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WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

BIENNIAL REPORT

1986 – 1987

FOR THE PERIOD 1 JULY 1985 TO 30 JUNE 1987

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
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INTRODUCTION

The Agency's main interests continue to be cancer etiology and the generation and dissemination of information useful for the prevention of human cancer. The maintenance of scientific expertise permits the Agency to identify new findings in basic research, while at the same time assuring its credibility in fulfilling its catalytic and coordinating role at the international level. The Agency monitors how new findings in basic research can be applied in public health and, in particular, in developing approaches to bridging the gap between investigations of genetic factors and studies of environmental factors in the causation of human cancer.

Furthermore, the Agency maintains complete scientific independence and objectivity, assisted by the commitment of its staff and by the continuous support of a large number of scientists all over the world who collaborate readily with the Agency.

It is with great satisfaction that the Agency can now count among its members two states with long-standing records of achievements in cancer research: Finland, which joined in 1986, and Norway, which joined in 1987. This brings the total number of members to 14: Australia, Belgium, Canada, Federal Republic of Germany, Finland, France, Italy, Japan, The Netherlands, Norway, Sweden, the UK, the USA and the USSR.

The last two years have seen considerable expansion of the activities of the Agency, in particular in the area of epidemiology, on the descriptive and analytical sides, as well as in the area of laboratory-based molecular and metabolic epidemiology. Tangible evidence of this increased activity is provided by the number of individual and collective publications of Agency staff or as a result of Agency-sponsored meetings. Additional evidence of the increasing interest that the Agency is eliciting on the international scene is shown by the large number of visiting scientists, fellows, lecturers and visitors who come yearly to Lyon.

With the adoption of a regular system for reviewing the Agency's activities, a number of projects are considered in detail either by a Scientific Council Peer Review Sub-committee, which convenes jointly with external consultants in September, or by the Scientific Council at its plenary session in January of each year. In the two years (June 1985 to June 1987) that have elapsed since the last full *Annual Report,* the following projects were reviewed: the fellowships programme; the project on the formation and repair of DNA alkylation; the development of techniques for detecting exposure to aflatoxins in body fluids; the registry of persons exposed to phenoxyacetic acid herbicides; the manuals of selected methods of analysis of environmental carcinogens; the investigation on drug-related cancers; the proposal for a programme on genetic predisposition to cancer; the project to investigate cervical cancer in relation to male sexual behaviour and papilloma virus infection; a study on passive smoking; assistance to cancer registry systems in developing countries; the investigation of intragastric nitrosation and stomach cancer; studies on laryngeal cancer; studies on workplace exposures; field studies of oesophageal and liver cancer; and investigation of precancerous lesions in stomach cancer.

The Scientific Council also considered several new projects and programme increases that may be implemented should funds become available.

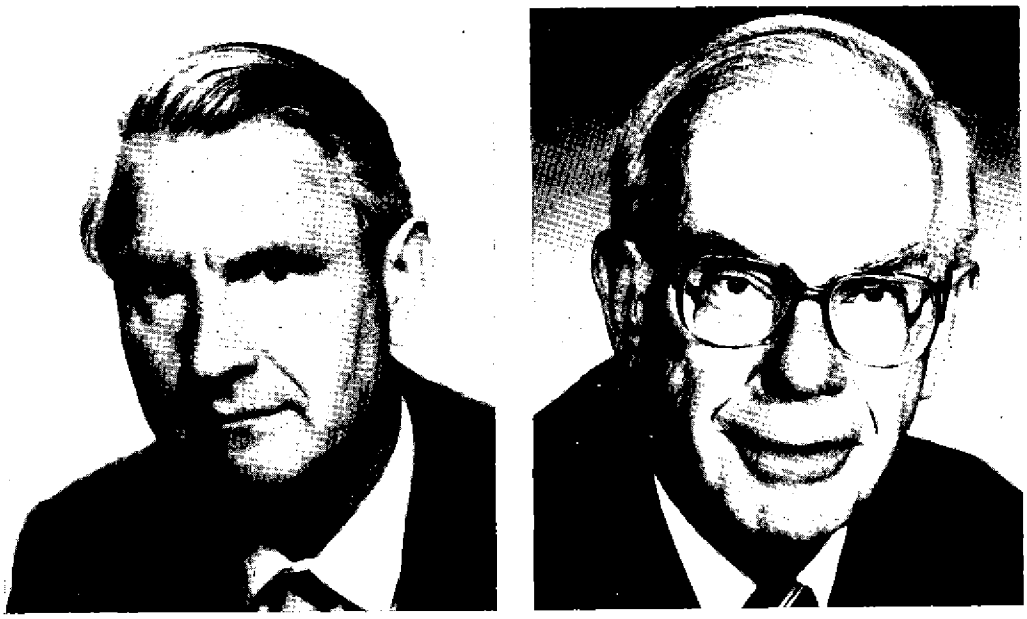
Fig. 1a. New members of the Scientific Council, 1986–1989

Professor L. L. Griciute

Professor R. Monier

Professor R. Simard

Fig. 1b. New members of the Scientific Council, 1987–1990



Professor F. De Waard

Professor S. Graham

Below, some of the achivements of the past two years are described briefly:

Descriptive epidemiology

The fifth volume of *Cancer Incidence in Five Continents*, covering data from 112 registries all over the world, will be published this year. This volume will contain no data from Africa, since no African registry was able to send in information. This unfortunate event provides convincing evidence of the urgent necessity to extend support to registration facilities in developing countries to prevent their collapse and to improve the quality of the information they provide. At present, the Agency is assisting ten national cancer registries in Africa, South America, Asia and Oceania. The first volume of *Cancer Occurrence in Developing Countries*, containing information from 75 centres in four continents, was published in 1986, and a second edition of this work is envisaged within the next two to three years.

Of particular relevance is an investigation of the international incidence of childhood cancer, a collaborative effort comprising data obtained from 72 registries. A monograph will be published in 1988; the available data indicate that the incidence of several types of childhood cancer varies between different regions of the world more widely than had hitherto been assumed.

The most recent Agency assessment of the global cancer burden indicates that 6.35 million new cancer cases occurred in 1980. Stomach cancer was still the most frequent cancer in 1980, but, since its rates are declining in most countries and the rates for lung cancer are rising persistently, the latter may now be the most frequent type in the world. This survey provides useful indications of priorities for cancer prevention and control in both developed and

developing regions of the world. For instance, the most frequent cancer in developing countries is cervical cancer, which ranks only tenth in the developed countries; colon and rectum is the second most frequent site of cancer in developed countries, but ranks only eighth in other parts of the world.

Occupational and environmental hazards

The large international collaborative study on possible long-term hazards of man-made mineral fibres in production workers, carried out by the IARC with the support of the Joint European Medical Research Board, has been successfully completed. The results were presented at a symposium organized by the World Health Organization Regional Office for Europe in Copenhagen, and have been published. Elucidation of the mechanisms by which fibres of different physical and chemical characteristics exert their carcinogenic effects could permit better assessment of risks, even at low levels of exposure.

Studies are in progress of the possible cancer hazards of exposure to welding fumes, to vinyl chloride, to styrene and to silica. The register of individuals exposed to dioxin-contaminated substances has been completed and is now ready for use in follow-up studies.

Occupational risks of a very particular nature are those to which biological laboratory workers may be exposed. Following the recent occurrence of six cases of cancer in a scientific institute, the Agency was invited to participate in setting up an international study to assess possible excesses in cancer mortality and incidence in laboratory workers. The first step will be to identify any such excesses in research institutes in different countries. A large epidemiological study may be organized, following a feasibility study.

Although it is now generally accepted that passive inhalation of tobacco smoke constitutes a health hazard, as indicated by several epidemiological investigations, an accurate assessment of the risk depends on reliable measurement of exposure levels. The Agency therefore initiated a large, collaborative, methodological study, which is now nearing completion, in 13 centres in ten countries; analysis of the data is currently under way. The methodology developed in this investigation will be used in an international study of lung cancer in nonsmokers.

The first phase of the international collaborative study of second malignancies following cytostatic therapy has been completed. Clear excesses were seen for leukaemia following treatment for Hodgkin's disease and for ovarian cancer, and for non-Hodgkin's lymphoma and lung cancer following treatment for Hodgkin's disease. The collaborative group is now completing data collection for a series of case-control studies of second cancers at a number of sites; statistical analysis will be undertaken to compare the carcinogenic potency of different drugs and other effects. The problem of chemotherapy-induced malignancies was addressed at a symposium held in November 1985, the proceedings of which were published as *IARC Scientific Publications No. 78.*

Within a project aimed at assessing the role of DNA-damaging agents in the etiology of human cancers, the major emphasis is on the possible role of *N*-nitroso compounds. A project of particular interest is the development of methods for measuring DNA damage in chewers of betel quid with and without tobacco. Specific attention is paid to the compounds that are formed in the oral cavity following nitrosation of betel-nut constituents. An important contribution to understanding the role of endogenously-formed carcinogens has been the development of a method for measuring total *N*-nitroso compounds in body fluids and of immunological assays for detecting alkylated DNA base adducts excreted in urine.

The 9th International Meeting on *N*-Nitroso Compounds was held in September 1986 in Baden, Austria, under the patronage of the Austrian Ministry of Health and Environmental Protection, the proceedings of which will be published as *IARC Scientific Publications No. 84.*

IARC Monographs

Four volumes of *Monographs* were prepared during the period under review: on naturally-occurring synthetic food components, furocoumarins and ultraviolet radiation (Volume 40), on some halogenated compounds and pesticides (Volume 41), on silica and some silicate minerals (Volume 42), and on man-made mineral fibres and radon (in press, Volume 43). There was sufficient evidence for carcinogenicity in humans of erionite and talc containing asbestiform fibres, and of radon and its decay products. Two ad-hoc working groups reconsidered the criteria used for evaluating carcinogenicity within the IARC Monographs programme. In addition, in March 1987, an ad-hoc working group reconsidered and updated evaluations of carcinogenicity for all chemicals, groups of chemicals and complex exposures that had been considered in Volumes 1–42 of the *Monographs*. A previous group summarized and brought up to date the results of a broad spectrum of tests for genetic and related effects of selected chemicals included in Volumes 1–42, the outcome of which will appear as Supplement No. 6. The results of the March 1987 meeting will be published as Supplement No. 7.

Site-oriented studies

Studies carried out at the Agency several years ago demonstrated a positive correlation between exposure to aflatoxin and risk of hepatocellular carcinoma. An additional investigation in Swaziland, terminated recently, provides strong evidence for an association between aflatoxin consumption and liver cancer, even when infection with hepatitis B virus is taken into account. Investigations are under way in Thailand, Singapore and Spain to assess the role of the various risk factors for liver cancer — primarily hepatitis B viral infection and aflatoxins, but also parasites, smoking, alcohol, *N*-nitroso compounds and hormones.

The large intervention trial to study the role of hepatitis B virus infection in liver cancer is in progress in the Gambia with the financial support of the Italian Government and the collaboration of the UK Medical Research Council Unit in Fajara. This initiative will also contribute to maintaining and strengthening the expanded programme of immunization against the major childhood diseases in that country. Vaccination was begun in July 1986, and, since no adverse reaction to the vaccines used has been observed to date, the campaign is continuing as planned.

The large, collaborative case-control study in Colombia and Spain on cervical cancer is aimed at establishing to what extent male and female sexual behaviour contributes to the ten-fold differential risk between the two countries, and at verifying the role that human papilloma virus plays in the development of this tumour. Following a successful feasibility study, a full-scale investigation was started in nine provinces in Spain and in Cali, Colombia. Tissue and cell samples will be analysed systematically to determine the presence of human papilloma virus-DNA.

A case-control approach has been proposed to the study of the relationship between hormonal profile and breast cancer incidence in women. The study, which will be carried out among population groups in China and in Chinese and Caucasians in New York City, it based mainly on the 'free oestrogen' hypothesis.

The international study on cancer of the larynx and hypopharynx has been completed. A precise estimate was obtained of the risk of alcohol drinking for cancers at various subsites, and the study permits better understanding of the combined effects of alcohol and tobacco.

The investigation of cancers of the pancreas, bile duct and gall bladder is the most advanced of the several studies within the multi-centre case-control network (SEARCH). Active data collection — the most extensive ever made for pancreatic tumours — ended recently and analysis

has been initiated. The studies of brain tumours in children and in adults are under way, and the possibility of a project on breast and intestinal cancers in women is being explored.

Nutrition and cancer

The preliminary methodological study in Malmö (Sweden) to assess the feasibility of conducting a large-scale, long-term investigation on an adult population has been completed. It was shown that the methods envisaged for the collection of dietary information were useful and could be implemented reliably and that the collection of biological samples in parallel was feasible. The next step is to complete the design and logistical organization of the study, which will mean the enrolment of 30 000 individuals. Close collaboration between Swedish scientists and Agency staff is the necessary prerequisite to guarantee successful continuity of this large cohort study. The experimental aspect is being pursued within the Agency, concentrating on the mechanisms by which lipids are involved — causally or noncausally — in the induction of tumours at different sites. Results to date indicate that a high level of dietary fat, together with chronic exposure to a *N*-nitroso compound, can concur in producing oxidative stress, which could be the mechanism by which carcinogenic initiators act.

Genetics and cancer

Although most of the Agency's programmes have been devoted to the identification of environmental carcinogens, it recognizes that understanding of the mechanisms underlying individual susceptibility to carcinogenic risk factors will open the way to more efficient cancer prevention. Due to limited laboratory facilities, activities in this area have been orientated mainly towards the collection of biological samples and the establishment of cell lines suitable for molecular or linkage studies. Several projects, however, are being pursued in the Agency's own laboratories. Among these is a study of the recessive genetic disorder linked to the X-chromosone, which can lead to the development of malignant lymphoma following infection with Epstein–Barr virus. Another project is on multiple endocrine neoplasia type IIa, and a further study aims at verifying if the abnormal gene observed in the constitutional duplication of c-*myc*, identified in two cancer patients and their families, represented a cancer risk marker. Another project is the establishment of lymphoblastoid cell lines from individual members of families with multiple cases of breast cancer.

The Agency closely follows progress in the area of genetics to survey possibilities for active involvement and, in particular, for applying newly developed laboratory tests to epidemiological investigations.

A study of biochemical and metabolic parameters is being carried out to investigate the basis for individual susceptibility to tobacco smoke-induced cancers. Initial results indicate that pulmonary metabolizing enzymes are induced more frequently among smokers who have developed lung cancer than in cancer-free smokers. This observation, which would support a genetic determinant of susceptibility to lung cancer by tobacco, requires confirmatory evidence.

Mechanisms of carcinogenesis

Burkitt's lymphoma cells are a very useful tool for studying the consequences of chromosomal arrangements, and, in particular, the chromosomal translocation at the c-*myc* locus. In Burkitt's lymphoma cells characterized by the t(8;22), a specific Burkitt's lymphoma translocation, the c-*myc* locus appears to be rearranged by a cluster of somatic mutations, and not by chromosomal translocation. Results from recent studies indicate that the genome sequence coding for the Epstein–Barr nuclear antigen-2 activates the cellular genes associated with the

immortalization of lymphoid cells. New malignant lymphoid cell lines are also being established and karyotyped at the Agency.

Various antibodies against alkylated DNA nucleosides (O^6-methyldeoxyguanosine, O^4-methylthymidine and N^7-methyldeoxyguanosine) are now available, allowing highly sensitive, specific detection of these adducts in animals and in humans exposed to alkylating agents. It has been shown that these DNA adducts can be detected not only in liver and oesophagus but also in peripheral blood cell DNA, opening the way to easier assessment of human exposure to environmental alkylating agents. Studies of the possible link between DNA adducts in human oesophageal tissues and cellular oncogene activity address the possible relevance to the origin of oesophageal cancer of DNA polymorphism at the c-*mos* locus, observed in certain oesophageal cancer patients.

Considerable progress has been made in elucidating the role of cell-to-cell communication in tumour development. The phenomenon of selective (transformed *versus* nontransformed cells) intercellular communication is not confined to mesenchymal cells, as shown by the studies carried out in rat liver epithelial cells. Reconstruction experiments also suggest that growth of NIH 3T3 cells transformed by *myc* is inhibited by the communication with normal cells, and the tumour-promoting agents modulate this effect by blocking communication. These study results indicate that the phenomenon of selective communication among transformed cells (but not between transformed and nontransformed cells) through gap junctions could be exploited for selective killing of transformed cells.

Within the study on prenatal carcinogenesis, it was demonstrated that prenatal exposure to 7,12-dimethylbenz[*a*]anthracene induces a specific mutation at codon 61 of c-Ha-*ras*, which appears to be necessary for development of tumours following exposure postnatally to a promotor.

Data collection and research method

A second edition of the 1978 scientific publication *Cancer Registration and Its Techniques* is being prepared, as the first was found to be extremely useful in the setting up of cancer registries throughout the world. The new edition will include more information on the use of computers in registration, nevertheless retaining a description of card-filing techniques. A further contribution to descriptive epidemiology is the Agency's cancer mapping projects; these include mortality atlases for the European Economic Community and for Hungary, and an atlas of cancer incidence in the German Democratic Repulic. The Directories of on-going research in cancer epidemiology continue to provide scientists with information on studies being conducted throughout the world and promote contacts between scientists. Since 1985, the directories have included studies in the field of mutation epidemiology.

The Agency is investigating means of improving the statistical tools available in descriptive epidemiology as well as methods for estimating and, if possible, controlling measurement errors in the assessment of exposure variables in epidemiological studies.

The project for evaluating the usefulness of early detection programmes includes methods for screening cancers of the cervix, breast, colon and rectum, and nasopharynx. Results of studies on the first two sites have been published; a pilot study is under way to investigate the feasibility of evaluating screening for colorectal cancer, and another feasibility study is looking at methods for screening for nasopharyngeal cancer in China.

A useful contribution to the Agency's aim of providing standardized, validated methods to the international scientific community is the series of manuals, *Environmental Carcinogens: Methods of Analysis and Exposure Measurement.* A volume on methods for

measuring metals has been published, and volumes are being prepared on passive smoking, on a number of workplace contaminants and on indoor air in general. The next subject of high priority is biological monitoring techniques. With a similar aim, a publication has been issued on the conduct and use of long-term and short-term assays for detecting carcinogens. It is based on reports prepared by groups of experts convened at the Agency, and includes discussions of the relevance of short-term tests to the detection of carcinogens and their mechansims of action. Methods for detecting agents that damage DNA are to be the subject of a further Agency publication. The Agency also disseminates useful information for cancer research and other scientists on the destruction of carcinogenic wastes and safe handling of carcinogens in the laboratory. The most recent of this series of publications concerns cytostatic drugs.

In order to bring to the Agency's programmes an efficient computing and biostatistical support, its hardware was recently upgraded; the software is monitored continually and improved when necessary. An efficient bibliogaphical retrieval system has been evolved, and new data bases created for a variety of programmes.

Education and training

The Agency's fellowships programme was able to award 12 places in 1986 and 11 in 1987. An analysis of the programme since its inception in 1966 showed that the large majority of persons who received an Agency fellowship returned to their home country after the tenure of the fellowship and are still engaged in cancer research. The provision by the programme of basic training in cancer epidemiology, which is offered by very few other institutions, was instrumental to the development of epidemiology in a number of countries. The Agency's training courses have also contributed to this development. In the last two years, courses on cancer epidemiology were held in the German Democratic Republic, Kuala Lumpur, Morocco, the Federal Republic of Germany and at the Agency. A course on health monitoring of populations exposed to mutagens and carcinogens was held in Bombay, India.

The high standards of the Agency's publications series are maintained by ensuring that all proposals are reviewed by the Advisory Committee on Publications, set up in November 1985. Additionally, all manuscripts included in the proceedings of meetings published by the Agency now undergo peer review.

The regular budget for the biennium 1986–1987 was US$ 17 289 000.

On 30 June 1987, the Agency's staff consisted of 49 scientists, 49 technicians and 71 administrative and secretarial staff.

