This workshop offered the opportunity for potential scientific partners to review the current state of knowledge in the sector of high-Tc superconductivity, to report on recent research advances in their laboratories and to discuss areas of mutual interest with a view to establishing the bases for future joint research activities. The high level of the workshop's talks reflected the scientific excellence of the two coordinators, both world leaders in this modern science. The main themes developed pertained to basic as well as applied research. They included new insights into the superconductivity of cuprates, discussions on redox mechanisms in layered copper oxides, fluctuation effects, dissipation phenomena, chemistry, stoichiometry and electronic structure of high-Tc oxides, as well as applications to SQUIDs and other Josephson junction devices.

Workshops are cooperation initiatives aimed at promoting long-lasting relationships between the participants and the parent institutions. It is believed that the workshop on superconductivity will prove to have been extremely successful in generating further India-EC joint research projects.

Postdoctoral fellowships

K.B. Garg

Department of Physics,
University of Rajasthan,
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B. Raveau

Laboratoire CRISMAT (CNRS),
Institut des Sciences de la Matière et du Rayonnement,
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France.

Study of the electronic structure of the hole and electron oxide superconductors using high energy spectroscopies

Fellowship period: July - December 1991

A.K. Pradham

Cryogenic Engineering Centre,
Indian Institute of Technology,
Kharagpur 721 302,
India.

R.A. Cowley

Clarendon Laboratory,
Department of Physics,
Oxford University,
Oxford, OX1 3PU,
United Kingdom.

Synthesis, transport, magnetic and structural characterisation of high-temperature superconductors

Fellowship period: February 1991 - January 1992

P. Suryanarayana

Semiconductor Devices Area,
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Research Institute,
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India.

C. Bernard

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Ecole Nationale Supérieure
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38402 Saint-Martin d'Hères Cedex,
France.

Process development and characterisation of newer electronic materials for VLSI technology

Fellowship period: September 1990 - August 1991

Summary

As the feature size of VLSI circuits continues to shrink below one micron, unmet requirements have driven industry to find alternative methods to achieve acceptable RC delay in interconnection paths and to manage wafer topography issues. Because CVD tungsten and tungsten disilicide meet the requirements and offer etch barrier protection for head lead contacts and diffusion barriers together with improved electromigration resistance, the level of interest in this technology has mushroomed. CVD tungsten films processed by fluorine-based reactive gases (WF_6) have some problems of encroachment and tunnel formation due to the highly reactive nature of these gases. The aim of the present study is to develop a new process technology based on chlorine reactive gases (WCl_4 etc.) which could overcome the problems of tunnel formation and encroachment at the Si/W and WSi_2 interfaces.

In the present study, thermodynamic simulation has been carried out to optimise the process parameters prior to experimental work. That simulation was based on minimising the total Gibbs free energy of the whole chemical system. A low pressure CVD reactor has been designed and fabricated which has an in-situ chlorination facility. Generation of tungsten chloride gases (WCl_4 etc) by in situ chlorination has been confirmed by mass spectroscopy studies. Tungsten silicide films were grown using 1% silane in argon, chlorine, hydrogen and argon. On the basis of TD calculations, a typical set of experiments was carried out at a fixed temperature (600°C) varying the silane to chlorine gas ratio (Rx). The grown films were characterised by studying their electrical resistivity, surface and interface texture, structural properties and phase composition. Films grown below Rx<1 show two main phases: WSi_2 and W_5Si_3. The as-grown films have a smooth surface and interface, however the sheet resistance is high. Increase in chlorine partial pressure (Rx>1) in the system results in selective deposition of pure tungsten. The TD simulation results are well supported by the present experimental study. Further studies are in progress to optimise the process parameters.

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Council of Scientifical Industry
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Singleton Park,
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United Kingdom.