L. Tomatis, M.D.
Director

I. STUDIES ON ETIOLOGY AND PREVENTION

1. STUDIES OF GEOGRAPHICAL DISTRIBUTION, TIME TRENDS AND SPECIAL GROUPS

(*a*) *Cancer Incidence in Five Continents, Vol. V* (Dr C.S. Muir, Miss S. Whelan, Mr M. Smans and Miss F. Casset; in collaboration with Dr J.A.H. Waterhouse and Miss J. Powell, Birmingham and West Midlands Regional Cancer Registry, UK: and Dr T.M. Mack, University of Southern California, Department of Family & Preventive Medicine, Los Angeles, CA, USA)

A total of 112 registries have contributed material to Volume V of the series, and data from 105 of these, covering 137 populations in 36 countries, will be published (see Fig. 2), with a separate analysis by urban/rural residence for 11 of them. For the first time, no African registry has been able to send data: the increase in coverage since Volume IV (which included 79 registries and 103 populations)is due largely to the greater number of registries from Central and South America, and from Asia. New contributors include Fortaleza and Porto Alegre in Brazil, Costa Rica, Martinique, the Chinese registry of Tianjin, three new Indian registries, Kuwait, and Rizal in the Philippines. National data for Canada, England and Wales, and Scotland are also being published.

The aim of the *Cancer Incidence in Five Continents* series is to present in standard format comparable data from as many parts of the world as possible. While every encouragement is given to registries to submit data, it is editorial practice to assess the data and not to include them if registration is demonstrated to be incomplete; this has been the case for a very small number of the registries that sent data for Volume V.

An innovation is the addition of a footnote indicating registries that cover populations in which less than 90% of persons are of a single ethnic group, given that the validity of some comparisons between cancer incidence rates depends upon assumptions about the homogeneity of the genetic composition and about past and present cultural practices of the populations compared.

The main tables present the data in much the same format as in Volume IV[1], with the addition of columns giving the cumulative rates for ages 0–64 and 0–74, and the relative frequency of the age-standardized rate for each site.

In general, the data in Volume V show that lung cancer incidence has continued to rise in most countries in people of each sex. In some countries, rates of cancers of the large bowel and female breast are still on the increase, being stable elsewhere; in most countries, stomach cancer incidence continues to fall. In many registries, rates for lymphoma (ICD 202) have risen sharply; such rises have usually been accompanied by a fall in the rates for ICD 200, which suggests that there may have been changes in the nomenclature employed for these tumours. Rates for multiple myeloma and for connective tissue tumours have increased considerably in many registries; to some extent, this may be due to improved histological-diagnosis and to the

[1] Waterhouse, J., Muir, C., Shanmugaratnam, K. & Powell, J., eds (1982) *Cancer Incidence in Five Continents, Volume IV (IARC Scientific Publications No. 42)*, Lyon, International Agency for Research on Cancer

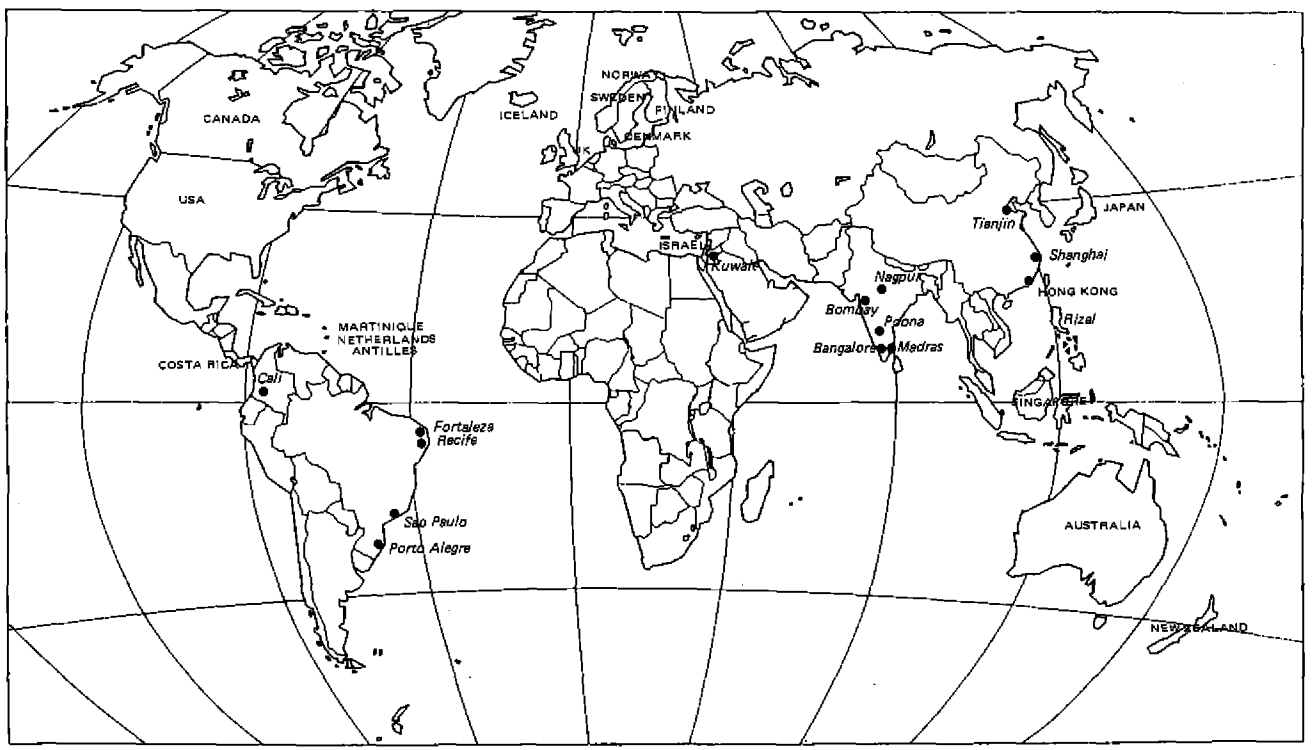


Fig. 2. Registries that have contributed material for Volume V of *Cancer Incidence in Five Continents*

inclusion of peripheral nerve malignancies in the ICD-9 classification, which has been used for the tabulations in the volume.

Volume V will be published towards the end of 1987.

(*b*) *Global burden of cancer* (Dr D.M. Parkin and Dr C.S. Muir; in collaboration with Dr E. Läärä, University of Oulu, Finland)

In a previous study, we estimated the number of new cases of cancer at 12 common sites occurring in the world in 1975[2]. This exercise has been repeated using more recent data on incidence, mortality and relative frequency of cancer for the period centering on 1980, and new estimates have been produced for 16 different cancer sites[3]. The total number of new cancer cases in the world was estimated to be 6.35 million; the ten most common tumours in males and females are shown in the Table 1.

Table 1. The most frequent cancers, 1980

Rank	Males			Females		
	Site	No. ($\times 10^3$)	%	Site	No. ($\times 10^3$)	%
1	Lung	513.6	15.8	Breast	572.1	18.4
2	Stomach	408.8	12.6	Cervix	465.6	15.0
3	Colon/Rectum	286.2	8.8	Colon/Rectum	285.9	9.2
4	Mouth/Pharynx	257.3	7.9	Stomach	260.6	8.4
5	Prostate	235.8	7.3	Corpus uteri	148.8	4.8
6	Oesophagus	202.1	6.2	Lung	146.9	4.7
7	Liver	171.7	5.3	Ovary	137.6	4.4
8	Bladder	167.7	5.2	Mouth/Pharynx	121.2	3.9
9	Lymphoma	139.9	4.3	Oesophagus	108.2	3.5
10	Leukaemia	106.9	3.3	Lymphoma	98.0	3.2

It is proposed to use data on recent changes in morbidity and mortality rates from some common tumours to predict the likely changes in occurrence up to the year 2000 and beyond. We shall use the results of a study of time trends in incidence and mortality (see I.1.*d*). Most of the data were obtained from the developed countries of the world. Projections of the future cancer burden in developing countries involve assumptions about the probable shape of age-incidence curves for different cancers, population projections by age and sex, and such information as exists about current cross-sectional trends in incidence and mortality.

(*c*) *Cancer in developing countries*

(i) *Cancer occurrence in developing countries* (Dr D.M. Parkin)

Data from 75 centres in Africa, America, Asia and Oceania were published as a monograph in 1986[4]. Each entry comprises standard tables for males and females, showing numbers of

[2] Parkin, D.M., Stjernswärd, J. & Muir, C.S. (1984) *Bull. World Health Organ.*, *62*, 163–182

[3] Parkin, D.M., Läärä, E. & Muir, C.S. (1987) *Int. J. Cancer* (in press)

[4] Parkin, D.M., ed. (1986) *Cancer Occurrence in Developing Countries* (*IARC Scientific Publications No. 75*), Lyon, International Agency for Research on Cancer

cancer cases by site and age, together with crude and age-standardized frequencies and, whenever possible, estimated rates of incidence. A brief description of each centre, together with a commentary on the results, accompanies the sets of tables.

(ii) *Support to cancer registries* (Dr. D. M. Parkin and Dr C.S. Muir)

The Unit of Descriptive Epidemiology continues to support and encourage cancer registration activites, especially in centres in Africa, Asia, Oceania and Central America.

Fiji (Principal investigator, Dr I. Seruvatu, Pathology Department, CWM Hospital, Suva): The collaborative research agreement with the Ministry of Health (DEB/81/023) has been renewed, and a visit was made in 1986 to advise on updating and coordination of registration activities. Arrangements were made to transfer data collected since 1984 to computer medium for analysis.

India As in previous years[5], the Agency acted as a consultant to the cancer registries supported by the Indian Council of Medical Research (Dr U.K. Luthra, New Delhi). A review was conducted at the Cancer Institute (WIA), Madras, on 19–22 November 1985 (Dr V. Shanta), and site visits were paid to clincial campuses of the Cancer Institute and to the hosptial records department, as well as to the cancer registries.

Comments made previously concerning the necessity to improve quality control had clearly been given careful consideration, notably with regard to detection of erroneous information at the level of the registry. Mr R. Skeet, director of the newly created Thames Cancer Registry (UK), gave a course on quality control. It was noted that a cancer registry has been established in Bhopal, which will extend geographical coverage in India and provide a means of follow-up for the cohort study of the population exposed to methyl isocyanate during the accident at the Union Carbide plant.

Gambia: As part of the Gambia hepatitis immunization study (I.3.*a*.v), a cancer registry covering the entire population of the Gambia has been established. The main objective is to identify new cases of liver cancer, but the surveillance system is being used to identify all other types of cancer as well.

Results for the first six months of operation have been analysed and show equivalent annual age-standardized incidence rates of 90.8 per 10^5 (males) and 53.1 per 10^5 (females). The incidence rates for liver cancer in males are some 50% higher than those previously recorded in West Africa (in Dakar, Senegal).

Mali (Principal investigator, Professor S. Bayo): Financial support to enhance the activites of the recently founded registry, which covers the area of the capital, Bamako, has been given *via* a collaborative research agreement (DEP/87/02). This should permit more exhaustive data collection. The cancer registry clerk was provided with a four-week training programme in Strasbourg (Dr. P. Shaffer) and Lyon, France.

Gabon (Principal investigator, Dr P. Walter): The histopathology-based registry in Gabon has been used as a demonstration project for the microcomputer system 'CANREG' (III.1.*b*.i) under a collaborative research agreement. Further analysis of data from this registry is expected in 1987.

Zimbabwe (Principal investigator, Progessor C. Chetsanga, Harare): A collaborative research agreement with the newly founded registry in Harare provided funds for the purchase of a

[5] IARC (1985) *Annual Report 1985*, Lyon, pp. 11–12

microcomputer. A visit in March 1986 allowed a review of procedures and installation of the 'CANREG' software (III.1.*b*.i).

In September 1986, the former director of the Bulawayo Cancer Registry, Dr M. Skinner, visited IARC to review the data collected by that registry over 1963–1977. The file was carefully checked, and some preliminary analyses performed; however, for more than 1000 cases, it was necessary to check the recorded information in the original registry files in Bulawayo. The data set has now been corrected, and a full analysis will be carried out in 1987.

Rwanda (Principal investigator, Dr P. Ngendahayo: An analysis of data on cases recorded in the department of anatomopathology over a three-year period has been published[6]. The establishment of a collaborative research agreement has allowed employment of a full-time registrar, which will make it possible to extend the scope of data collection.

Costa Rica: During a visit of Dr R. Sierra (University of Costa Rica), the data from the national cancer registry of that country were carefully reviewed and analysed. A monograph is in preparation which will include information on the incidences of the major cancers and their variation within the provinces and regions of Costa Rica, and some recent trends in cancer mortality rates.

Thailand: Support to the national cancer registry in Bangkok has included the provision of consultant advice on computerization of the registry and analysis of geographic variations in the incidence of liver cancer[7]. During a visit to the university hospital in Khon Kaen, discussions were held concerning the conversion of the hospital-based registry to cover the entire population of the province, and a collaborative research agreement (DEP/87/04: principal investigator, Dr Vanchai Vatanasapt) has been established to permit training of registry personnel.

Philippines: Consultant support was provided for computerization of the central tumour registry in Manila. This registry covers the central part of the area of Metro-Manila, the remainder being covered by the Rizal Tumour Registry. Information of 8800 cases recorded in the latter centre between 1978 and 1982 has been transferred to machine-readable form, and the data from both centres will be analysed in July 1987 during the visit to Lyon of Dr D. Esteban. In addition, it planned to make use of the experience of the two registries to produce a set of training manuals adapted for use by cancer registrars working in urban areas of developing countries.

(*d*) *Time trends* (Dr D.M. Parkin, Dr J. Estève, Dr M. Coleman, Mr P. Damiecki and Miss F. Casset)

With the publication of Volume V of *Cancer Incidence in Five Continents*, data will be available in IARC to permit study of changes in incidence in several cancer registries over time periods ranging from 15 to 25 years. In addition, mortality data from 39 countries for periods from 20 to 35 years are available through WHO. A meeting was held at IARC on 1–3 April 1987 of specialists who have contributed to the practical analysis of time series data: Dr D.G. Clayton, University of Leicester, UK: Dr S. Devesa, National Cancer Institute, USA: Dr T. Hakulinen, Finnish Cancer Registry, Finland; Dr A. Lopez, WHO, Geneva; Dr C. Osmond, Medical Research Council, UK: Dr E. Schifflers, University of Namur, Belgium). Planning was completed for a comprehensive analysis of the time trends in incidence and/or mortality of 28

[6] Ngendahayo, P. & Parkin, D.M. (1986) *Bull. Cancer, 73*, 155–164

[7] Srivatanakul, P., Sontipong, S., Chotiwan, P. & Parkin, D.M. (1987) *J. Gastroenterol. Hepatol.* (submitted for publication)

different cancers. This will include a presentation of trends by period (date of incidence or death) and birth cohort (year of birth), with a general indication of the goodness of fit of the relevant models.

The work will be carried out mainly in 1988, and will be published in the form of a monograph, dealing in summary form with 28 cancer sites.

In addition, further work on certain sites of special interest is anticipated, where temporal trends throw light upon the relative importance of changes in exposure to environmental agents in different areas (e.g., oesophageal cancer, cervical cancer).

(*e*) *Migrant populations* (Dr D.M. Parkin and Dr J. Kaldor)

Studies of migrant populations are of particular interest in estimating the relative contributions of genetic and environmental factors in cancer etiology.

(i) *Cancer incidence in Jewish migrants to Israel* (Dr D.M. Parkin, Dr J. Kaldor and Mr A. Bieber; in collaboration with Dr R. Steinitz and Dr L. Katz, Israel Center for Registration of Cancer and Allied Diseases, Jerusalem; and Dr J. Young, National Cancer Institute, Bethesda, MD, USA, DEB/83/19)

The Israel Cancer Registry records the place of birth of all cases registered since 1960, and, for immigrants to Israel, the year of immigration. The Israel Bureau of Statistics publishes annual estimates of population by county of origin, and for continent of origin estimates are also provided by period of migration. It is thus possible to calculate incidence rates of different cancers in migrants from several countries or regions, and to examine how these rates vary with duration of residence of Israel. The registrations have been checked against the population register of Israel to complete missing data on country of origin and period of migration, for cancer cases registered in the 20 years 1961–1980.

The data will be published as a monograph in which incidence rates will be compared between different migrant groups and between migrants to Israel and those in the country of origin. Log-linear modelling methods have been used to examine the importance of changes in risk due to duration of residence in Israel (or age at time of migration) in the face of underlying temporal trends in incidence (see Table 2).

Table 2. Relative risk of malignant melanoma (adjusted for age and sex in Jewish migrants to Israel

Continent of birth	Period of diagnosis (adjusted for duration of stay)			
	1961–1966	1967–1971	1972–1976	1977–1980
Asia	0.34	0.29	0.48	0.29
Africa	0.42	0.83	0.92	0.81
Europe	1.0	1.5	1.6	1.4
	Duration of stay in Israel (years) (adjusted for period of diagnosis)			
	0–9	10–19	20–29	30+
Asia	1.0	1.1	1.8	2.5
Africa	1.0	0.4	0.4	1.5[a]
Europe	1.0	1.1	1.2	1.8

[a] Not significant

(ii) *Studies on Italian migrant populations* (Dr D.M. Parkin and Dr J. Kaldor; in collaboration with Dr E. Buiatti and Dr M. Geddes, Tuscan Cancer Registry, Florence, Italy)

Two collaborative studies on Italian migrant populations are planned to start in 1987. The first concerns the populations of individuals born in southern Italy and resident in four northern provinces (Turin, Genoa, Bologna and Florence). It takes the form of a case-control study, in which the cases comprise deaths from 18 major cancers and controls (two per case) are individuals matched by sex, age and residence, drawn from the population registries for the four districts. The hypothesized protective effect of being born in southern Italy will be estimated in relation to age at migration (or duration of residence in the north), to the specific area of birth, and to other routinely recorded demographic variables (occupation, level of education).

The second study is an investigation of incidence and/or mortality rates of cancer in populations born in Italy but resident in other countries. The objective of the study is to compare rates for cancers at the major sites in these migrant populations (a) with each other, (b) with the locally-born, and (c) with those obtaining in Italy. Whenever data are available on time of migration, the effect of duration of residence in the new host country will be studied. The countries to be studied are Argentina, Australia, Brazil, Canada, Switzerland, the UK, Uruguay and the USA. Depending on availability, both mortality and incidence data will be used, and the analysis will involve a comparison of age-adjusted rates, if appropriate census denominators are available, or of proportionate morbidity/mortality ratios if they are not.

A planning meeting to finalize the protocols for these studies was held in Florence in March 1987, and it is intended that the two studies will start later in the year.

(*f*) *Long-term autopsy study on cancer mortality in Trieste* (Dr E. Riboli, Dr A.J. Sasco and Dr. R. Saracci; in collaboration with Dr G. Stanta, Dr M. Delendi and Professor L. Giarelli, Institute of Pathological Anatomy, University of Trieste, Italy)

From the end of the 1960s onwards, the Institute of Pathological Anatomy, under the direction of Professor L. Giarelli, steadily increased the number of autopsies performed on patients who had died in local hospitals. The proportion of all deaths coming to autopsy rose from 30% in early 1970 to 50% in 1975, and to 70% in recent years. For all subjects autopsied, diagnoses are available based on clinical data and on the diagnosis made at autopsy.

The work jointly undertaken consists in the retrieval and analysis of all data on deaths for which there was a diagnosis of cancer, either at the clinical level or at autopsy, or both. The data are being analysed, focusing on the rate of concordance and on the types of errors in cases of disagreement between the two diagnoses. Preliminary reports on specific findings regarding early gastric cancer and breast cancer have been published[8,9].

An extensive analysis of data on over 30 000 autopsies is now in progress, focused on false-positive and false-negative clinical diagnoses of cancers, on time trends of 'autopsy-corrected' cancer morality and on the effect of the implementation of new diagnostic techniques on the rates of error.

(*g*) *Analysis of data from the Singapore Cancer Registry* (Dr N. Day and Miss D. Magnin; in collaboration with Professor K. Shanmugaratnam and Dr H.P. Lee, Singapore Cancer Registry)

As a follow-up to the publication *Cancer Incidence in Singapore 1968–1977*, a second monograph covering the years 1968–1982 will be in print by the end of 1987[10]. Special attention

[8] Giarelli, L., Stanta, G., Delendi, M., Sasco, A.J. & Riboli, E. (1986) *Lancet, ii*, 864

[9] Stanta, G., Sasco, A.J., Riboli, E., Cocchi, A. & Rossilti, P. (1986) *Lancet, i*, 624

[10] Lee, H.P., Day, N.E. & Shanmugaratnam, K., eds (1987) *Trends in Cancer Incidence in Singapore 1986–1982 (IARC Scientific Publications No. 91)*, Lyon, International Agency for Research on Cancer (in press)

Table 3. Trends in cancer incidence in Singapore: average percent annual change in cumulative rates

Site	Male		Female	
	% change	χ^2 for trend 1 df	% change	χ^2 for trend 1 df
Oesophagus	−3.6	33.9	−6.1	35.7
Stomach	−2.3	34.6	−1.5	7.6
Colon	2.8	17.5	5.0	57.9
Rectum	2.5	13.3	4.1	27.3
Lung	2.4	57.1	2.3	18.8
Skin	2.5	7.9	3.4	13.5
Prostate	4.9	18.6	—	—
Breast	—	—	3.1	45.9
Ovary	—	—	3.4	18.4

has been paid to time trends over that period. The major changes are increasing incidences of cancers of the large bowel, lung and skin (nonmelanoma) in people of each sex; increases in prostatic cancer among men and in cancers of the breast and ovary among women; and decreases in the incidences of cancers of the oesophagus and stomach (Table 3). For cancers of oesophagus and breast, the percent changes are much higher in young people (less than 50 years old) than in older people, suggesting a cohort effect.

2. DETERMINATION OF ENVIRONMENTAL AND OCCUPATIONAL HAZARDS

(*a*) *Carcinogenic risk of inhalable particles* (Dr L. Simonato, Dr R. Saracci, Dr A.C. Fletcher and Ms R. Winkelmann)

(i) *Man-made mineral fibre production* (Dr L. Simonato, Dr A.C. Fletcher, Dr R. Saracci, Ms R. Winkelmann, Ms B. Charnay and Dr J. Estève; in collaboration with Dr A. Andersen, Institute for Epidemiological Research, Oslo; Dr P.A. Bertazzi, Institute of Occupational Medicine, Milan, Italy; Mr J. Cherrie and Mr J. Dodgson, Institute of Occupational Medicine, Edinburgh, Scotland, UK: Ms J. Claude and Dr R.R. Frentzel-Beyme, Institute for Documentation, Information and Statistics, Heidelberg, Federal Republic of Germany; Professor M. Gardner and Dr P. Winter, Southampton General Hospital, Southampton, UK: Dr O. Møller Jensen and Dr J. Olsen, Danish Cancer Registry, Copenhagen; Dr L. Teppo, Finnish Cancer Registry, Helsinki; and Dr P. Westerholm, Swedish Trade Union Confederation, Stockholm)

The full results of the European cohort study of man-made mineral fibre (MMMF) production workers which were described in the 1985 *Annual Report*[11] were presented at at international symposium on man-made mineral fibres held at WHO/EURO in Copenhagen in

[11] IARC (1985) *Annual Report 1985*, Lyon, pp. 15–17

October 1986 and cosponsored by IARC and the Joint European Medical Research Board (JEMRB). Publication of these results includes a special supplement to the *Scandinavian Journal of Work Environment and Health* and a forthcoming special issue of the *Annals of Occupational Hygiene*. An attempt to estimate past levels of exposure to airborne fibres will be carried out by an ad-hoc working group coordinated by the Institute of Occupational Medicine in Edinburgh, UK. The results of this exercise, if suitable, may be used to investigate the presence of a dose-response relationship between cumulative fibre dose and lung cancer mortality in the cohort.

(ii) *Man-made mineral fibre use* (Dr A.C. Fletcher; in collaboration with Dr G. Engholm, Dr A. Englund and Mr H. Lowing, Bygghälsan, The Swedish Foundation for Occupational Safety and Health in the Construction Industry, Stockholm)

A cohort of 135 037 Swedish construction workers were interviewed in 1971–1974 and followed prospectively for approximately ten years. By the end of 1983, 7356 deaths had been identified, including 440 cases of lung cancer and 23 of pleural mesothelioma. Using cohort and nested case-control techniques, the incident lung cancer cases were analysed in relation to exposure to MMMF and asbestos, by both self-reported exposure and job-exposure matrix approaches. The results of these analyses were presented at the international symposium on man-made mineral fibres held at WHO/EURO in Copenhagen in October 1986. Potential exposure to both MMMF and asbestos was found to be associated with excess lung cancer risk, the effect being much more pronounced for asbestos. However, most individuals with potential MMMF exposure had also had potential asbestos exposure, and there was only limited power to assess the effect of each exposure independently.

(iii) *Mesothelioma in central Turkey* (Dr L. Simonato and Dr R. Saracci; in collaboration with Dr Y.I. Baris and Dr M. Artvinli, Department of Chest Diseases, Hacettepe University, Ankara, DEB/82/014: Professor J. Skidmore and Dr C. Wagner, MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, Wales, UK: and Dr F. Pooley, Department of Mineral Sciences, University of Cardiff, Wales, UK)

The results of the four-year environmental, radiological and epidemiological investigation in central Cappadocia have been summarized and evaluated in a scientific publication[12].

The mortality rates in three affected and in one control village are presented in Table 4. The mortality pattern follows the distribution of exposure to erionite fibres assessed by both environmental sampling and lung tissue analysis. Animal data support the human evidence which points at erionite fibres as a potent carcinogen.

(iv) *Silica and lung cancer* (Dr L. Simonato, Dr R. Saracci, Dr A.C. Fletcher and Ms R. Winkelmann)

The results of studies carried out by members of the working group were presented at a meeting held in Lyon in December 1986. The data definitely indicate an excess of lung cancer among workers compensated for silicosis, while the evidence for workers exposed to silica is less clearcut. Results were not yet available for a cohort of slate quarry workers in the German Democratic Republic or for a cohort of pottery workers in the UK. The full results of all the

[12] Baris, I., Simonato, L., Artvinli, M., Pooley, F., Saracci, R., Skidmore, J. & Wagner, C. (1987) *Int. J. Cancer, 39*, 10–17

Table 4. Age- and sex-specific mortality rates and overall standardized rates per 1000 persons–years by sex and by village in central Turkey[a]

Age range	Cause[b]	Karain		Karlik		Sarihidir		Tuzkoy	
		Males	Females	Males	Females	Males	Females	Males	Females
20–29	A	13.5 (2)	0.0	0.0	0.0	0.0	0.0	2.3 (2)	1.2 (2)
	B	6.8 (1)	0.0	0.0	0.0	0.0	0.0	1.1 (1)	0.0
30–39	A	50.0 (5)	10.4 (1)	0.0	0.0	0.0	0.0	9.3 (4)	4.4 (2)
	B	50.0 (5)	0.0	0.0	0.0	0.0	0.0	7.0 (3)	2.2 (1)
40–49	A	10.0 (1)	46.5 (8)	9.3 (1)	0.0	23.0 (2)	0.0	5.9 (1)	8.3 (3)
	B	10.0 (1)	40.7 (7)	0.0	0.0	0.0	0.0	2.9 (1)	5.6 (2)
50–59	A	15.3 (3)	22.7 (4)	0.0	19.2 (2)	61.7 (5)	14.5 (1)	41.7 (11)	9.0 (3)
	B	15.3 (3)	0.0	0.0	0.0	49.4 (4)	14.5 (1)	18.9 (5)	6.0 (2)
60–69	A	111.1 (8)	47.6 (4)	50.0 (4)	52.1 (5)	88.9 (4)	27.8 (2)	32.8 (6)	49.4 (8)
	B	55.6 (4)	23.8 (2)	0.0	0.0	44.4 (2)	13.9 (1)	0.0	12.3 (2)
70+	A	125.0 (6)	222.2 (8)	27.8 (1)	125.0 (3)	76.9 (3)	466.7 (7)	69.0 (6)	47.6 (3)
	B	0.0	0.0	0.0	0.0	25.6 (1)	0.0	0.0	31.7 (2)
Standard rate/1000 persons–years									
A		34.7	28.1	6.7	12.8	26.8	21.1	16.9	11.3
B		20.2	10.9	0.0	0.0	13.5	3.9	5.2	4.9
A–B		14.5	17.2	6.7	12.8	13.3	17.2	11.7	6.4

[a] In parentheses, number of cases

[b] A, all causes; B, malignant mesothelioma (pleural or peritoneal), lung cancer and laryngeal cancer

studies, which should be available by the end of 1987, will constitute the body of an IARC Scientific Publication to be published by the end of 1988.

(*b*) *Carcinogenic risk of occupational exposure to chemicals* (Dr L. Simonato, Dr. R. Saracci, Dr A.C. Fletcher and Ms R. Winkelmann)

(i) *Exposure to welding fumes* (Dr A.C. Fletcher, Dr L. Simonato, Dr R. Saracci, Ms R. Winkelmann and Ms N. Charnay; in collaboration with WHO/EURO, the Commission of the European Communities and the Danish Welding Institute)

Following the WHO/EURO conference on welding fumes held in Copenhagen in February 1985, the Occupational Cancer Programme organized the first meeting of national collaborators in Lyon in November 1986. The working group agreed to conduct a multicentric cohort study coordinated by the Agency. Some characteristics of the working population are reported in Table 5.

A reconstruction of past exposure to welding fumes will be attempted for use in analysis of the cohort. The study will be carried out in collaboration with WHO/EURO, which is coordinating similar studies on morbidity and monitoring of working populations exposed to welding fumes. The data from the national collaborators should reach the Agency by the end of 1987. Data processing and quality control will be performed during the first half of 1988. Results should be available by the end of 1988.

Table 5. Summary of data for each national cohort of welders in a multicentre cohort study[a]

Country	Size of cohort		Type of cohort
	Stainless	Mild	
Denmark	3228	2040	Welders plus other workers from 90 factories, employed >1 year
Finland	—	1689	Welders in four machine shops and five shipyards employed >1 year; controls are plate machinists and pipefitters
France	440	600	Welders in four factories employed at beginning of 1976; about three age matched controls from same factories per welder
Germany, Federal Republic of	1221	—	Welders in 25 factories employed before 1970
Italy	(600)	(250)	Welders in two factories
Sweden	234	208	Welders in eight factories identified by colleagues as employed >5 years
UK	(600)	(600)	Welders in 18 (perhaps up to 25) factories employed >5 years before 1970
Total	6323	5387	

[a] In parentheses, estimates

(ii) *Exposure to vinyl chloride monomer* (Dr L. Simonato, Dr R. Saracci, Ms R. Winkelmann and Ms N. Charnay)

A group of epidemiologists involved in studies on vinyl chloride monomer met in Lyon in February 1987 to assess the feasibility of pooling the European studies on vinyl chloride workers. In view of the possible contribution from existing data bases to understanding the effects of this well-known carcinogen that are still to be clarified (i.e., sites at risk other than liver, characteristics of dose-response relationship, it was decided to undertake a multicentre cohort study under the coordination of the Agency. Some characteristics of the study population are shown in Table 6.

Table 6. Study population of workers exposed to vinyl chloride (VC) monomer[a]

Country	Type of production	No. of plants	Production starts (year)	Size of population	End of follow-up (year)
France	VC production and polymerization	12	NA	1 100	1988
Germany, Federal Republic of, and Austria	VC production and PVC[b] processing	NA	NA	7 021	NA
Italy	Mainly polymerization	9	1956–1972	5 491	1983–1984
Norway	VC production and polymerization	1	1951	1 200	1984
Spain	VC production and PVC processing	1	1970	700	1984
Sweden	VC production and polymerization	1	1940	771	1971
	PVC processing	4	1945	1 970	1974
UK	Polymerization	9	1940	7 000	On-going
Total		37		29 260	

[a] NA, not available

[b] Polyvinyl chloride

The data should be received at the Agency by the end of 1987. Processing will take place in the first half of 1988, and the analysis will be performed in the second half of the same year.

Within the framework of the study, the feasibility of involving laboratory research has been explored. Dr A. Barbin from the Unit of Environmental Carcinogens and Host Factors is developing a method for measuring vinyl chloride metabolites and DNA base adducts excreted in the urine of exposed persons. This method could be applied to a sample of the study population. The possibility of collecting tissue samples from cases of liver angiosarcoma for the purpose of studying oncogene activation will also be explored.

(iii) *Exposure to styrene* (Dr L. Simonato, Dr R. Saracci and Ms R. Winkelmann)

The Occupational Cancer Programme is exploring the feasibility of conducting a multicentre cohort study in Europe on workers exposed to styrene. Some preliminary estimates of the study population characteristics are presented in Table 7. A meeting of the potential collaborators will be organized at the Agency before the end of the year in collaboration with the Commission of the European Communities.

Table 7. Study population of workers exposed to styrene[a]

Country	Type of production production[b]	No. of plants	Production start (year)	Size of population	End of follow-up (year)
Denmark	GRP	100–150	1950	NA	1986
Finland	GRP	160	–1960	2 209	On-going
Germany, Federal Republic of	Production and polymerization	1	1931	1 960	1975
Italy	Styrene–butadiene	1	NA	NA	NA
Norway	GRP	NA	NA	NA	NA
Sweden	GRP	16	1950–1975	~1 500	1976
UK	Production and polymerization	1	1940	624	1978
	GRP	51	1950	1 897	On-going
	GRP	8	1953–1968	5 434	1984
Total		338–388		13 624	

[a] NA, not available
[b] GRP, glass-reinforced plastics

(iv) *Exposure to dioxin-contaminated substances* (Dr R. Saracci and Dr E. Johnson)

The Agency has established an international register of persons occupationally exposed to phenoxyacetic acid herbicides and chlorophenols. These herbicides are likely to be contaminated with dioxins, including 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD). The aim of the register is to assemble cohorts of exposed workers to be followed up for mortality and morbidity, particularly cancer incidence, in order to investigate the possible health effects of exposure to these herbicides. The cohorts have been enrolled in the register from several countries in Europe, North America, Australia and New Zealand and are of two main types: (1) workers exposed during the production and formulation of the herbicides and (2) workers exposed during the use of these substances, for example, sprayers. The register is near completion and will ultimately include over 20 000 workers. The editing of the data base is in progress as well as planning of investigations on mortality and cancer incidence.

(v) *International study of cancer risk in biological laboratory research workers* (Dr A.J. Sasco, Dr C.S. Muir and Dr R. Saracci)

The recent occurrence of five cases of rare cancers at the Pasteur Institute in Paris led in May 1986 to the setting up of a commission of enquiry under the chairmanship of Professor Jean Bernard; the Agency was invited to participate in the work of the Commission. Subsequently, a sixth case has come to light. Almost immediately, it became obvious that any relationship between laboratory work and specific cancers would be hard to establish with certainty, and impossible to refute, if only one research centre were studied. The Agency was therefore asked to explore the possibility of conducting an international collaborative study in a number of research centres around the world.

A preliminary meeting was held at the Agency in February 1987 to discuss the feasibility of such a study. It was attended by 16 outside participants, representing eight countries (Canada, the Federal Republic of Germany, France, Italy, the Netherlands, Sweden, the UK and the

USA). Three other countries (Denmark, Finland, Israel) were not able to send representatives to the meeting but expressed interest.

The objectives of the proposed study are to assess for biology research workers any possible excess cancer mortality and/or incidence, overall and site-specific, with particular attention to histology and age at cancer occurrence. Emphasis will be placed on exposures deriving from work in the new field of biotechnology (molecular biology, recombinant DNA genetics), but this emphasis does not exclude exposures to other agents (chemical carcinogens, mutagens, radiation) also present in the laboratory setting, the effects of which on human health (under the conditions of laboratory work) have not been fully evaluated. The study will be limited to public research laboratories of sufficient size that have been in existence for at least ten years. An effort will be made to separate out subcohorts based on type of scientific activity and unexposed persons.

As a first step, a retrospective approach is used. Data sources can be identified from information provided at the meeting, and a retrospective study will be conducted in a reasonable amount of time (two years) in Denmark, Finland, Sweden and the UK, if funds can be secured. Elsewhere, while the retrospective approach would be feasible, cohort assembly might be slow and expensive. Nonetheless, in most countries, cohorts could be established now and followed up prospectively. Once cases are identified, a case-control study nested within the cohort can provide an efficient design for better information on exposures. Mortality appears the most easily accessible outcome, although problems exist for using such an approach in the Federal Republic of Germany and in France. Incidence may be used in the UK, Canada and the Nordic countries.

The second part of 1987 and the beginning of 1988 will be devoted to a detailed feasibility study, with definition of the study protocol for the retrospective cohort study (identification procedures, ways of classifying subcohorts, and ways of assessing outcome).

(vi) *Occupational cancer review* (Dr L. Simonato, Dr R. Saracci and Mrs A. Zitouni)

Published studies investigating carcinogenic risk in the occupational environment are collected on a continuous basis using a computerized system. A list of occupations and industries involving increased risk of cancer is updated regularly.

(c) *Smoking and cancer*

(i) *Passive smoking and respiratory cancers* (Dr E. Riboli, Dr R. Saracci; in collaboration with Dr S. Preston-Martin, Department of Preventive and Family Medicine, University of Southern California, Los Angeles, CA, USA; Dr N.J. Haley, Clinical Biochemistry, Naylor Dana Institute for Disease Prevention, American Health Foundation, New York, NY, USA, DEB/85/01; Dr E.T.H. Fontham, Pathology Department, Louisiana State University Medical Center, New Orleans, LA, USA, DEB/85/03; Dr Y.T. Gao, Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China; Dr S.K. Jindal, Department of Chest Diseases, Postgraduate Institute of Medical Education and Research, Chandigarh, India; Dr L. LeMarchand, Cancer Center of Hawaii, University of Hawaii, Honolulu, HI, USA; Dr L.C. Koo, Department of Community Medicine, University of Hong Kong, Hong Kong, DEB/85/12; Dr J.D. Burch, Epidemiology Unit, National Cancer Institute of Canada, Toronto, Ontario, Canada, DEB/85/18; Dr N. Segnan, Unit of Epidemiology, Health Office, Turin, Italy, DEB/85/04; Dr H. Shimizu, Tohoku University School of Medicine, Sendai, Japan; Dr G. Stanta, Institute of Pathology, Trieste, Italy, DEB/85/22;

Dr D. Trichopoulos, Department of Hygiene and Epidemiology, School of Medicine, Athens, DEB/85/02; Dr A. Wu, Department of Preventive and Family Medicine, University of Southern California, Los Angeles, CA, USA, DEB/85/11; Dr W. Zatonski, Unit of Epidemiology, Institute of Oncology, Warsaw, DEB/85/13; and Dr H. Becher, Bremen Institute for Research in Preventive and Social Medicine, Bremen, Federal Republic of Germany, DEB/85/38)

The programme on passive smoking was started following a meeting of an ad-hoc working group, which advised that, first, methodological investigations be developed to improve measurement of exposure to environmental tobacco smoke (ETS) and, as a second step, multicentre epidemiological studies be instituted to investigate the relation between passive smoking and respiratory cancer.

A collaborative methodological study was started in 1985 in 13 centres located in ten countries (Canada, China, The Federal Republic of Germany, Greece, Hong Kong, India, Italy, Japan, Poland and the USA). The aims of the study are:

(1) to investigate among healthy nonsmokers the pattern of exposure to ETS, with particular emphasis on the different sources, places and times of exposure;

(2) to explore the relations(s) of urinary cotinine/creatinine and thiocyanate/creatinine ratios with self-reported recent exposure; and

(3) to explore variations of (1) and (2) in different countries.

Data collection was completed towards the end of 1986, and analysis of data from all the centres is now being carried out at the Agency. A total of 1400 subjects were interviewed and gave urine samples. Subject selection was planned in order that there be about 100 subjects per centre, half of them married to a smoker and half to a nonsmoker. Where possible, these two subgroups were then subdivided into women going out to work and those staying at home[13]. The statistical analysis will focus on the one hand on comparisons of indicators of exposure derived from the different sections of the questionnaire, and on the other hand on the relation between quantitative indexes of exposure (duration, number of cigarettes, indoor air volume) and urinary cotinine levels.

The analyses conducted so far indicate that, in general, there is a fairly good correlation between biological markers and recent exposure both in terms of duration (time spent in a smoky environment) and intensity (number of cigarettes smoked in the presence of the subject). The comparison between individual exposure as derived form very general questions (i.e., Do you live with a smoker? Do you work with smokers?) *versus* the exposure that can be estimated from detailed questions on ETS at home, at work, in vehicles and in public places suggests that crude indicators (as used in previous studies) can lead to substantial misclassification of exposure to ETS. This study provides valuable information and practical tools for improving the measurement of exposure to ETS in future epidemiological studies.