Development of constitutive models and solution strategies for solution of coupled problems of rock engineering

Fellowship period: August 1990 - January 1991

The objective of the research was to study new topics, locate new areas of application and to collect the available knowledge in these areas. The research areas concerned were:

- Elastoplastic and elastoviscoplastic finite elements analysis.
- Constitutive modelling of materials like rock, soil and concrete.
- Thermal finite element analysis.
- Software development.
- Extensive literature survey.

Such a study was bigger and more far-reaching than could be completed in the six months period of the fellowship; no results or definite conclusions could thus be reached.

A.V. Moharir

Nuclear Research Laboratory,
Indian Agricultural Research Institute,
New Delhi 110 012,
India.

P. Kiekens

Laboratorium de Meulemeester voor
Technologie der Textielstoffen,
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9052 Zwijnaarde, Gent,
Belgium.

Studies on the structure of cotton fibres

Fellowship period: October 1990 - March 1991

Summary

The relationship between true-spiral angle and strength of fibres in varieties of all the four commercial species of cotton has been reviewed afresh. It has been concluded that, contrary to popular belief, the true-spiral angle in different varieties and species of cotton is not constant. Further, the best parameters for characterisation of cotton for strength have been identified to be the Hermans crystallite orientation factor (obtained from X-ray diffraction) and the average angle of crystalline orientation deduced from it. The average angle also measures the spirality of cellulose crystallites to the fibre axis more faithfully than the 40, 50 and 75% X-ray angles. Since strength of cotton is heritable and environmentally the most stable fibre property, it is strongly recommended that only the above identified parameters, rather than the X-ray angles, be used in characterisation of genotypes in breeding cotton for strength. This is important in view of the demand by the new spinning technology (open-end spinning) for a shift in cotton breeding priority from increased length to increased strength of fibres.

In the light of the recent developments in the field of cotton cellulose, several high-resolution instrumental techniques have been used to take an integrated look at the origin and growth of the enigmatic 'reversal extinction bands' in native cotton fibres. It has been argued that the origin and growth of these bands, as seen under crossed polarised-light, can also be explained on the basis of relative phase shifts in the microfibrillar wave pattern of crystalline cellulose deposited within individual diurnal secondary layers of a developing cotton fibre. Observation of these bands in *in vitro* ovule-cultured cotton indicates that their number along the length of a fibre increases and their spacing decreases with maturity of the fibre.

Convolution twists/cm measured on fibres of the same cotton varieties grown at five different agroclimatic locations appear to decrease with increase in the latitude of the place of growth of the cotton. The number of convolutions is known to have a negative affect on the cellulose crystallite orientation and the strength of fibres. In view of the present results, it would be interesting to investigate further if there is an increase in crystallite orientation and a fall in convolution number the higher the latitude at which the cotton is grown. This would help to resolve the issue of the extent of the contribution of convolutions to modifying the crystallite orientation and the tensile strength of fibres.

Publications

Moharir, A.V. and Kiekens, P. An integrated look at the origin and growth of extinction bands in native cotton fibres. Submitted to *Journal of the Textile Institute.*

Moharir, A.V. X-ray orientation in never-dried cotton fibres: a review. Submitted to *Journal of Applied Polymer Science.*

Moharir, A.V. and Kiekens, P. Convolutions and crystalline orientation in native cotton fibres. Submitted to *Journal of Applied Polymer Science.*

Moharir, A.V.; Louwagie, J.; Van Langenhove, L. and Kiekens, P. The correlation between X-ray orientation parameters and strength of fibres in native cotton. Submitted to *Journal of Applied Polymer Science.*

8 PHYSICAL, MATHEMATICAL AND ENGINEERING SCIENCES

Postdoctoral fellowships

A. Roy Chowdhury

High Energy Physics Division,
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Calcutta 700 032,
West Bengal, India.

B. Fuchssteiner

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Universität Gesamthochschule
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Postfach 1621,
4790 Paderborn, Germany.

On the study of complete integrability of non-linear evolutionary systems

Fellowship period: September 1990 - January 1991

Summary

The research work conducted at the University of Paderborn can be divided into two main categories: the work already commenced in India, which was further developed in Paderborn; and work done in collaboration with B. Fuchssteiner.