At present, contact is being made with potential collaborators for an international study on lung cancer in nonsmokers, to be started during 1988 with a first group of collaborating centres.

(ii) *Smoking habits of French adolescents* (Dr A.J. Sasco; in collaboration with Ms M. Jambon, Association de Lutte Etudiante contre le Cancer, Lyon, France)

A study is being carried out to evaluate the smoking habits of school children between the ages of 11 and 18. A total of 2600 pupils aged 11–15 in 16 representative lower secondary schools

[13] Riboli, E. (1987) *Toxicol. Lett., 35,* 19–27

in Lyon and the surrounding area answered a detailed questionnaire describing their smoking habits, their reasons for smoking and their attitudes towards health education. The same type of information will now be sought from pupils at upper secondary schools (aged 15–18 years).

(iii) *Tobacco smoking interactions* (Dr R. Saracci)

A review of published studies on the main agents that interact with tobacco smoking in cancer etiology (asbestos, radon, nickel, arsenic, chloromethylether, aromatic amines, alcohol) has been prepared, outlining the implications for public health and for the study of interactions in general[14].

(*d*) *Second malignancies following chemotherapy* (Dr J. Kaldor, Dr N.E. Day, Mrs A. Arslan and Mrs B. Kajo; in collaboration with Dr P. Band, Cancer Control Agency of British Columbia, Vancouver, BC, Canada; Dr R. Cartwright, Yorkshire Regional Cancer Organization, Leeds, UK: Professor N.W. Choi, Manitoba Cancer Treatment and Research Foundation, Winnipeg, Manitoba, Canada; Dr E.A. Clarke and Dr N. Kreiger, Ontario Cancer Treatment and Research Foundation, Toronto, Ontario, Canada; Dr J. Cuzick, Imperial Cancer Research Fund, London; Dr N.E. Day, Medical Research Council, Cambridge, UK (from September 1986); Mr A. Decker and Dr Z. Péter, National Institute of Oncology, Budapest; Dr M. Fiorentino, Padua Civic Hospital, Padua, Italy; Dr P. Fraser, London School of Hygiene and Tropical Medicine, London; Dr P. Ghadirian, Cancer Institute of Montreal, Quebec, Canada; Dr M. Hakama and Dr S. Karjalainen, Finnish Cancer Registry, Helsinki; Dr M. Henry-Amar, Gustave Roussy Institute, Villejuif, France; Dr M. Koch, Cancer Registry, Edmonton, Alberta, Canada; Dr F. Langmark, Norwegian Cancer Registry, Oslo, Dr W.H. Mehnert, National Cancer Registry, Berlin-Johannisthal; Dr F. Neal, Weston Park Hospital, Sheffield, UK: Dr F. Petterson, Karolinska Hospital, Stockholm; Dr R. Pfeiffer, University Clinic, Essen, Federal Republic of Germany; Dr I. Pleško, Cancer Research Institute, Bratislava, Czechoslovakia; Dr V. Pompe-Kirn, Cancer Registry of Slovenia, Ljubljana, Yugoslavia; Dr P. Prior, Birmingham Cancer Registry, Birmingham, UK: Dr S. Smith and Dr M. Stovall, University of Texas, Houston, TX, USA; Dr H.H. Storm, Danish Cancer Registry)

The international collaborative group studying second cancer in relation to cytostatic therapy[15] has completed the first phase of its work — a cohort study of subsequent cancer incidence in patients already diagnosed with testicular cancer, ovarian cancer or Hodgkin's disease. Eleven population-based cancer registries tabulated observed and expected numbers, based on general population rates, among 130 000 individuals who were followed up for over 560 000 person-years. Table 8 gives the number of person-years of follow-up, broken down by time since the diagnosis of the first cancer. The overall relative risks for second cancer were 1.3, 1.2 and 1.8 for the three index cancers, respectively.

Table 9 gives relative risks for the second cancer sites that were prominently in excess. As expected, leukaemia, particularly of the acute and nonlymphocytic subtypes, following Hodgkin's disease and ovarian cancer was the most striking excess, and the fact that it could be detected so clearly in this study was a confirmation of the utility of cancer registries in work of this kind. The excess of non-Hodgkin's lymphoma following Hodgkin's disease was also

[14] Saracci, R. (1987) *Epidemiol. Rev., 9* (in press)

[15] Kaldor, J., Day, N.E., Band, P., Choi, N.W., Clarke, E.A., Coleman, M.P., Hakama, M., Koch, M., Langmark, F., Neal, F.E., Petterson, F., Pompe-Kirn, V., Prior, P. & Storm, H.H. (1987) *Int. J. Cancer, 39,* 571–585

Table 8. Person–years at total risk for developing a second primary cancer, by site of first cancer and time since its diagnosis

First cancer	Time since diagnosis of the first cancer (years)								
	<1	1–4	5–9	10–14	15–19	20–24	25–29	30+	Total[a]
Testicular cancer	11 598	34 029	25 986	14 581	7 079	2 532	762	122	124 801
Ovarian cancer	54 458	99 190	61 289	31 423	13 893	3 995	846	104	302 385
Hodgkin's disease									
Males	11 718	28 819	17 656	7 598	2 914	609	138	18	78 139
Females	8 114	20 976	13 987	6 494	2 429	625	217	46	59 262

[a] Subtotals do not include Denmark, whereas totals do.

consistent with previous observations. Lung cancer following Hodgkin's disease had been reported earlier, but it has never been so clearly documented as in this study. Other possibly treatment-related excesses were of bladder cancer following ovarian cancer, nonmelanoma skin cancer following Hodgkin's disease, cancer of the bone following Hodgkin's disease (in women) and cancer of the connective tissue following ovarian cancer.

In addition to therapy for the first cancer, overdiagnosis, misclassification of metastases, misdiagnosis of the first tumour, confounding by other risk factors, and the role of the first cancer itself must be considered as possible explanations for risks of second cancers occurring in excess of the expectation based on general population rates.

Table 9. Elevated relative risks of second cancer (observed number of cases)

Second cancer	First cancer			
	Testicular cancer	Ovarian cancer	Hodgkin's disease	
			Males	Females
Leukaemia	1.7 (18)*	2.5 (94)**	10.3 (69)**	10.9 (37)**
Acute leukaemia	2.0 (9)	4.1 (68)**	17.5 (53)**	15.8 (27)**
Non-Hodgkin's lymphoma	2.7 (28)**	1.3 (45)	3.0 (15)**	3.1 (9)**
Bladder cancer	1.1 (30)	1.7 (75)**	1.2 (19)	2.2 (8)
Lung cancer	1.0 (75)	1.2 (104)	1.9 (89)**	2.2 (17)**
Connective tissue cancer	2.0 (7)*	3.1 (25)**	0.7 (1)	3.6 (3)
Bone cancer	0.8 (1)	2.5 (7)*	1.3 (1)	10.6 (4)**
Nonmelanoma skin cancer	1.3 (82)	1.1 (125)	2.3 (37)**	2.1 (19)**

* $p < 0.05$

** $p < 0.01$

Table 10. Number of second cancer cases abstracted as of 30 June, 1987

First cancer	Second cancer	No. of cases
Hodgkin's disease	Leukaemia	76
	Lung	73
Ovarian cancer	Leukaemia	58
	Bladder	38
Testicular cancer	Leukaemia	8
	Bladder	25

The collaborative group, which, in addition to the 11 population-based registries who participated in the cohort study, includes several major European hospitals and some further registries, has nearly completed data collection for a series of case-control studies. The primary goal of these studies is to answer specific questions on the role of chemotherapy in the etiology of second cancers. Table 10 shows which second cancer sites are being considered, and how many cases had been registered for the study as of 30 June 1987. For each case and three controls, matched by age, year of diagnosis, site of first cancer and survival time to the case, detailed abstraction therapy records for the first cancer is taking place. The name of each drug, the duration, dosage and intensity of treatment, and the route by which it was administered are noted. Organ radiation doses are being estimated from the radiotherapy records of each patient. For the leukaemia cases and matched controls, information on bone-marrow toxicity resulting from chemotherapy for the first cancer is also being abstracted.

The statistical analysis of these data is expected to begin in July 1987. The analyses will compare the carcinogenic potency of different drugs, which is already known to vary be several orders of magnitude[16], examine the effect of treatment, intensity and dose, and examine temporal factors such as the age at which exposure occurs, and the duration of risk following the start and cessation of therapy.

One of the participants in the collaborative group, the Cancer Registry of the German Democratic Republic, has completed and published the results of a case-control study of leukaemia following breast and ovarian cancer[17,18]. Because cyclophosphamide was used almost exclusively as a chemotherapeutic agent in the treatment of these tumours during the study period, it was possible to estimate its leukemogenic effect independently of that of other cytostatic agents. Table 11 is a summary of treatment details for cases and controls in the study, and Table 12 shows the relationship between total dose of cyclophosphamide and relative risk of leukaemia.

In conjunction with the German Cancer Research Centre, a meeting was held in November 1985 in Heidelberg, to bring together clinicians, laboratory scientists, and epidemiologists concerned by the problem of chemotherapy-induced malignancies. The proceedings of the meeting were edited and published as an IARC Scientific Publication[19].

[16] Kaldor, J., Day, N.E. & Hemminki, K. (Submitted for publication)

[17] Haas, J.F., Kittlemann, B., Mehnert, W.H., Staneczek, W., Möhner, M., Kaldor, J. & Day, N.E. (1987) *Br. J. Cancer, 55*, 213–218

[18] Mehnert, W.H., Haas, J.F., Kittlemann, B., Staneczek, W., Möhner, M., Kaldor, J. & Day, N.E. (1986) In: Schmähl, D. & Kaldor, J., eds, *Carcinogenicity of Alkylating Cytostatic Drugs (IARC Scientific Publications No. 78)*, Lyon, International Agency for Research on Cancer, pp. 203–221

[19] Schmähl, D. & Kaldor, J., eds (1986) *Carcinogenicity of Alkylating Cytostatic Drugs (IARC Scientific Publications No. 78)*, Lyon, International Agency for Research on Cancer

Table 11. Leukaemia following ovarian and breast cancer in the German Democratic Republic

	Ovarian cancer	Breast cancer
Number of cases of		
Second leukaemia	12	93
Chemotherapy (%)	83	17
Number of Controls	48	185
Chemotherapy (%)	31	11
Relative risk of leukaemia associated with		
Chemotherapy	15.7	1.7
Cyclophosphamide	7.6	1.3

Further studies of the long-term effects of chemotherapy are being planned in collaboration with clinical centres participating in the European Organization for Research and Treatment of Cancer. In addition to continuing the investigation of the relationship between risk of second cancer and specific aspects of therapy, these studies will explore the role of short-term measures of drug toxicity, such as myelosuppression and chromosomal rearrangements, in predicting long-term risk.

Table 12. Leukaemia following cyclophosphamide

Total dose (g)	Ovarian cancer		Breast cancer		Combined relative risk
	Leukaemia cases	Matched controls	Leukaemia cases	Matched controls	
0	1	23	56	132	1.0
<10	3	3	6	15	1.5
10–29	3	3	0	2	3.3
30+	2	6	3	1	7.3
Unknown	1	1	4	1	10.9

(*e*) *Collaborative studies on in-vivo formation of N-nitroso compounds in human subjects* (Dr H. Ohshima, Dr B. Pignatelli, Dr G. Maru, Dr J. Nair, Mr C. Malaveille, Dr M. Friesen, Miss M.-C. Bourgade, Miss S. Calmels, Mrs I. Brouet, Mrs F. Ciroussel, Dr N. Muñoz, Dr J. Kaldor and Dr H. Bartsch; in collaboration with external institutions as cited below)

Following initial feasibility studies, eight investigations on endogenous formation of *N*-nitroso compounds (NOC) in humans are currently under way.

(i) *Precancerous lesions of the stomach* (in collaboration with Professor M. Crespi, Dr V. Casale and Dr V. Ramazotti, Regina Elena Institute, Rome; and Dr H. Leclerc, National Institute for Health and Medical Research, Villeneuve d'Ascq, France)

Subjects included in these studies are patients: (1) with chronic atrophic gastritis with and without intestinal metaplasia; (2) with pernicious anaemia; and (3) who have undergone partial gastrectomy. The endpoints analysed include gastroscopy, collection of fasting gastric juice, histopathological evaluation of biopsy samples and the measurement of nitrosated amino acids in urine after application of the *N*-nitrosoproline (NPRO) test.

Results[20] on patient group 1 indicate that gastric juices collected from these patients have higher pH values, more bacteria and higher concentrations of nitrite than those of control subjects. However, patients, as compared to healthy controls, excreted no apparent excess of NPRO. The urinary level of NPRO was dependent of gastric pH, and maximal yields were seen at about pH 2. There was no correlation between bacterial count and urinary NPRO level. Bacterial strains isolated from the gastric juice of patients with chronic atrophic gastritis were found to form nitrosamines *in vitro* at pH 7 from precursors[21].

Because of the inherent limitations of the NPRO test to measure nitrosation at neutral pH, and as recent applications of several methods for group determination of total NOC gave rise to contradictory findings, a rapid and potentially more reliable analytical method for total NOC in gastric juice has been developed. This procedure is now being applied for the analyses of gastric juice samples collected from subjects with precancerous conditions (see III.3.*h*.iii), together with measurement of DNA damage in the gastric mucosa, determined in biopsies taken for diagnostic purposes from the same patient.

(ii) *Study in high- and low-incidence areas for gastric cancer in Japan*[22] (in collaboration with Professor S. Kamiyama, Akita University, School of Medicine, Akita, Japan; and Dr W. Zatonski, Institute of Oncology, Warsaw)

Three different samples of 24-h urine were collected from each of 104 inhabitants of high-risk (Akita) and low-risk (Iwate) areas for stomach cancer in northern Japan, according to the following protocols: (1) when they were undosed, (2) after ingestion of proline three times a day and (3) after ingestion of proline together with vitamin C, three times a day. These samples were analysed for *N*-nitrosamino acids, nitrate and chloride ions as indices of the exposure. The median values of NPRO and *N*-nitroso-2-methylthiazolidine 4-carboxylic acid (NMTCA) excreted in the urine of undosed subjects were not different between the two areas; however, that of *N*-nitrosothiazolidine 4-carboxylic acid (NTCA) was significantly higher in subjects from the high-risk area. Salt intake, estimated from the level of chloride in the urine, did not differ in the two areas. After intake of proline, the NPRO level increased significantly only in subjects from the high-risk area, but not in those in the low-risk area; intake of vitamin C inhibited this increase of NPRO and lowered the levels of other nitrosamino acids only in the high-risk subjects. In contrast, the urinary level of nitrate was higher in subjects from the low-risk area, compared with those of the high-risk area; nitrate levels were found to correlate well with the amounts of vegetables consumed.

These results indicate that, although nitrate intake by subjects in the high-risk area is lower, their potential for intragastric nitrosation is higher, suggesting the occurrence of some inhibitory factors for nitrosation in the diet of inhabitants of the low-risk area. These data also show that the determination of nitrate/nitrite in saliva, urine and gastric juice is not sufficient to predict the complex nitrosation reaction occurring in the human body. Data from a similar study, now

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[20] Crespi, M., Ohshima, H., Ramazotti, V., Muñoz, N., Grassi, A. Casale, V., Leclerc, N., Calmels, S., Cattoen, C., Kaldor, J. & Bartsch H. (1987) In: Bartsch, H., O'Neill, I. & Schulte-Hermann, R., eds, *Relevance of N-Nitroso Compounds to Human Cancer: Exposure and Mechanisms* (*IARC Scientific Publications No. 84*), Lyon, International Agency for Research on Cancer, pp. 511–517

[21] Calmels, S., Ohshima, H., Crespi, M., Leclerc, H., Catteon, C. & Bartsch, H. (1987) In: Bartsch, H., O'Neill, I. & Schulte-Hermann, R., eds, *Relevance of N-Nitroso Compounds to Human Cancer: Exposure and Mechanisms* (*IARC Scientific Publications No. 84*), Lyon, International Agency for Research on Cancer, pp. 391–395

[22] Kamiyama, S., Ohshima, H., Shimada, A., Saito, N., Bourgade, M.-C., Ziegler, P. & Bartsch, H. (1987) In: Bartsch, H., O'Neill, I. & Shulte-Hermann, R., eds, *Relevance of N-Nitroso Compounds to Human Cancer: Exposure and Mechanisms* (*IARC Scientific Publications No. 84*), Lyon, International Agency for Research on Cancer, pp. 497–502

completed conducted on inhabitants of rural and urban areas of Poland, are currently under statistical evaluation.

(iii) *Correlation study on urinary excretion on* N-*nitroso compounds and cancer mortality in China*

Following a recently completed study[23] in Lin-xian and Fan-xian, high- and low-risk areas for oesophageal cancer in northern China, urine samples were collected from about 1000 subjects in 26 counties all over China, selected on the basis of mortality rates from cancers of the oesophagus, stomach and liver. The urine samples were analysed for nitrate/nitrite, *N*-nitrosamino acids and thioethers[24]. Samples of 12-h overnight urine were collected from approximately 40 male adults in each of the 26 counties. Two urine specimens were collected from each subject, one after a loading dose of proline and ascorbic acid, another after a loading dose of proline only. *N*-Nitrosamino acids and nitrite/nitrate in pooled urine samples were analysed and correlated with cancer mortality per 100 000 male subjects in the truncated age range 35–64. Preliminary results show no clear correlation of stomach cancer or liver cancer with nitrosation potential (as measured by urinary level or NPRO after the proline load test or of nitrate). For oesophageal cancer, there was a trend for mortality rates to be associated positively with nitrosation potential and negatively with background ascorbate levels present in plasma. These results further support the hypothesis that exposure to endogenously formed *N*-nitroso compounds may be associated with increased risks of cancers at certain sites, but the compounds involved remain to be identified. Collaborative studies are planned.

(iv) *Studies on betel-quid chewers and on betel-quid ingredients* (in collaboration with Dr S.V. Bhide, Cancer Research Institute, Tata Memorial Center, Bombay, India; and Dr U. Mohr, Hanover Medical School, Hanover, Federal Republic of Germany)

(1) In order to study whether tobacco-specific nitrosamines (TSNA) and areca nut-specific nitrosamines (ASNA) are formed *in vivo* in the oral cavity during the chewing of betel quid, quids supplemented with proline or with proline and ascorbic acid were chewed by volunteers, and the saliva samples were analysed for NPRO, TSNA and ASNA[25].

When results were expressed as a ratio of NPRO (ng/ml) to nicotine (mg/ml), all chewers of betel quid with tobacco (BQT) had an increased NPRO content after chewing BQT with proline. In chewers of betel quid without tobacco (BQ), when the results were expressed as a ratio of NPRO (ng/ml) to arecoline (mg/ml), a similar increase in NPRO content was observed. These findings suggest that *N*-nitroso compounds are formed in the oral cavity during chewing of BQT or BQ. The presence of ascorbic acid inhibited the increased nitrosation of proline in only four out of ten BQT chewers and five out of ten BQ chewers, while in the rest of the samples its presence enhanced the levels of NPRO. These results are in good agreement with our previous data[26], in which certain nitrosamines were shown to be formed in the saliva while chewing BQT/BQ.

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[23] Lu, S.H., Ohshima, H., Fu, H.M., Tian, Y., Li, F.M., Blettner, M., Wahrendorf, J. & Bartsch, H. (1986) *Cancer Res., 46,* 1485–1491

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[25] Nair, J., Ohshima, H., Pignatelli, B., Friesen, M., Malaveille, C., Calmels, S. & Bartsch, H. (1986) In: Hoffmann, D. & Harris, C.C., eds, *New Aspects of Tobacco Carcinogenesis* (*Banbury Report 23*), Cold Spring Harbor, NY, CSH Press, pp. 45–61

[26] Nair, J., Ohshima, H., Friesen, M., Croisy, A. Bhide, S.V. & Bartsch, H. (1985) *Carcinogenesis, 6,* 295–303

(2) A long-term bioassay on hamsters[27] fed areca-nut powder with or without nitrite in the drinking-water has been evaluated histologically. Results showed a higher incidence of lymphomas in males treated with areca nut plus nitrite. Urine analyses of the latter treatment group revealed the presence of *N*-nitrosonipecotic acid, a major urinary metabolite of ASNA, indicating that areca nut alkaloids undergo nitrosation *in vivo*. One such nitrosation product, 3-(methylnitrosamino)propionitrile, occurs in the saliva of betel-quid chewers and is a powerful alkylating carcinogen in rats[28].

(3) *Effects of areca-nut extract and related compounds in cultured human buccal cells*[29] (Dr J. Nair and Dr H. Bartsch; in collaboration with Dr K. Sundqvist, Dr J.M. Dypbukt and Dr R.C. Grafström, Karolinska Institute, Stockholm)

Effects of aqueous areca-nut extract and several areca nut-specific alkaloids and *N*-nitroso compounds were investigated in cultured human buccal epithelial cells and fibroblasts. The extract decreased both colony forming efficiency and clonal growth rate of epithelial cells to less than 50% at 10 mg/ml. Exposure to higher concentrations also caused both dose-dependent depletion of thiols and formation of DNA single-strand breaks. Of eight betel nut-associated compounds investigated, 3-(methylnitrosamino)propionaldehyde was the most potent on a molar basis and significantly decreased both cellular survival and thiol content and also caused DNA damage in buccal cells at concentrations between 0.1 and 0.3 mM. More than ten-fold higher concentrations of arecoline, guvacoline of *N*-nitrosoguvacoline were required to cause similar effects. Arecaidine, guvacine, *N*-nitrosoguvacine or 3-(*N*-nitrosomethylamino) propionitrile up to 6 mM did not affect the cells. The induction of cytotoxic and genetoxic effects by extracts of several betel nut-specific compounds may be of importance for understanding the relationship between betel chewing and carcinogenesis in the human buccal epithelium and for identifying the active constitutents involved.

(4) *Formation of reactive oxygen species and of 8-hydroxydeoxyguanosine in DNA* in vitro *with betel-quid ingredients* (Dr U. Nair, Dr J. Nair, Mrs V. Bussachini-Griot and Dr M. Friesen; in collaboration with Mr R.A. Floyd, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA)

The formation of reactive oxygen species from betel-quid ingredients (areca nut, catechu and tobacco) was studied using a chemiluminescence technique[30]. Aqueous extracts of areca nut and catechu were capable of generating superoxide anion and hydrogen peroxide at pHs greater than 9.5. The formation of O_2^- was enhanced by Fe^{2+}, Fe^{3+} and Cu^{2+} but inhibited by Mn^{2+}. Tobacco extract failed to generate reactive oxygen species under similar conditions. Saliva was found to inhibit both O_2^- and hydrogen peroxide formation from betel-quid ingredients. Upon incubation of DNA at alkaline pH with areca nut extract and Fe^{3+} or catechu, 8-hydroxydeoxyguanosine was formed.

To investigate the significance of oxidative DNA damage in the etiology of oral cancer in betel-quid chewers, the nature of DNA base damage resulting from such oxidative attack is now being studied in exfoliated buccal epithelial cells of chewers of BQ and BQT. For this purpose, a high-performance liquid chromatography (HPLC) method using electrochemical detection is being used to measure 8-hydroxydeoxyguanosine in DNA as a measure of oxidative damage by reactive oxygen species such as O_2^-, $^{\cdot}OH$ or hydrogen peroxide.

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[30] Nair, U., Floyd, R.A., Nair, J., Bussachini, V., Friesen, M. & Bartsch, H. (1987) *Chem.-biol. Interact.* (in press)

(5) *DNA damage as a marker of exposure to betel quid/tobacco* (Dr G. Maru, Dr M. Friesen, Mr C. Malaveille, Dr H. Ohshima, Dr J. Kaldor and Dr H. Bartsch; in collaboration with Dr S.V. Bhide and Dr J. Nair, Cancer Research Institute, Tata Memorial Center, Bombay, India; Dr R.C. Grafström and Dr K. Sundqvist, Karolinska Institute, Stockholm; and Dr G. Obe, Free University of Berlin, Berlin (West), Federal Republic of Germany; supported by NIH Grant 1U01 CA 43176-01)

With the aim of developing, validating and applying methods for detecting DNA damage as biolgical markers of carcinogen exposure in chewers of BQT, a collaborative programme has been initiated in which DNA from in-vitro reactions with TSNA, ASNA, areca-nut alkaloids and aqueous extracts of tobacco/areca-nut, from the tissues of treated animals (rats and hamsters), from cultured human buccal epithelial cells/explants treated *in vitro* and from exfoliated buccal epithelial cells from habitual chewers of BQ, BQT and tobacco and from nonchewers/ nonsmokers, will be analysed for carcinogen-DNA adducts, oxidative DNA base damage, DNA strand breaks and micronuclei.

Tissues from treated animals and exfoliated buccal mucosa cells from about 97 volunteers with one of these habits have been collected and are being processed for isolation of DNA. Smears of exfoliated buccal epithelial cells from 23 volunteers with one of the habits are being processed for evaluation of frequency of micronuclei.

(v) *Studies on bladder cancer patients* (in collaboration with Dr P. Vincent, Croix-Rousse Hospital, Lyon, France; Dr N.M. El-Torkey, Cairo University, Cairo; and Dr M. Ramses, Tanta, Egypt)

Several epidemiological studies have suggested that infection of the urinary tract may be a risk factor for cancer of the bladder. Although nitrosamine formation *in vivo* in the bladder has been hypothesized to be associated with the risk, no extensive study has been carried out on the occurrence and formation of *N*-nitroso compounds in relation to the type and count of bacterial flora present in infected urine.

Therefore, urine samples from 31 patients with urinary-tract infections and from 31 controls were analysed for volatile nitrosamines, nitrosamino acids, total *N*-nitroso compounds as a group, and nitrite/nitrate[31]. The concentration of *N*-nitrosodimethylamine was significantly elevated in urines infected with *Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae*. The levels of nitrite, NPRO and total *N*-nitroso compounds, when expressed as the amount per mol creatinine, were also significantly increased in patients with bacteriuria. Twelve out of 14 isolated bacterial strains were capable of catalysing nitrosation of morpholine at neutral pH. These results suggest that *N*-nitroso compounds can be formed *in vivo* in infected bladders, which could explain the association between urinary-tract infections and increased risk for bladder cancer.

Bilharziasis, an endemic disease in the Nile Valley of Egypt, has been associated with an increased risk for bladder cancer in that area. In order to examine the hypothesis that bacterial flora in the urine contribute to the production of nitrite and carcinogenic *N*-nitrosamines, about 80 urine samples were collected from the following groups: (1) subjects with *Schistosoma haematobium* infection, (2) subjects with bacteriuria, (3) subjects with both bacteriuria and *S. haematobium* infection and (4) healthy control subjects. Samples were analysed for bacterial count, presence of *S. haematobium* ova, nitrate/nitite, volatile *N*-nitrosamines, *N*-nitrosamino

[31] Ohshima, H., Calmels, S., Pignatelli, B., Vincent, P. & Bartsch, H. (1987) In: Bartsch, H., O'Neill, I. & Schulte-Hermann, R., eds, *Relevance of N-Nitroso Compounds to Human Cancer: Exposure and Mechanisms (IARC Scientific Publications No. 84)*, Lyon, International Agency for Research on Cancer, pp. 384–390

acids and mutagens. Although the urine samples of patients infected with bacteria and *S. haematobium* contained higher levels of volatile nitrosamines, the results were not statistically significant. While the mutagenicity of the urines was not significantly different among the four groups, it correlated well with smoking status, revealing the confounding nature of smoking, which could not be controlled for in this study.

In a feasibility study, exfoliated urothelial cells were collected from subjects from the four groups defined above and evaluated for the presence of micronucleated cells (in collaboration with Dr H.F. Stich, Vancouver, BC, Canada). While the limited number of samples precluded any statistical analysis of the results, the practical use of this method was confirmed for use in future studies.

(vi) *Bacterial formation of N-nitrosamines* (Miss S. Calmels and Dr H. Ohshima; in collaboration with Dr H.S. Rosenkranz, Case Western Reserve University, Cleveland, OH, USA: Professor A.R. Gounot, Claude-Bernard University, Lyon, France; and Dr M. Chippaux, National Centre for Scientific Research, Marseille, France)

Catalysis of nitrosation by bacteria at neutral pH has been demonstrated unequivocally[32,33], but its biochemical properties and clinical relevance to humans have not been studied. Screening of bacteria isolated from human subjects for nitrosating activity has therefore been continued. Eleven out of 30 bacterial strains isolated from gastric juice collected from patients with chronic atrophic gastritis or duodenal ulcers exhibited nitrosating activity (range, 1–308 nmol *N*-nitrosomorpholine formed per mg protein per hour). Twelve out of 14 strains isolated from patients with urinary-tract infections were also shown to catalyse nitrosation of morpholine (range, 2–86 nmol *N*-nitrosomorpholine formed per mg protein per hour)

In order to characterize the mechanism of bacterial nitrosation further, the effect of culture conditions on the induction of nitrosating activity has been investigated in *E. coli*, *Proteus morganii* and *Pseudomonas aeruginosa*. In *E. coli* and *P. morganii* (1) the nitrosating activity was induced under anaerobiosis in the presence of nitrate, (2) the induction of both nitrosating and nitrate-reductase activities are possibly correlated, and (3), when nitrate was replaced by nitrite in the culture medium, there was no further induction of the nitrosating activity. In contrast, in *P. aeruginosa*, the nitrosating activity was induced by nitrite as well as by nitrate, suggesting that an additional pathway may be involved in the bacterial nitrosation[34]. The implication of nitrate-reductase in bacterial nitrosation was further confirmed by analysing defined *E. coli* mutants in which the structural genes of nitrate/nitrite reductase had been deleted. *E. coli nar GH1* strain, deficient for nitrate reductase, did not exhibit nitrosating activity, while the loss of NADH-, formate- or glucose-dependent nitrite reductase activities did not affect the ability of *E. coli* to catalyse nitrosation[35].

Work is in progress to characterize the mechanism of nitrate reductase-catalysed nitrosation and to identify nitrosatable amino substrates that show differences in substrate specificity for the nitrosation enzyme(s) in *E. coli*, *P. morganii* and *P. aeruginosa*.

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[32] Calmels, S., Ohshima, H., Vincent, P., Gounot, A.-M. & Bartsch, H. (1985) *Carcinogenesis, 6,* 911–915
[33] Leach, S., Challis, B., Cook, A., Hill, M. & Thompson, M. (1985) *Biochem. Soc. Trans., 249,* 5321
[34] Calmels, S., Ohshima, H., Rosenkranz, H. S., McCoy E. & Bartsch, H. (1987) *Carcinogenesis* (in press)
[35] Calmels, S., Ohshima, H. & Bartsch, H. (1987) (submitted for publication)

(vii) *Studies on liver cancer patients* (Dr H. Ohshima, Dr D. Shuker and Dr M. Parkin; in collaboration with Dr N. Habib, Paris Hospital, Villejuif, France; Dr S.H. Chan and Dr C.J. Oon, Department of Medicine, Signapore General Hospital, Singapore; and Dr P. Srivatanakul, National Cancer Institute, Bangkok)

Although hepatitis B infection and aflatoxins have been associated with cancer of the liver, exposure to *N*-nitrosamines (many of which are hepatocarcinogens in experimental animals) in connection with hepatitis B infection has not been studied in liver cancer patients. Recently, it was shown that activated macrophages can synthesize nitrate/nitrite and nitrosating agents, which can form nitrosamines[36,37]; such macrophage-mediated nitrosation reactions could be of importance in infection and inflammatory diseases.

In order to assess exposure to *N*-nitroso compounds from endogenous and exogenous sources, urine samples are being collected in Singapore and Thailand from (1) hepatitis B surface antigen (HBsAg) carriers with active hepatitis, (2) HBsAg carriers without active hepatitis and (3) controls. Samples will be analysed for nitrate and *N*-nitrosamino acids and for the presence of alkylated purines.

The association of hepatocellular carcinoma with cirrhosis is well recognized, although the etiological and pathological role of cirrhosis is not clear. Following our initial findings that the amounts of NPRO and NMTCA excreted by cirrhosis patients are about three-fold higher than those of controls, more urine samples are being collected from these patients. The NPRO test is being applied to determine whether more *N*-nitrosamines are formed endogenously.

(viii) *Identification of new nitrosamino acids in human urine* (Dr H. Ohshima, Dr M. Friesen, Mrs L. Garren, Miss M.-C. Bourgade and Dr H. Bartsch)

We reported previously the occurrence in human urine of NPRO and *N*-nitrososarcosine and, more recently, two sulphur-containing *N*-nitrosamino acids, NTCA and its 2-methyl derivative[38,39]. Three new *N*-nitrosamino acids have now been identified in human urine: 3-(*N*-nitroso-*N*-methylamino)propionic acid, *N*-nitrosoacetidine 2-carboxylic acid and *N*-nitrosotetrahydro-4*H*-1,3-thiazine 4-carboxylic acid.

(ix) *Identification of precursor amino compounds of directly-acting mutagenic N-nitroso compounds in food* (Dr H. Ohshima, Mrs I. Brouet, Mr C. Malaveille, Mrs A. Hautefeuille, Dr M. Friesen and Dr H. Bartsch)

Directly-acting *N*-nitroso compounds, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, have been reported to induce cancer in the glandular stomach of experimental animals. Human exposure to such compounds resulting from their formation in the stomach *in vivo* could be an important risk factor for stomach cancer. Several groups have reported that Japanese fish, soya sauce, pickled Chinese cabbage and fava beans yield directly-acting mutagens on treatment with nitrite. We recently found that a strong directly-acting mutagen is produced by nitrosation of various smoked fish products, the consumption of which has been associated with an increased

[36] Hibbs, J.B., Taintor, J.R. & Vavrin, Z. (1987) *Science, 235,* 473

[37] Stuehr, D.J. & Marletta, M.A. (1985) *Proc. natl Acad. Sci. USA, 82,* 7738

[38] Ohshima, H., O'Neill, I.K., Friesen, M., Bériziat, J.-C. & Bartsch, H. (1984) *J. Cancer Res. clin. Oncol., 108,* 121–128

[39] Nair, J., Ohshima, H., Pignatelli, B., Friesen, M., Malaveille, C., Calmels, S. & Bartsch, H. (1986) In: Hoffmann, D. & Harris, C.C., eds, *New Aspects of Tobacco Carcinogenesis* (*Banbury Report 23*), Cold Spring Harbor, NY, CSH Press, pp. 45–62

risk of stomach cancer in northern Europe. Isolation and structural identification of the responsible precursor amino compounds in these foods are underway.

(x) *Ninth international meeting on* N-*nitroso compounds: exposures, mechanisms and relevance to human cancer* (Dr H. Bartsch & Dr I. O'Neill)

The ninth international meeting on *N*-nitroso compounds was held in Baden, Austria, 1–5 September 1986, under the patronage of the Austrian Ministry of Health and Environmental Protection; it was organized by IARC and cosponsored by the National Cancer Institute of the USA and a number of industrial companies.

The meeting was attended by 200 participants from 24 countries, who presented papers and review lectures focusing on *N*-nitroso compounds and their precursors, as summarized in a meeting report[40]. The proceedings of this meeting were published in 1987 as *IARC Scientific Publications No. 84: Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms*, edited by H. Bartsch, I. O'Neill and R. Schulte-Hermann. The proceedings of the eighth meeting are available as *IARC Scientific Publications No. 57*. The next meeting will be held in September 1989 in Beijing, under the title '*N*-Nitroso Compounds, Mycotoxins and Tobacco Smoke: Relevance to Human Cancer'. It will be organized together with the Chinese Academy for Preventive Medicine and Medical Sciences.

(*f*) *Characterization of biologically active substances in complex mixtures of environmental origin: pyrolysis products of opium and their possible role in oesophageal cancer in Iran* (Dr M. Friesen, Dr I.K. O'Neill, Mr C. Malaveille, Mrs L. Garren, Mrs A. Hautefeuille, Dr N.E. Day and Dr H. Bartsch)

Previous epidemiological and laboratory studies[41–43] have indicated an association between the ingestion of opium pyrolysates, dietary deficiencies and a high incidence of oesophageal cancer in north-eastern Iran. Nine of the most abundant mutagenic compounds present in morphine pyrolysates have been characterized: all are heterocyclic substituted hydroxyphenanthrenes.

Mutagenicity in *Salmonella typhimurium* TA98 in the presence of rat liver homogenates ranged over four orders of magnitude, one of the compounds being 1000 times more active than benzo[*a*]pyrene. The compounds are converted by a rat-liver microsomal preparation into phenols and dihydrodiols, implicating arene oxides as the ultimate mutagens. Their formation and reaction were shown by trapping experiments *in vitro* with ethanethiol and subsequent characterization of the ethyl sulphide reaction products. Biological activity of the compounds is dependent on the structure of the heterocyclic ring, suggesting that carbocations, resonance-stabilized as quinone or quinimine methides, may be their ultimate reactive metabolites.

These studies, now terminated, lend additional support to the role of opium pyrolysate as an etiological factor in oesophageal cancer in north-eastern Iran.

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[40] Bartsch, H. & O'Neill, I.K. (1987) *Cancer Res.* (in press)

[41] Friesen, M., O'Neill, I.K., Malaveille, C., Garren, L., Hautefeuille, A., Cabral, J.R.P., Galendo, D., Lasne, C., Sala, M., Chouroulinkov, I., Mohr, U., Turusov, V., Day, N.E. & Bartsch, H. (1985) *Mutat. Res., 150,* 177–191

[42] Ghadirian, P., Stein, G.F., Gorodetzky, C., Roberfroid, M.B., Mahon, C.A.T., Bartsch, H. & Day, N.E. (1985) *Int. J. Cancer, 35,* 593–597

[43] Friesen, M., O'Neill, I.K., Malaveille, C., Garren, L., Hautefeuille, A. & Bartsch, H. (1987) *Carcinogenesis, 8,* 1423–1432

(g) *Ochratoxin A in relation to nephropathy and bladder cancer* (Dr M. Castegnaro, Mr J.-C. Bérézíat, Ms J. Michelon, Dr L. Broussolle and Dr H. Bartsch; in collaboration with Dr I.N. Chernozemsky, Dr T. Petkova and Dr I. Nikolov, Institute of Oncology, Sofia; and Dr J. Idle, St Mary's Hospital Medical School, London)

(i) *Field and clinical studies*

A symposium, held during the 14th UICC International Cancer Congress in Budapest (August 1986), summarized current evidence that ochratoxin A plays an etiological role in Balkan endemic nephropathy (BEN) and in tumours of the urinary tract (UST). A meeting report has been published[44].

The nephrotoxic mycotoxin ochratoxin A has been found in cereals produced and stored locally at higher levels in an area with high incidence of BEN and UST in the Vratza district of Bulgaria than in an area with lower incidences[45]. In a pilot study, it was found that 65% of the cereal and beans consumed by affected families in the high-incidence area was contaminated with ochratoxin A whereas only 19% of samples from unaffected households were so contaminated[44]. Further sampling and analysis are in progress to confirm these findings.

A total of 312 blood samples have been collected from people living in areas with and without BEN and UST and analysed for their content of ochratoxin A (Table 13)[46]. Patients had ochratoxin A in their serum more often and at higher levels than healthy persons from healthy families in affected or unaffected villages. These results support the results of food analyses.

A method for analysis of ochratoxin A and its 4-hydroxy metabolite in urine is being improved to achieve the extremely low detection limit required. Samples of urine have been collected from the same groups from whom blood samples were taken.

Many environmental carcinogens require oxidative metabolism by cytochrome P450-dependent monoxygenases to exert their carcinogenic or toxic properties. Use of debrisoquine as a probe drug to investigate the oxidative metabolic competence of individuals has thus been carried out on 691 subjects from the following groups: (1) healthy subjects from villages free of BEN and UST, (2) healthy subjects from villages affected by BEN and UST, (3) subjects with a positive diagnosis of BEN, and (4) subjects suspected of suffering from BEN. The results, presented in Table 14, confirm those of pilot study[47]: in the BEN-positive population, fewer subjects have impaired metabolism (metabolic ratio, >3), and more subjects have high oxidative capacity (metabolic ratio, <0.8) than the healthy population from BEN and non-BEN areas.