In the first category, papers were completed on the following subjects:

1. Anisotropic affine lie algebra AKS theorem and integrable system: (with P. Guha).
2. On the bi-Hamiltonian structure of a coupled system whose reduction is the KN equation: (with S. Purkait).

In the second category, the topic being pursued was the quantization of non-linear systems.

Lastly, a computation was started on the unstable non-linear Schrodinger equation. This will be completed by W. Oevel of the Department of Mathematical Sciences, University of Loughborough, England, which was visited at the end of the fellowship period.

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J. Linderberg

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8000 Aarhus C., Denmark.

Theoretical atomic and molecular physics

Fellowship period: February 1990 - July 1991

D. Home

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F. Selleri

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M.A.B. Whitaker

Department of Pure and Applied Physics,
The Queen's University of Belfast,
Belfast BT7 1NN,
United Kingdom.

Investigation of some foundational problems in non-relativistic quantum mechanisms

Fellowship period: November 1990 - October 1991

Summary

The hitherto unexplored implications of the experimentally verified Aharonov-Bohm effect have been analysed from the standpoint of local realism.

A forthcoming review of the Einstein-Podolsky-Rosen (EPR) paradox provides a concise and critical account of all the significant studies made on issues related to the EPR paradox since its advent more than fifty five years ago. Directions for future studies are also indicated.

Finally, the quantum mechanical results for decaying, oscillating and regenerating neutral kaons have been shown to be compatible with treatments based on realism. This finding has significance in the context of the proposed F* factory project at Frascati, Italy.

Publications

Home, D. and Selleri, F.(1991) The Aharonov-Bohm effect from the point of view of local realism. *In* The Wave-Particle Duality, Plenum Press, New York.

Home, D. and Selleri, F.(1991) Quantum theory and the paradox of nonlocality. *Revista del Nuovo Cimento*, in press.

A. Lahiri

National Information System for Science and Technology, Department of Scientific and Industrial Research, Ministry of Science and Technology, New Mehrauli Road, New Delhi 110 016, India.

B. Mahon

Information Technology Applications S.A., 2 rue A. Borschette, Boîte Postale 262, 2012 Luxembourg, Luxembourg.

Development of an Indian model for database services

Fellowship period: October 1990 - March 1991

Summary

In India, serious efforts have been made during the plan period 1985-1990 to provide data networking for government departments and agencies, for public commercial use and for academic research institutions. Several specialised networks exist for banks, steel industries, railways, oil, thermal power etc. Institutions working in frontier areas of science and technology have been connected through e-mail facilities. With the establishment of the gateway system in Bombay and digital communication facilities between major cities, users in India can now get easy access to databases outside the country. Attempts, though isolated, are also being made to acquire databases on magnetic or optical media for generation of local services. To summarise, in the last five years, although fully operational networks or datahosts may have seen only limited growth there has nevertheless been a perceptible change in preparedness.

Complementary activities, especially those on indigenous database development, did not develop as they should however. A quick survey may put the number of databases at about three hundred, unfortunately none is anywhere close to the international standard. The use of non-standard formats and software, a neglect of vocabulary control, inadequate data collection and preparation facilities have all impeded database development in the country. Foremost has been a lack of vision, confidence and motivation. This has been particularly disappointing since factors like the availability of subject knowledge, information handling experience, computer hardware and software skills and low manpower costs could potentially foster the healthy growth of a database production industry.

Given the above situation in India today, the broad objective of this research programme is to evolve a model for the whole range of database generation and service activities in the context of the economy of a developing country. Specific study components will include *inter alia*:

- Database production and maintenance, including the use of various standards, formats, software, thesauri etc.
- Installation of databases on national and international networks.
- Alternative database service delivery mechanisms: direct delivery by database producer; installation of database on one or multiple hosts; provision of access through gateways and with the help of technology transfer agencies, information brokers and other such intermediaries.
- Marketing practices of existing databases, datahosts, gateways and intermediaries.