(ii) *Drug metabolism in rat strains phenotyped as poor and extensive metabolizers of debrisoquine and ochratoxin A* (Dr M. Castegnaro, Dr M. Ahotupa, Dr E. Hietanen, Dr P. Arvela, Mr C. Malaveille, Miss A.-M Camus and Mr L. Broussole)

Debrisoquine 4-hydroxylation *in vivo* has been proposed as a probe to assess individual oxidative drug metabolizing capacity and to a classify humans into poor metabolizers (PM) and extensive metabolizers (EM). PM appear to be at lower risk than the EM phenotype for certain types of environmental cancer. To understand the underlying mechanism, female rats of the DA

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[44] Castegnaro, M., Bartsch, H. & Chernozemsky, I. (1987) *Cancer Res., 47*, 3608–3609

[45] Petkova-Bocharova, T. & Castegnaro, M. (1985) *Food Addit. Contamin., 2*, 267–270

[46] Petkova-Bocharova, T., Chernozemsky, I. & Castegnaro, M. (1987) *Food Addit. Contamin.* (in press)

[47] Richtie, J.C., Crothers, M.J., Idle, L.R., Grieg, J.B., Connors, T.A., Nikolov, I.G. & Chernozemsky, I.N. (1983) In: Strahinie, S. & Stefanovie, V., eds, *Proceedings of the 5th Symposium on Endemic Balkan Nephropathy (Current Research in Endemic Balkan Nephropathy)*, Sofia, Institute of Oncology, pp. 23–27

Table 13. Ochratoxin A in blood samples from people living in areas with and without endemic nephropathy in Bulgaria

Group[a]		No. of persons assayed	Ochratoxin A-positive cases						Mean concentration ±SD
			Total		With 1–2 ng/g		With 2 ng/g		
			No.	%	No.	%	No.	%	
I	Patients with UST and/or EN	61	16	26.3[b]	9	14.8	7	11.5	20.3 ± 9.7
II	Healthy persons in families with UST and/or EN patients	63	10	15.8	6	9.5	4	6.3	14.5 ± 7.6
III	Healthy persons from 'healthy families' in endemic villages	63	7	11.1[b]	5	7.9	2	3.2	12.5 ± 3.5
IV	Healthy persons from unaffected villages in endemic area	60	7	11.6[b]	5	8.3	2	3.3	15.0 ± 4.2
V	Healthy persons from villages in nonendemic area	65	5	7.7[b]	4	6.2	1	1.5	10.01

[a] UST, urinary system tumours; EN, endemic nephropathy
[b] The differences between groups I and III and IV and V are statistically significant

Table 14. Percentage of subjects with Balkan endemic nephropathy with exceptional metabolic ratios

Metabolic ratio	Subject group[a]			
	1	2	3	4
<0.8	65.7	61.4	71.8	70.2
>3.0	11.3	11.6	2.7	9.7
>10.0	2.9	5.8	0.9	3.2

[a] 1, healthy subjects from villages free of Balkan endemic nephropathy (BEN) or urinary system tumours (UST); 2, healthy subjects from villages affected by BEN and UST; 3, subjects with a positive diagnosis of BEN; 4, subjects suspected of suffering from BEN

and Lewis strains have been phenotyped as PM and EM, respectively. We showed previously that PM and EM strains also differ also with respect to ochratoxin A metabolizing capacity *in vitro*[48], now found *in vivo*. This is the first report that a naturally occurring carcinogen shows polymorphism in drug oxidation. Another probe drug, spartein, exhibits a similar pattern of polymorphic metabolism, and assays therefore have been set up for the same purpose.

Since in-vitro hepatic and renal ochratoxin A 4-hydroxylase activity was much lower in DA

[48] Hietanen, E., Malaveille, C., Camus, A.-M., Béréziat, J.-C., Brun, G., Castegnaro, M., Michelon, J., Idle, J.R. & Bartsch, H. (1986) *Drug Metab. Disposition, 14,* 118–126

than in Lewis rats[49], an in-vivo study has been carried out using the same rat strains. Rats were treated with 1.5 mg/kg body weight of ochratoxin A, five times a week for eight weeks. Urine collection was performed at weeks 1, 3, 5, 7 and 8, and ochratoxin A and its 4-hydroxy metabolite were analysed. The ratio of the parent compound and its metabolite excreted was at all times greater in DA than in Lewis rats, confirming the results of the in-vitro study: female DA rats have a two- to four-fold lower 4-hydroxylase activity than Lewis rats.

(iii) *Mechanism of ochratoxin A-induced toxicity and possible carcinogencity*[50] (Dr A.D. Rahimtula, Mr J.-C. Béréziat, Mrs V. Bussacchini-Griot and Dr H. Bartsch)

The mechanism of the toxicity of ochratoxin A is not known; however, induction of oxidative stress and covalent binding to critical tissue macromolecules are likely possibilities. We have observed that incubation of ochratoxin A with rat liver microsomes and NADPH results in a large increase in lipid peroxidation and a substantial level of covalent binding to microsomal proteins. Cytochrome P450 was shown not to be involved in these processes. The onset of lipid peroxidation was also accompanied by the specific formation of (4*S*)-4-hydroxyochratoxin A, which could, in principle, serve as an index of lipid peroxidation. Inclusion of the antioxidant butylated hydroxytoluene abolished the lipid peroxidation and greatly reduced the level of protein binding and (4*S*)-4-hydroxyochratoxin A formation. Furthermore, the efficiency of several ochratoxin A analogues to enhance lipid peroxidation correlated precisely with their known toxicities in chicks. Also, administration of ochratoxin A to rats resulted in enhanced lipid peroxidation *in vivo* as evidenced by a several-fold increase in the rate of ethane exhalation.

The involvement of oxidative damage induced by ochratoxin A is consistent with the fact that, in preliminary experiments, we have observed a significant increase (two fold) in the level of 8-hydroxydeoxyguanosine in DNA isolated from kidneys of ochratoxin A-treated rats. It has been shown previously that deoxyguanosine residues in DNA are hydroxylated at the C-8 position *in vitro* and *in vivo* to produce 8-hydroxydeoxyguanosine by various agents that produce oxygen radicals. In keeping with this hypothesis is the fact that we have been unable to show the presence of ochratoxin A-DNA adducts in kidneys or livers of treated animals when analysed by the ^{32}P-postlabelling method developed by Randerath and coworkers for polycyclic aromatic hydrocarbon-DNA adducts.

Lewis (EM) and DA (PM) rat strains show genetic polymorphism for the gene regulating debrisoquine-4-hydroxylase in a manner analogous to human drug metabolism. Ochratoxin A has been shown to be metabolized more efficiently by Lewis rats *in vivo* and by their microsomes *in vitro*. Furthermore, administration of ochratoxin A led to a substantial elevation in the rate of ethane exhalation in Lewis but not in DA rats. These results suggest that lipid peroxidation may play a role in the observed toxicity of ochratoxin A, while the type of cytochrome P450 present may moderate this effect. Experiments currently in progress are designed to test this hypothesis.

(iv) *Analgesic-associated renal papillary necrosis and renal pelvic and ureteral/urothelial hyperplasia and carcinoma* (Principal investigator: Dr P.H. Bach, Robens Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford, UK; DEC/83/03)

The association of renal papillary necrosis and upper urothelial carcinoma with use of analgesics and nonsteroidal anti-inflammatory drugs is well established. The purpose of the

[49] Hietanen, E., Bartsch, H., Castegnaro, M., Malaveille, C., Michelon, J. & Broussolle, L. (1985) *J. Pharmacol. Clin., 4,* 71–78

[50] Rahimtula, A.D., Béréziat, J.-C., Bussacchini-Griot, V. & Bartsch, H. (submitted for publication)

present investigations is to use model papillotoxic compounds as molecular probes for studying renal papillary necrosis and its relation to urothelia carcinoma. Progress has been made in establishing models of upper urothelia carcinoma using the carcinogen *N*-nitrosobutyl(4-hydroxybutyl)amine. This model has a great number of pathological features that are similar to those in human analgesic abusers[51]. Using these animal models, the nephrotoxic compound ochratoxin A is now being investigated.

(*h*) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* (Dr H. Vainio, Dr L. Shuker, Ms L. Haroun, Mr J. Wilbourn, Dr A. Tossavainen, Mrs C. Partensky and Mrs I. Peterschmitt. The following members of other units have contributed to this programme: Dr N. Day, Dr J. Estève, Dr C.S. Muir, Dr E. Riboli, Dr R. Saracci and Dr L. Simonato for expertise in epidemiology; Dr H. Bartsch, Dr J.R.P. Cabral, Dr R. Montesano and Dr H. Yamasaki in experimental pathology, toxicology and mutagenesis; Dr M. Friesen and Dr I.K. O'Neill in analytical chemistry; Dr E. Cardis and Dr J. Kaldor in statistical aspects of data analysis; and Mrs E. Heseltine in editorial matters)

The main objectives of the IARC Monographs Programme have remained unchanged, although the coverage has been expanded to include exposures to agents other than chemicals, such as radiation. To reflect this widened scope the title of the series has been changed to the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

During the period 1 July 1985 to 30 June 1987, Volumes 40–43, and Supplements 6 and 7 were published or in press. Furthermore, the preamble to the monographs was revised and published as *IARC Internal Technical Report 87/001*.

Volume 40

In many recent studies, diet has been examined as a possible factor in human cancer. Thus, some naturally occurring and synthetic food components were considered in Volume 40. The Working Group considered that there was sufficient evidence for the carcinogenicity in experimental animals for butylated hydroxyanisole, potassium bromate, bracken fern, Glu-P-1 (2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]-imidazole), Glu-P-2 (2-aminodipyrido[1,2-*a*:3′,2′-d]-imidazole, MeAαC (2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole), AαC (2-amino-9*H*-pyrido-[2,3-*b*]indole) and IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline). For butylated hydroxtoluene, benzyl acetate, citrinin and ptaquiloside (present in bracken fern), it was considered that there was *limited evidence* of carcinogenicity in experimental animals. The experimental data were considered to provide *inadequate evidence* for the carcinogenicity of MeIQ (2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline), shikimic acid (present in bracken fern), rugulosin and patulin. Although there is extensive human exposure to some of these compounds, studies on carcinogenicity to humans were available only for bracken fern, for which the data were considered to provide *inadequate evidence* of carcinogenicity in humans.

Some furocoumarins (psoralens and angelicins) were considered which are used clinically in conjunction with ultra-violet A radiation (UVA) in the treatment of skin diseases. The data from experimental animals were considered to provide *sufficient evidence* for the carcinogenicity of 5-methoxypsoralen in combination with UVA and *limited evidence* for that of angelicin, 5-methylangelicin and 4,5′-dimethylangelicin in combination with UVA. It was considered that there was *no evidence* of carcinogenicity to experimental animals of 3-carboxypsoralen in combination with UVA. For the remaining furocoumarins, the data were judged to provide

[51] Bach, P.H. & Bridges, J.W. (1985) *CRC crit. Rev. Toxicol., 15*, 331–439

inadequate evidence of carcinogenicity or were not available to make an evaluation. Epidemiological data were either not available or provided *inadequate evidence* for the carcinogenicity of any of the furocoumarins in humans.

An appendix on ultra-violet radiation provides a brief overview of the experimental data relevant to carcinogenicity and an analysis of available case reports and epidemiological studies of carcinogenicity in humans.

Volume 41

This volume comprises monographs on some halogenated compounds and exposures to pesticides. A major concern with this group of compounds is their widespread distribution and, in many cases, persistence in the environment, in addition to occupational exposures. There was judged to be *sufficient evidence* for the carcinogenicity in experimental animals of dichloromethane, 1,3-dichloropropene (technical grade), polybrominated biphenyls and amitrole, and *limited evidence* for the carcinogenicity of 1,1,1,2-tetrachloroethane, pentachloroethane, 1,2-dichloropropane, bis(2-chloro-1-methylethyl)ether, methyl bromide, methyl iodide, chlorofluoromethane, chlorodifluoromethane and 2-chloro-1,1,1-trifluoroethane. The data provided *inadquate evidence* for the carcinogenicity of methyl chloride. Epidemiological data were either not available or provided *inadequate evidence* for the carcinogenicity of these compounds in humans.

The carcinogenic risks of occupational exposures to chlorophenols and chlorophenoxy herbicides were re-evaluated; for both exposures, the available data were considered to provide *limited evidence* of carcinogenicity in humans.

Volume 42

Six monographs, one on silica and five on silicate minerals, are included in Volume 42 of the *IARC Monographs*. The most common form of silica is quartz, which is one of the most abundant minerals in the Earth's crust. The silicate minerals wollastonite, attapulgite, sepiolite, talc and erionite occur to various extents in fibrous forms.

There was judged to be *sufficient evidence* for the carcinogenicity in experimental animals of crystalline silica and of erionite, and *limited evidence* for that of wollastonite and of attapulgite. The data were considered to provide *inadequate evidence* for the carcinogenicity of amorphous silica, sepiolite and talc. Epidemiological data were judged to provide *sufficient evidence* for carcinogenicity in humans of erionite and of talc containing asbestiform fibres, and *limited evidence* for that of crystalline silica. Epidemiological data were considered to provide *inadequate evidence* for the carcinogenicity of amorphous silica, wollastonite, attapulgite and talc not containing asbestiform fibres; no data were available to evaluate the carcinogenicity of sepiolite in humans.

Preamble to IARC Monographs

In preparation for the updating of Supplement 4 to the *IARC Monographs* in March 1987, an ad-hoc group was convened in October 1986 to examine the Preamble to the *Monographs*, to bring it up to date with regard to current scientific knowledge, and to advise the IARC about whether future working groups should make overall evaluations of carcinogenicity to humans.

Six months prior to the meeting, the Preamble to Volume 38 of the *Monographs* was sent to the members of the Working Group, asking for their comments and proposals for changes or additions. Further comments were received from academia, governmental agencies and industrial organizations. The Secretariat then prepared a modified Preamble, taking these

comments into consideration; in addition, the format and layout of the Preamble were altered. This document was sent to all participants one month before the meeting, for their consideration, and during the meeting it was used as a first draft. It was discussed first in plenary session, then more specifically in three subgroups, on animal pathology, toxicology and genetic effects, and epidemiology. The revised drafts were then collated, rediscussed and adopted in a further plenary session.

The Working Group also discussed the proposal that all future IARC working groups make an overall evaluation of carcinogenicity to humans in each monograph; i.e., that such overall evaluations not be restricted to meetings to prepare supplements. This proposal was accepted in principle, and the criteria for making the overall evaluations were briefly discussed. However, because of insufficient time to produce an optimal version, it was decided that this last section of the Preamble, 'overall evaluations', be discussed by a further, smaller working group. A group of experts was thus convened in January 1987. The Group confirmed that an overall evaluation should be made by future working groups during the preparation of monographs on individual agents. The Working Group finalized the proceedings for making 'overall evaluations', and recommended that agents be placed into one of four groups: Group 1 comprises those evaluated as carcinogenic for humans; this category is used only when epidemiological studies provide *sufficient evidence* of a causal association between the agent and cancer. Group 2 comprises agents for which, at one extreme, the degree of evidence is almost sufficient, as well as agents for which, at the other extreme, there are inadequate or no human data but for which there is convincing evidence of carcinogenicity in experimental animals. Agents are assigned to either 2A (probably carcinogenic to humans) or 2B (possibly carcinogenic to humans) on the basis of epidemiological, experimental and other relevant data, including data on genetic and related effects. Classification of an agent into Group 3 indicates that the data available do not allow an evaluation of its carcinogenicity to humans. Group 4 comprises agents that were considered to be probably not carcinogenic to humans.

The results of these two meetings are reproduced as the Preamble to the *IARC Monographs* and as *IARC Internal Technical Report 87/001*.

Supplement 6

In December 1986, a working group was convened at the IARC to summarize and bring up to date the findings from tests for genetic and related effects in experimental systems, and from studies of DNA damage and chromosomal effects in humans, for approximately 200 agents that had been evaluated in Volumes 1–42 of the *Monographs* and for which some data on carcinogenicity in humans were available. The results of such tests were summarized by endpoint for whole mammals, cultured mammalian cells and nonmammalian systems. The conclusions of the Working Group for each agent were given in four forms:

(1) *Summary*: The activity of the agent or exposure at each endpoint that was considered by the Group to be significant is described, with emphasis on studies of DNA damage and chromosomal effects in humans and studies in animals *in vivo*.

(2) *Tabular summary*: The activity of the agent is indicated by phylogenetic group (prokaryotes, lower eukaryotes, plants, insects, animal cells *in vitro*, human cells *in vitro*, animals *in vivo* and humans *in vivo*) according to different endpoints (DNA damage, mitotic recombination, gene mutation, sister chromatid exchanges, micronuclei, chromosomal aberrations, aneuploidy, dominant lethality, cell transformation and inhibition of intercellular communication).

(3) *Activity profile listing*: The activity of the agent in each individual study considered, as assessed by the Working Group, is given, with the dose used and the reference.

(4) *Activity profile plot*: The activity of the agent at each endpoint is displayed quantitatively in a bar graph according to phylogenetic order.

A full report of this meeting will be published as Supplement 6 to the *IARC Monographs*. The conclusions of the Working Group were also made available to the March 1987 meeting to assist in their evaluation of the carcinogenic risk of these compounds to humans, as described below.

Supplement 7

A working Group was convened in March 1987 with two principal aims. The first was to summarize and bring up to date the data on carcinogenicity in humans and in experimental animals for all agents that had been evaluated in Volumes 1–42 of the *Monographs* and for which some data on carcinogenicity in humans were available. The second was to make overall evaluations of carcinogenicity to humans for all agents evaluated in Volumes 1–42 of the *Monographs*.

For the agents for which information on carcinogenicity in humans was available, brief summaries were prepared of the experimental and epidemiological studies of carcinogenicity, and evaluations of degree of evidence (*sufficient, limited, inadequate,* or *evidence suggesting lack of carcinogenicity*) were adopted. Summaries were also available of the results of short-term tests for genetic and related effects (prepared by the Working Group that met in December 1986; see above). Overall evaluations of carcinogenicity to humans were then made on the basis of the combined evidence from humans and experimental systems. For the remaining agents (those agents for which in general no information on carcinogenicity to humans was available), overall evaluations were generally made on the basis of the summary and evaluation in the published monographs. All agents in this group for which there is *sufficient evidence* of carcinogenicity in experimental animals were re-evaluated by the Working Group. Where additional published data were available that could affect the current evaluation of carcinogenicity in experimental animals, new summaries and evaluations were prepared, and these were used in making the overall evaluations.

Of the more than 600 agents or groups of agents evaluated by the Working Group, 50 were identified as being causally associated with human cancer and were therefore assigned to Group 1. The Working Group concluded that 37 agents are *probably carcinogenic to humans* (Group 2A) and 159 agents are *possibly carcinogenic to humans* (Group 2B). Evidence permitting a classification of Group 4, *probably not carcinogenic to humans*, was available for only one compound. The remining agents could not be classified as to their carcinogenicity to humans (Group 3). The agents classified in Groups 1 and 2A are listed in Table 15. The conclusions of this meeting will be published as Supplement 7 to the *IARC Monographs*.

Volume 43

An IARC Working Group was convened in June 1987 to evaluate the carcinogenic risks of exposures to man-made mineral fibres and radon; the conclusions will be published in Volume 43 of the *IARC Monographs*. More than five million tonnes of man-made mineral fibres are produced annually throughout the world. Glass-fibre products comprise over 50% of the total. Most glasswool, rockwool and slagwool is used for thermal and acoustical insulation in the construction industry. Glass filaments are used mainly as textiles and as reinforcement materials in plastics. Ceramic fibres are being produced in increasingly large quantities for high-temperature insulation and in specialty products.

Table 15. *Conclusions of the IARC Working Group to prepare Supplement 7 to the* Monographs

Group 1. The Working Group concluded that the following 50 agents are carcinogenic to humans:

Aflatoxins
Aluminium production
4-Aminobiphenyl
Analgesic mixtures containing phenacetin
Arsenic and arsenic compounds[a]
Asbestos
Auramine, manufacture of
Azathioprine
Benzene
Benzidine
Betel quid with tobacco
N,N-Bis(2-chloroethyl)-2-naphthylamine (Chlornaphazine)
Bis(chloromethyl)ether and chloromethyl methyl ether (technical-grade)
Boot and shoe manufacture and repair
1,4-Butanediol dimethanesulphonate (Myleran)
Chlorambucil
1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl CCNU)
Chromium compounds, hexavalent[a]
Coal gasification
Coal-tar pitches
Coal-tars
Coke production
Cyclophosphamide
Diethylstilboestrol
Erionite
Furniture and cabinet making
Haematite mining, underground, with exposure to radon
Iron and steel founding
Isopropyl alcohol manufacture, strong-acid process
Magenta, manufacture of
Melphalan
8-Methoxypsoralen (Methoxsalen) plus ultraviolet radiation
Mineral oils, untreated and mildly treated
MOPP (combined therapy with nitrogen mustard, vincristine, procarbazine and prednisone) and other combined chemotherapy including alkylating agents
Mustard gas (Sulphur mustard)
2-Naphthylamine
Nickel and nickel compounds[a]
Oestrogen replacement therapy
Oestrogens, nonsteroidal[a]
Oestrogens, steroidal[a]
Oral contraceptives, combined[b]
Oral contraceptives, sequential
The rubber industry
Shale-oils
Soots
Talc containing asbestiform fibres
Tobacco products, smokeless
Tobacco smoke
Treosulphan
Vinyl chloride

[a] This evaluation applies to the group of agents as a whole and not necessarily to all individual agents within the group

[b] There is also conclusive evidence that these agents protect against cancers of the ovary and endometrium

Table 15 *(continued)*

Group 2A. The Working Group concluded that the following 37 agents are probably carcinogenic to humans:

Acrylonitrile
Adriamycin
Androgenic (anabolic) steroids
Benz[*a*]anthracene
Benzidine-based dyes
Benzo[*a*]pyrene
Beryllium and beryllium compounds
Bischloroethyl nitrosourea (BCNU)
Cadmium and cadmium compounds
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)
Cisplatin
Creosotes
Dibenz[*a,h*]anthracene
Diethyl sulphate
Dimethylcarbamoyl chloride
Dimethyl sulphate
Epichlorohydrin
Ethylene dibromide
Ethylene oxide
N-Ethyl-*N*-nitrosourea
Formaldehyde
5-Methoxypsoralen
4,4'-Methylene bis(2-chloroaniline) (MOCA)
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine
N-Methyl-*N*-nitrosourea
Nitogen mustard
N-Nitrosodiethylamine
N-Nitrosodimethylamine
Phenacetin
Polychlorinated biphenyls
Procarbazine hydrochloride
Propylene oxide
Silica, crystalline
Styrene oxide
Tris(1-aziridinyl)phosphine sulphide (Thiotepa)
Tris(2,3-dibromopropyl) phosphate
Vinyl bromide

Radon and its decay products are ubiquitous in soil, water and air. Particularly high levels occur in regions of the world where uranium-containing rocks, such as granite, occur. Radon in the ground, groundwater and building materials can enter working and living spaces and disintegrate into its decay products. Humans are exposed to higher concentrations of radon and its short-lived decay products in confined air spaces, particularly in underground work areas, such as mines, and in certain buildings, than in outdoor air.

The overall evaluations were that glasswool, rockwool, slagwool and ceramic fibres are *possibly carcinogenic to humans* (Group 2B), that glass filaments are *not classifiable as to their carcinogenicity to humans* (Group 3) and that radon and its decay products *are carcinogenic to humans* (Group 1).

3. SITE-ORIENTED STUDIES

(*a*) *Etiological studies on liver cancer*

(i) *Aflatoxin and hepatitis B virus studies in Swaziland* (Dr F.X. Bosch, Dr N. Muñoz, Dr J. Kaldor and Dr M. Parkin; in collaboration with Dr F.G. Peers, Mbabane, Swaziland; Dr A. Linsell, Nairobi; and Dr M. Pluijmen, Hulsberg, The Netherlands; UNEP/IARC FP/0107.78-03(1391))

A study was carried out in Swaziland to assess the relationship between aflatoxin exposure, hepatitis B virus (HBV) infection, and the incidence of hepatocellular carcinoma (HCC), which is the most commonly occurring malignancy among males in Swaziland. Data collection was conducted from July 1982 to June 1983, analysis of the results was finalized during 1986, and a report has just been published[52]. The levels of aflatoxin intake were evaluated in dietary samples from households across the country and crop samples taken from representative farms. Table 16

Table 16. Estimated exposure to total aflatoxins and aflatoxin B_1 to different food types and estimated total exposure per man per day to total aflatoxin in the four topographical regions (number of positive samples in parentheses)

Topographical region	Aflatoxin type	Maize (μg/kg)	Sauce (μg/kg)	Peanuts (μg/kg)	Beer (μg/l)	Sour porridge (μg/kg)	Total[a] (μg)
High veld	Total	0.09 (5)	2.00 (4)	47.95 (7)	0.57 (2)	0.54 (3)	3.1
	B_1	0.04 (4)	0.99 (4)	17.56 (7)	0.09 (1)	0.29 (3)	1.0
Middle veld	Total	0.26 (9)	3.84 (9)	279.13 (13)	0.65 (4)	0.12 (2)	10.7
	B_1	0.18 (9)	1.66 (9)	47.21 (13)	0.28 (3)	0.10 (2)	2.8
Low veld	Total	0.79 (13)	9.76 (14)	298.15 (12)	0.69 (6)	1.15 (4)	17.5
	B_1	0.28 (12)	5.06 (14)	145.13 (12)	0.51 (6)	0.52 (4)	8.9
Lubombo	Total	0.33 (3)	2.06 (4)	147.94 (3)	0.35 (1)	0.10 (2)	9.5
	B_1	0.09 (3)	1.43 (4)	20.94 (3)	0.23 (1)	0.06 (2)	2.3

[a] Estimated total exposure per man per day, based on a mean of all samples collected of 664 g maize, 227 g sauce twice per day, 2 l beer (estimate) and 200 g sour porridge (estimate) and a quantity of peanuts estimated per region, as described in the text

shows the estimated exposure to aflatoxins (total and B_1) from different foodstuffs and the estimated exposure per man per day in four broad topographical regions. Across these four regions, there was a more than five-fold variation in the estimated daily intake of aflatoxin, ranging from 3.1 to 17.5 mg. Similar estimates were obtained from crop samples. The prevalence of HBV markers was estimated from the serum of blood donors and is shown in Table 17. The proportion of HBV-exposed individuals was very high (86% in men), but varied relatively little by geographic region; the prevalence of carriers of the surface antigen was 23% in men, and varied from 21 to 28%. Liver cancer incidence was recorded for the years 1979–1983 through a national system of cancer registration. Table 18 shows the number of cases registered in males aged 15–64 and the relative risks for the four main topographical areas of the country. HCC incidence varied over a five-fold range and was stronly associated with estimated levels of aflatoxin. In a multivariate statistical analysis involving ten smaller subregions, aflatoxin exposure emerged as a more important determinant of the variation in liver cancer incidence than the prevalence of hepatitis infection.

[52] Peers, F., Bosch, X., Kaldor, J., Linsell, A. & Pluijmen, M. (1987) *Int. J. Cancer, 39,* 545–553

Table 17. Numbers and proportions of persons ever exposed to HBV and carriers of HBsAg among blood donors by sex and topographical area

Topographical region	Number of subjects		HBV-exposed[a]				HBsAg carriers			
	Males	Females	Males		Females		Males		Females	
			No.	%	No.	%	No.	%	No.	%
High veld	569	517	488	85.8	397	76.8	118	20.7	78	15.1
Middle veld	698	619	598	85.7	454	73.7	151	21.6	82	13.2
Low veld	261	225	231	88.5	187	82.7	72	27.6	38	16.9
Lubombo	96	62	87	90.6	47	77.0	27	28.1	13	21.0
Total	1624	1423	1404	86.5	1085	76.2	368	22.7	211	14.7

[a] HBsAg+ and/or HBsAb+ and/or HBcAb+

An important component of the project was to assess the impact of the Rural Development Areas (RDA) programme implemented in Swaziland over the past 15 years. This assessment required a comparison of results from the study with those obtained in a previous dietary survey carried out in 1972–1973 in Swaziland by the Agency.

Under the RDA programme, all nationally-owned land (about 50% of the country) was allocated to one of four levels of intervention. Although it was not possible to define the exact extent to which the plans were followed in any given area, the categories give a broad indication of improvements that may have taken place since the first study. We therefore reallocated all the food samples obtained in both the 1972–1973 survey and the 1982–1983 survey, according to the level of intervention, on a scale of 1 to 4. The overall frequency of aflatoxin contamination was lower in the last survey (4.7 *versus* 6.6% $p < 0.01$). There has been a substantial decrease in the frequency of contamination of the combined sauce and maize, which constitutes the 'main meal', (3.4 *versus* 6.7% $p < 0.01$). Peanuts were more frequently contaminated in the recent survey (21.2% *versus* 12.0%; $p < 0.001$). For regions 3 and 4, in which the highest level of intervention

Table 18. Number of male HCC cases aged 15–64 in Swaziland and relative risk by age group and topography (1976 male populations in parentheses)

Topographical region	Age group				Relative risk
	15–34 (63 890)	35–49 (29 536)	50–64 (14 618)	15–64	
High veld (34 187)	1	1	4	6	1.0
Middle veld (40 017)	7	5	5	17	2.42
Low veld (29 322)	8	9	10	27	5.25
Lubombo (4518)	0	0	2	2	2.52
Total	16	15	21	52[a]	—
Relative risk	1.0	2.03	5.74	—	

[a] Excludes ten of the 62 cases included in the registry due to lack of information on either age or place of residence

was applied, the proportion of total samples contaminated was markedly lower in the second survey than in the first (RDA region 3: 4.6% *versus* 8.7% ($p < 0.01$); RDA region 4: 3.2 *versus* 7.9% ($p < 0.05$). The two other RDA regions showed no such difference. Although there is a possibility that the results could be explained by climatic and topographic interactions, they provide suggestive evidence that the RDA programme has had some success in reducing exposure to dietary aflatoxins.

(ii) *Cohort studies on hepatitis B virus, aflatoxin and other risk factors* (Dr N. Muñoz, Dr F.X. Bosch and Dr J. Estève; in collaboration with Professor W.O. Phoon, Dr N.P. Fong and Dr J. Lee, Department of Social Medicine and Public Health, University of Singapore; and Dr P. Srivatanakul and P. Punthumchiuda, National Cancer Institute, Bangkok)

In Singapore, a total of 14 853 Chinese males in the age group 35–65 years have been admitted to this cohort. Among 13 422 tested for HBsAg, 1082 (8%) were found positive (Table 19). Nineteen HCC cases have been identified by linking this cohort to the Cancer Registry records up to December 1985, but all of them except two were diagnosed within a year of admission to the cohort.

Since the increased risk for HCC among HBsAg carriers has been confirmed in several cohort studies in various population groups, the main value of the cohort study in Singapore would be to evaluate possible interactions between HBV and other putative risk factors such as aflatoxin, *N*-nitroso compounds and hormones, exposure to which can be assessed only by prospective monitoring. However, results of a pilot study indicate that exposure to aflatoxin, as measured by urinary levels of aflatoxin B_1, is relatively low in Singapore. It was then decided to extend this study to other high-risk populations for HCC with known high exposures to aflatoxin, such as in Thailand.

Thailand offers special advantages for this study, as HCC is one of the most common cancers among males, the prevalence of HBsAg is high (about 10%), and high exposure to aflatoxin has been documented. In Bangkok, a cohort of 3000 HBsAg-positive men over 30 years of age has been identified from blood banks and during a serological survey carried out among army personnel. On study entry, a questionnaire will be completed for each subject to obtain information on smoking, drinking and dietary habits, and specifically on the intake of foodstuffs known or suspected to contain aflatoxin and *N*-nitroso compounds. Physical and ultrasound examinations designed to identify signs and symptoms of liver disease will be performed, and serum and urine specimens will be collected and stored at −70°C. Yearly follow-up of each subject will be organized for a five-year period. During these follow-up visits, physical and ultrasound examinations will be performed, and serum and urine specimens will be collected. It

Table 19. Cohort study of HBsAg carriers in Singapore

Source of cohort members	No. of serum specimens		Serum secimens positive for HBsAg		No. of subjects interviewed
	Collected	Tested	No.	%	
Hospitals	10 493	9 036	904	10.0	7 095
Blood bank	3 378	3 378	85	2.5	3 340
Others	982	981	93	9.5	785
Total	14 853	13 422	1 082	8.1	12 030

is estimated that at the end of the five-year period, 40–50 HCC cases and a similar or even higher number of cases of chronic hepatitis and cirrhosis will be identified in this cohort. Early cases of HCC are expected to be detected by yearly determinations of α-fetoprotein and ultrasound examination of the hepatobiliary system.

Appropriate controls will be selected for each case, and exposure to the relevant risk factors will be assessed for cases and controls. The relevant risk factors include aflatoxin, *N*-nitroso compounds, male hormones, smoking and alcohol. Exposure will be assessed by questionnaire and by measuring the corresponding compounds (adducts or metabolites) in serum and urine.

(iii) *Hepatitis B virus and liver cancer in the Philippines* (Dr N. Muñoz, Dr F.X. Bosch, Dr J. Estève and Miss M. Blettner; in collaboration with Dr A. Lingao, Dr J. Lao, Dr G. Viterbo, Dr E. Domingo and Dr M.A. Lansang, Liver Study Group of the Philippines, Manila)

The analysis of data from this study has been completed, and a report is being submitted for publication[53]. The results showed no statistically significant difference in the prevalence of HBsAg between parents and older siblings of HCC patients and parents and siblings of population controls. However, there was an increased risk associated with total HBV infection of both mother and father of HCC patients as compared with those of population controls. When the parents and older siblings of HCC patients were compared with the parents and older siblings of asymptomatic HBsAg carriers, no difference was observed in the prevalence rates of HBsAg, nor in the prevalence of total HBV infection (see Table 20). These findings suggest that the fact of having a mother or father positive for HBV markers increases the chance of being a HBsAg carrier but not necessarily of developing HCC. Therefore, the HBsAg carrier state conveys the same risk of HCC, independently of the presumed age at HBV infection. Thus, the results of our study did not confirm the findings of a similar study in Senegal which suggested that early HBV infection of offspring by HBsAg carrier mothers is an important factor in the development of HCC and that fathers and siblings of HCC patients have a lower prevalence of anti-HBs than the fathers and siblings of control patients.

(iv) *HBsAq-positive blood donors in Catalonia: a feasiblity study* (Dr F.X. Bosch and Dr N. Muñoz; in collaboration with Dr M. Casas, Dr M.C. Rodriguez and Dr J. Cuervo, Municipal Health Department, Barcelona, Spain; Dr J.M. Hernandez, Blood Bank of the Residencia Vall d'Hebro, Barcelona, Spain; and Dr M. Gallen, Hospital del MAR, Barcelona, Spain)

Mortality data from the region of Catalonia in Spain suggest that it is a relatively high-risk area for liver cancer within the context of the western industrialized world: death rates in 1985 were 11.2 among males and 9.0 among females. A feasibility study has been initiated to assemble a cohort of HBsAg-positive blood donors and to link their names with local death certificate files to determine liver cancer risk. Data are being obtained from five major blood banks operating in the area. Of the 2515 HBsAg-positive blood donors identified, 1802 (72%) have been traced: 1782 were alive in 1985, 20 had died and 713 were still being traced. Identified causes of death include four cancers of the gastrointestinal tract, three lung cancers, two cirrhoses of the liver, seven cardiovascular diseases and four other causes.

Complete follow-up and analysis of the results are expected to be terminated in 1987.

[53] Muñoz, N., Lingao, A., Lao, J., Estève, J., Viterbo, G. Domingo, E., Lansang, M.A., Bosch, F.X. & Blettner, M. (1987) (submitted for publication)

Table 20. HBV profile among parents and older siblings of HCC cases and controls

Pairs considered	HBsAg status of pairs					HBV exposure[a] of pairs				
	+/+	+/−	−/+	−/−	RR (95% CI)	+/+	+/−	−/+	−/−	RR (95% CI)
Mothers of cases and of population controls	0	5	2	26	2.5 (0.4–26.3)	17	12	0	4	(3.5–∞)
Fathers of cases and of population controls	1	4	3	25	1.3 (0.2–9.1)	18	11	1	3	11 (1.6–473)
Older siblings of cases and of population controls	0	8	4	21	2.0 (0.5–9.1)	3	11	8	11	1.4 (0.5–3.9)
Mothers of cases and of asymptomatic HBsAg carriers (AC)	1	2	4	26	0.5 (0.04–3.5)	24	5	3	1	1.7 (0.3–10.7)
Fathers of cases and of AC controls	0	5	6	22	0.8 (0.2–3.3)	24	5	4	0	1.2 (0.3–6.3)
Older siblings of cases and of AC controls	2	6	5	20	1.2 (0.3–5.0)	6	8	11	8	0.7 (0.2–2.0)

[a] As measured by the presence of any HBV serological marker

(v) *Intervention studies using hepatitis B virus vaccine: the Gambia hepatitis intervention study* (Dr C.S. Muir, Dr A. Hall, Dr H. Inskip, Dr F. Loik, Dr F.X. Bosch, Dr N. Day, Dr N. Muñoz, Dr M. Parkin, Dr J. Estève and Dr R. Montesano; in collaboration with Dr P.G. Smith, London School of Hygiene and Tropical Medicine, London; Dr B. Greenwood and Dr H. Whittle, Medical Research Council, Fajara, Gambia; DIR/86/01)

The Gambia hepatitis intervention study is designed to evaluate the effectiveness of HBV vaccination in the prevention of chronic liver disease and HCC in a population at high risk (Fig. 3). The study, funded by the Department of Cooperation and Development of the Ministry of Foreign Affairs in Italy, in collaboration with the Medical Research Council Unit operating in Fajara, also assists the Government of the Gambia in maintaining a strong Expanded Programme of Immunization (EPI).

The first meeting of the steering committee for the study was held in the Gambia in February 1986, with Dr F. Oldfield, Director of Medical Services, Gambia, in the chair. The committee was composed of representatives of the Gambian government, the UK Medical Research Coucil, the Italian government and the Agency. Also present were representatives of UNICEF, WHO, The Centre for Control of Childhood Diseases and five consultants to the Agency. The most relevant changes introduced by the steering committee meeting to the main protocol concerned the definition of the groups selected for detailed studies on response to HBV vaccination: group 1 will consist of the first 250 newborns who present to each of the four vaccination teams

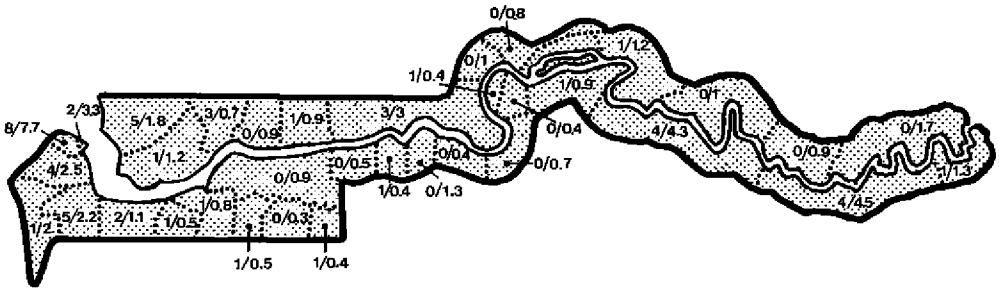

Fig. 3. Observed and expected numbers of cases of hepatocellular carcinoma in the Gambia by district, 1986

initiating HBV vaccination; group 2 will consist of one cross-sectional survey at the end of phase 1 of the study covering nonimmunized children aged 0 to 5.

The study plan called for the progressive incorporation over a four-year period of HBV vaccine delivery, following a period of personnel recruitment and training, into the regular EPI vaccination schedule. To ensure that group 1 is representative of the country as a whole, the Gambia was stratified into four geographical zones of roughly equal population size. In each zone, a health centre was chosen at random to be the first to deliver HBV in that zone. The first dose of HBV vaccine was given at Brikama on 23 July 1986. Meanwhile, all children presenting to EPI teams at Banjulunding, Gunjur, Serekunda and Sibanor were also registered. HBV vaccination started in the second zone, at Dankunku and Kudang, during October 1986. Vaccination in the third zone, at Yorobawal and Badjakunda, began on 10 February 1987. HBV vaccination began at Essau in April 1987. On the completion of vaccination in zone 4, the entire nation will have been covered by the registration system, and HBV vaccine will have been incorporated into the EPI schedule at six of the 26 centres, i.e., for about 25% of the population. Following the introduction of HBV vaccination into zone 4, one centre in each time period will change over from 'control' status (i.e., current EPI vaccines only) to HBV immunization in addition to EPI vaccines.