- Privatisation of database generation and services.
- Education and training requirements for database services.

Based on information so gathered and on the experience of leading database producers and database use-facilitators in the EEC countries and in India, an attempt will be made to specify the design of a set of database service facilities suited to the Indian environment.

Activities in the first four months of the fellowship have helped to develop the concept of an integrated information project with distributed database production and services linked to a gateway system for "one window" access by a national and international community of users.

A discussion paper outlining the integrated service concept is currently under study by experts. The detailed report giving the goals to be set, strategies to be adopted and various technical aspects of the concept is being prepared.

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Ultrasonic estimations of heavy metals in biological media

Fellowship period: December 1990 - November 1991

Summary

During the first three months of the project, literature on ultrasound velocity measurements in solution has been scanned and is now being prepared for publication as a review article or a book. It is hoped that this will provide a valuable benchmark for workers in this field.

An ultrasound interferometer for the measurement of ultrasound velocity has been ordered and is on its way from India. A precision displacement measurement system is also being procured. It is intended to use these to provide a careful assessment of the accuracy of the Indian ultrasound interferometer, which is widely used in chemistry departments in India but with a varying quoted accuracy. This analysis will not only provide a vital reference for workers in the field in India but will permit optimisation of an experimental system for the present project; to investigate the use of ultrasonic techniques for detecting inorganic ions in biological media.

T.S.R.R.K. Rao

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E. Behrends

Fachbereich Mathematik,
Freie Universität Berlin,
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1000 Berlin 33, Germany.

A study of banach spaces that are L-ideals in their bidual

Fellowship period: January - December 1991

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This volume presents a summary of international scientific cooperation activities with India for which a financial commitment was made during the period 1988 to 1990. It covers 10 joint research projects, 33 post-doctoral fellowships and 3 workshops which conform to the patterns described in the foreword except for some of the early research projects.

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. In addition, it should be noted that there is a bias in the distribution of activities towards fellowships and workshops which are essentially preparatory activities. It is thus to be expected that there will shortly be an expansion in joint research projects as research proposals are generated from these preparatory activities.

The objective of compiling this volume is to show what has been achieved in the framework of international scientific cooperation with India. The strength of these achievements lies with their firm 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 Indian and European, 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 India and the European Communities.

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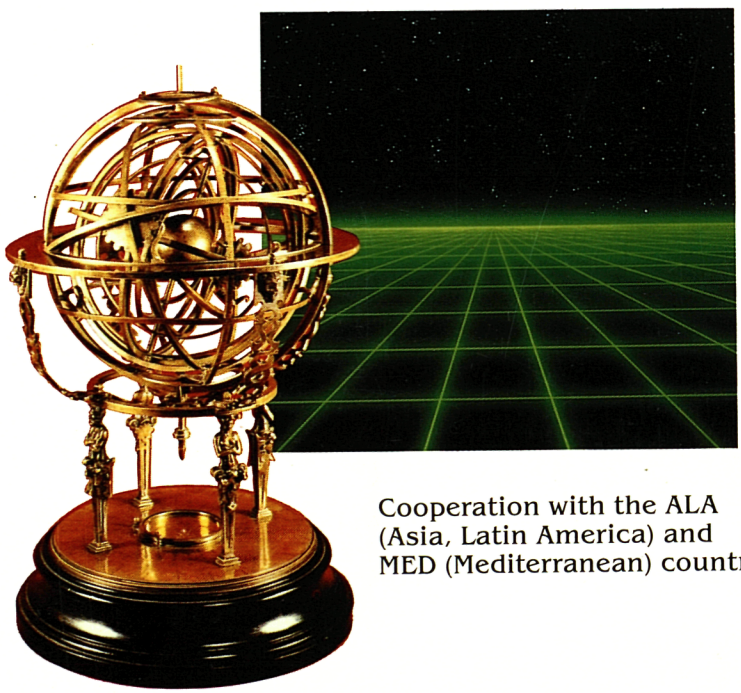

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