No adverse reaction to the HBV vaccine has been observed to date. All vaccine has been delivered by sterile needles and syringes, and particular attention to sterility has been emphasized in EPI training courses and during supervision.

The second meeting of the steering committee was held on 2 March 1987, chaired by Dr H. N'jie, Director of Medical Services, the Gambia. The WHO representative in the Gambia, Dr T. Tshabalala, was elected as a new member of the committee. The most relevant points discussed at the meeting were:

- the logistic changes that might be required in the original study design due to the expansion of the EPI system in the Gambia (from 17 to over 40 vaccination teams) and the need to improve vaccination coverage;
- the finding that newborns and infants in the Gambia come to vaccination at later ages than was anticipated in the protocol, and therefore a fraction of them might have already been infected with HBV at the time of first HBV vaccination;
- the successful implementation of the cancer registry. Since July 1986, and for a six-month period, 161 cases have been registered. The age-standardized incidence rates for all sites was 90.8 per 100 000 males per year and 53.1 for females. Liver cancer was the most frequently registered cancer in males (age-standardized rate, 38.6) and was in second rank in females (age-standardized rate 11.8).

Several ancillary studies were outlined to be implemented within the next two to three years. These projects were peer-reviewed at a meeting held in Lyon in May 1987. In summary these are: a study on the prevalence of HBV infection in the families of neonates receiving HBV vaccine and its influence on the response to vaccination; three sero-epidemiological studies on (1) immunological causes of non-response to HBV vaccine; (2) epidemiology of hepatitis δ virus infection; (3) epidemiology of human retrovirus infections; (4) an intervention study to evaluate the contribution of arthropods to the transmission of HBV between children in the Gamia; (5) a case-control study to evaluate the etiology of chronic liver diseases in the Gambia; (6) a study to determine the genetic contribution to the immune response to vaccines; (7) a pilot study on liver function tests and serum markers of HBV in women using contraceptive drugs; (8) a case-control study to evaluate the protective efficacy of BCG vaccine in the Gambia; (9) a study on mortality records from Banjul; and (10) two studies on aflatoxin exposure and metabolism.

These projects are in various stages of development.

(vi) *Liver cancer etiology in Thailand* (Dr D.M. Parkin, Dr H. Bartsch, Dr R. Montesano and Dr N. Muñoz; in collaboration with Dr P. Srivatanakul, National Cancer Institute, Bangkok)

A preliminary descriptive analysis of liver cancer in Thailand has shown considerable regional variation, particulary for cholangiocarcinoma, which comprises about 40% of registered cases[54]. The prevalence of risk factors will be studied in a sample of the normal adult population (markers of infection with HBV and *Opisthorchis viverrini* and urinary aflatoxin and *N*-nitrosamines) in four areas of the country with wide variations in the risk of HCC and cholangiocarcinoma.

A case-control study of residents of north-east Thailand is planned, to investigate risk factors for HCC and cholangiocarcinoma, in which 100 cases of HCC and 100 cases of cholangiocarcinoma will be recruited in two hospitals in north-eastern Thailand and in Bangkok; controls matched for age, sex and residence will be drawn from patients attending the same hospitals. Cases and controls will be interviewed in relation to dietary history, smoking, alcohol and contraceptive use, and specimens obtained for investigation of markers of infection with HBV and *O. viverrini*. Blood specimens will be stored for later analysis of albumin-bound aflatoxin. Pilot studies were carried out in 1986, and the main investigations will be performed in 1987 and 1988.

(*b*) *Cancers of the gastrointestinal tract*

(i) *Precancerous lesions of the oesophagus in China* (Dr N. Muñoz, Dr J. Wahrendorf, Dr F.X. Bosch and Dr G.T. O'Conor; in collaboration with Dr Lu Jian-Bang, Professor Shen Chuin, Dr Yang Guan-Rei and Dr Qu Song-Lang, Henan Medical College and Henan Cancer Institute, Henan, China; Professor M. Crespi and Dr A. Grassi, Regina Elena Institute, Rome; Dr D. Thurnham, Dudley Road Hospital, Birmingham, UK; Dr M. Hambidge, University of Colorado, Denver, CO, USA; Dr P. Correa, Louisiana State University Medical Center, New Orleans, LA, USA; Dr M. Hayashi, National Institute of Hygienic Sciences, Tokyo; and Dr M. Lipkin, Memorial Sloan-Kettering Cancer Center, New York, NY, USA)

A randomized double-blind intervention trial was carried out in Huixian province, China,

[54] Srivatanakul, P., Sontipong, S., Chotiwan, P. & Parkin, D.M. (1987) *Gastroenterol. Hepatol.* (submitted for publication)

Table 21. Distribution of study subjects at final investigation by frequency of micronucleated cells in oesophageal mucosa

Treatment group	Total no. of subjects	Percentage of micronucleated cells							
		0	0.2	0.4	0.6	0.8	1.0	≥1.2	Mean (±SD)
Placebo	43	13	11	8	8	0	3	0	0.31 (±0.29)
Vitamins	40	13	17	10	0	0	0	0	0.19 (±0.15)

from September 1983 to November 1984. The aim of the trial was to determine whether combined treatment with retinol, riboflavin and zinc could result, after one year, in a lower prevalence of precancerous lesions of the oesophagus in comparison with a group receiving a placebo. Analysis of the results of the trial showed that the combined treatment over 13.5 months did not affect the prevalence of histologically diagnosed precancerous lesions of the oesophagus[55]. Further analyses of the data collected during the trial were conducted in 1986 and 1987.

The finding of an increase in blood retinol levels in 40% of subjects in the placebo group and in 70% of subjects in the treatment group motivated an exploration of different retinol-related variables using logistic regression analysis. The most interesting result was that increases in blood retinol levels from the initial to the final survey were associated with a reduced prevalence of histologically diagnosed precancerous lesions of the oesophagus. However, no interaction was found between changes in retinol levels and treatment, suggesting that the effect of retinol on the prevalence of oesophageal lesions is independent of whether the improvement in retinol levels was caused by the administration of retinol or whether it was due to other sources.

A similar indication was obtained when riboflavin serum levels were analysed, but the association did not reach statistical significance. A report of these findings has been submitted for publication[56].

The above results suggest that the same dose of retinol applied over a longer period of time, or a higher dose (which, however would require closer clinical surveillance of the study participants), may affect regression of precancerous lesions of the oesophagus. This possibility is supported by the results of a more detailed analysis of the nutritional data collected during the intervention study, which have also been submitted for publication[57].

In addition, analysis of the data using two earlier endpoints, namely the prevalence of micronucleated cells as an indicator of chromosal aberrations and the pattern of cell proliferation of the oespohageal mucosa, was completed in a subsample of the study population. A statistically significant reduction ($p = 0.04$) was observed in the prevalence of micronuclei in oesophageal cells in the treatment group as compared to the placebo group, as shown in Table 21. However, no statistically significant difference was observed in the prevalence of micronuclei in the buccal mucosa cells before and after treatment nor between the treatment and the placebo group at the final examination. A paper describing these findings is in press[58].

[55] Muñoz, N., Wahrendorf, J., Lu, J.B., Crespi, M., Thurnham, D.I., Day, N.E., Zheng, H.J., Grassi, A., Li, W.Y., Liu, G.L., Lang, Y.Q., Zhang, C.Y., Zheng, S.F., Li, J.Y., Correa, P., O'Conor, G.T. & Bosch, F.X. (1985) *Lancet, ii,* 111–114

[56] Wahrendorf, J., Muñoz, N., Lu, J.B., Thurnham, D.I., Crespi, M. & Bosch, F.X. (submitted for publication)

[57] Thurnham, D., Muñoz, N., Wahrendorf, J., Hambridge, M., Lu, J.B., Zheng, S.F. & Crespi, M. (submitted for publication)

[58] Muñoz, N., Hayashi, M., Lu, J.B., Wahrendorf, J., Crespi, M. & Bosch, F.X. (1987) *J. natl Cancer Inst.* (in press)

Table 22. Number of labelled cells by columnar layer by treatment group

Treatment group	No. of subjects	Columnar layer								Total
		1	2	3	4	5	6	7	8	
Placebo	18	363	755	413	192	92	42	17	16	1890
Vitamins	18	444	914	397	135	45	13	7	7	1962

In order to investigate the pattern of cell proliferation, oesophageal biopsies taken from 58 subjects were incubated with tritiated thymidine. Biopsies from 36 subjects were suitable for evaluation, and the results (Table 22) indicate that the vitamin treatment reduced the abnormal cell proliferation in the upper layers of the epithelium. A report of these findings is being prepared for publication.

The overall results of these additional studies indicate that the vitamin treatment had a beneficial effect, manifested by a reduction in the number of micronuclei in oesophageal cells and a correction of the abnormal cell proliferation observed in the oesophageal mucosa of subjects in this high-risk population.

(ii) *Case-control studies of oesophageal cancer in high-risk populations of Latin America* (Dr N. Muñoz and Dr N.E. Day; in collaboration with Dr C. Vitora, Dr C. Saul and Dr N. Braga, Federal University of Pelotas, Brazil; Dr L. Barcelos and Dr D. Peccin, Secretariat of Health and Enironment, Porto Alegre, Brazil; Dr E. de Stefani, Hospital of Clinics and Dr A. Vasallo, Cancer Institute, Montevideo; Dr R. Castelletto, National University, La Plata, Argentina; and Dr J. Iscovich, San Martin Hospital, La Plata, Argentina)

In Latin America, the highest rates of oesophageal cancer are observed in an area which includes southern Brazil, Uruguay and north-eastern Argentina. These high-risk populations provide a unique opportunity for assessing the role of thermal injury on the causation of oesophageal cancer, because a considerable proportion of the population (about 80%) has the habit of drinking *maté*, a hot infusion made of *Ilex paraguayensis*; and there is a well-defined unexposed group (about 20%) who do not drink *maté* at all. To assess the role of thermal injury and of other known risk factors for oesophageal cancer, such as alcohol and tobacco, case-control studies are being carried out in these countries and an endoscopic survey has been carried out in Brazil.

An endoscopic survey was carried out in a group of Brazilian male workers, 30–59 years of age, to compare the prevalence of precursor lesions of oesophageal cancer in a group of heavy drinkers of *maté* with that in a group of non-drinkers or light drinkers. These two groups were matched for age, smoking habits and alcohol intake. *Maté* drinkers had 2.2 times more histologically confirmed chronic oesophagitis than non-drinkers. Table 23 summarizes the endoscopic and histological findings. A report of this study has been published[59].

In a case-control study, 171 cases and 342 age- and sex-matched controls recruited from hospitals in the State of Rio Grande do Sul in southern Brazil were interviewed during April 1985 to February 1986. The crude odds ratio for daily *maté* drinkers was 1.92 relative to those drinking less often than daily ($p = 0.006$). Other risk factors included the drinking of *cachaça* (a sugar-cane spirit), smoking, rural residence, low fruit consumption and high intake of meats, as shown in Tables 24 and 25. After adjustment for these variables through conditional logistic

[59] Muñoz, N., Victora, C.G., Crespi, M., Saul, C., Braga, N.M. & Correa, P. (1987) *Int. J. Cancer, 39*, 708–709

Table 23. Prevalence of endoscopic and histological lesions according to *maté* drinking in Pelotas, Brazil, 1985

Examination findings	*Maté* drinking			
	Drinkers		Non-drinkers	
	No.	%	No.	%
Endoscopic findings				
Mild or moderate oesophagitis	12	40	14	47
Leukoplakia	14	47	18	60
Whitish mucosa	19	63	13	43
Incontinent cardia	4	13	4	13
Elevated z-line	14	47	12	40
Gastritis or gastric ulcer	13	43	13	43
Duodenitis or duodenal ulcer	6	20	8	27
Histological findings				
Oesophagitis	13	43	6	20
Congestion	6	20	10	33
Clear-cell acanthosis	12	40	13	43
Gastritis	6	20	4	13
No. of subjects	30	100	30	100

Table 24. Multivariate conditional logistic regression analysis, final model, for oesophageal cancer incidence

Variable	Score (DF)	Odds ratio (CI)	*p*
Years of *cachaça* drinking (log) and daily amount drunk (0, 1–29, 30+ g/day)	54.21 (3)	[c]	<0.001[a]
Place of residence	15.35 (1)		<0.001[b]
(trend test)		1.89 (1.36–2.64)	<0.001
Large cities		1.00	
Small cities		1.82 (0.96–3.45)	0.06
Rural areas		3.62 (1.87–7.12)	<0.001
Smoking status	14.86 (1)		<0.001[a]
(trend test)		2.19 (1.54–3.10)	<0.001
Nonsmoker		1.00	
Ex-smoker		1.29 (0.61–2.73)	0.3
Current smoker		3.87 (1.88–7.98)	0.001
Frequency of eating fruits (log days/month +1)	8.84 (1)	0.66 (0.52–0.83)	0.001[a] 0.002
Frequency of eating meat (log days/month +1)	7.40 (1)	1.71 (1.15–2.54)	0.007[b] 0.008
Variable left out of the model:			
Frequency of *maté* drinking	1.46 (1)		0.11[a]
Less than daily			1.00
Daily	1.47 (0.87–2.50)		0.11

[a] One-sided test and 90% confidence intervals

[b] Two-sided test and 95% confidence intervals

[c] See Table 25

Table 25. Estimated joint effect of duration and daily amount of *cachaça* intake on oesophageal cancer incidence; fitted odds ratios relative to non-drinkers, after controlling for smoking, place of residence and ingestion of fruits and meat

Years of drinking	Daily intake of alcohol	
	1–29 g	30 g or more
20 years	2.22	4.80
30 years	3.68	7.96
40 years	5.26	11.40
50 years	6.95	15.06

Values obtained from the formulae:

For those drinking 1–29 g/day: odds ratio = $0.0527 \times \text{years}^{1.248}$

For those drinking 30+ g/day: odds ratio = $0.114 \times \text{years}^{1.248}$

regression, the odds ratio associated with daily *maté* drinking was reduced to 1.47 (90% CI, 0.87–2.50). Although the study failed to provide evidence of a strong association between *maté* drinking and oesophageal cancer, the cluster of high rates could be explained by relative risks of the magnitude observed. This is due to the fact that approximately 70% of adult males and 50% of females are daily drinkers. A report of this study has also been published[60].

On the basis of the experience gained in Brazil, the protocol and questionnaire were slightly modified, decreasing the upper age limit for cases from 80 to 75 years and collecting additional information on ways of preparing *maté* and on types of tobacco used, since both black and blond tobacco are used in Uruguay and Argentina.

In Uruguay, interviewing of cases and controls commenced in June 1985, and, up to June 1987, a total of 180 cases (134 males and 46 females) and 330 controls had been interviewed in the four main hospitals of Montevideo. It is estimated that about 80% of oesophageal cancer occurring in the whole country is diagnosed at these four hospitals. Data collection will continue until June 1988.

In Argentina, a pilot study was carried out between November 1985 and February 1986. Interview of cases and controls commenced in May 1986. Cases and controls are being ascertained from six main public hospitals and from private pathology laboratories. Up to June 1987, 70 cases and 130 controls had been interviewed. Data collection will continue until early 1989.

Should an increased risk of oesophageal cancer among *maté* drinkers be demonstrated by the collective results of all three studies, it remains to be explored whether the association can be accounted for by thermal injury or by the chemical nature of the infusion. In order to address this issue, two approaches are being considered: firstly, a fourth case-control study in Paraguay, where *maté* is drunk frequently, but cold; secondly, to carry out validation studies to evaluate

[60] Victora, C.G., Muñoz, N., Day, N.E., Barcelos, L.B., Peccin, D.A. & Braga, N.M. (1987) *Int. J. Cancer, 39,* 710–716

the accuracy and the precision of the perceived and reported information on the temperature at which *maté* is drunk.

(iii) *Stomach cancer*

(1) *Cohort study on chronic atrophic gastritis (CAG) and intestinal metaplasia (IM) in Slovenia* (Dr N. Muñoz; in collaboration with Dr I. Matko and Dr A. Jutersek, University Clinical Center of Ljubljana, Yugoslavia; and Dr M.I. Filipe, School of Medicine, Guy's Hospital, London)

A cohort study of subjects with CAG and IM carried out by the Agency in the early 1970s showed an increased risk for gastric cancer. However, only a minor fraction of individuals with these lesions would eventually develop stomach cancer; it is therefore of great importance to identify those subjects with IM who have the highest risk. Dr Filipe has recently proposed a classification of IM into three types, which are suspected to have different malignant potentials: type I or complete IM is characterized by the presence of mature absorptive cells, paneth and goblet cells secreting sialomucins; type II or incomplete IM is characterized by few or no absorptive cells, columnar cells secreting neutral or acid sialomucins and goblet cells secreting sialomucins and occasionally sulphomucins; type III or incomplete IM involves columnar cells secreting predominantly sulphomucins. Cross-sectional studies suggest that type III IM might have a higher malignant potential than the other two types.

To assess the risk of gastric cancer associated with these three types of IM, a cohort study was initiated in Slovenia in September 1985. Over 10 000 gastric biopsies taken between 1967 and 1976 were reviewed to identify those with IM. A total of 1650 patients in whom IM was found in one or more gastric biopsy were identified, and two sections of each paraffin block were cut and stained with Alcian blue/periodic acid-Schiff and with high iron-diamin/Alcian blue to identify sialomucins and sulphomucins. Histological evaluation of about 5000 slides from 1298 patients has been completed, and the preliminary results are shown in Table 26. The proportion of IM type III (28%) is higher than that reported from other European populations (about

Table 26. Distribution of intestinal metaplasia (IM) types among gastric biopsies in Slovenia

Year of diagnosis	IM-I	IM-II	IM-III	I or II and III	Total
1967	11	2	4	5	22
1968	5	1	4	2	12
1969	5	2	3	0	10
1970	13	5	8	2	28
1971	31	18	24	16	89
1972	72	27	33	14	146
1973	110	34	45	11	200
1974	143	62	39	17	261
1975	136	59	27	29	251
1976	148	56	39	36	279
Total No.	674	266	226	132	1298
%	51.9	20.5	17.4	10.2	100.0

10%). The slides of the remaining 352 subjects are being processed. The cases of gastric cancer and other malignant tumours occurring in this cohort will be identified by matching them to the Cancer Registry files.

(2) *Precancerous lesions of the stomach in Huixian, China* (Dr N. Muñoz; in collaboration with Dr Lu Jian-Bang, Dr Yang Guan-Rei and Dr Qu Song-Lang, Henan Cancer Institute and Henan Medical College, Henan, China; Professor M. Crespi and Dr A. Grassi, Institute Regina Elena, Rome; Dr D. Thurnham, Dudley Road Hospital, Birmingham, UK; Dr M. Hambidge, Medical Center, University of Colorado, Denver, CO, USA and Dr P. Correa, Louisiana State University Medical Center, New Orleans. LA, USA)

During the intervention study on precancerous lesions of the oesophagus carried out in Huixian (see I.3.*b*.i), gastroscopy was also performed on 248 of the 566 subjects on whom oesophagoscopy was done. Four biopsies were taken from the antral region 5 cm from the pylorus, and one from the fundus. The histological slides were read independently and blindly by three pathologists. As was the case for the oesophagus, no significant difference in the prevalence of the various precancerous lesions of the stomach was observed between the group that had received retinol, riboflavin and zinc for one year and the placebo group (Table 27).

(c) *Laryngeal and pharyngeal cancer in south-western Europe* (Dr A.J. Tuyns, Dr J. Estève, Dr E. Riboli and Mrs A. Arslan; in collaboration with Dr A. Zubiri, Cancer Registry of Zaragoza, Spain; Dr A. Del Moral and Dr N. Ascunce, Health Department of Navarra, Pamplona, Spain; Dr B. Terracini, Institute of Pathology, University of Turin, Italy; Dr F. Berrino, National Cancer Institute, Milan, Italy; Mr L. Raymond, Geneva Cancer Registry, Switzerland; Dr W. Lehmann, Geneva University Hospital, Switzerland; and Dr H. Sancho-Garnier and Dr E. Benhamou, Gustave Roussy Institute, Villejuif, France)

Table 27. Histological diagnosis of gastric lesions by treatment group in Huixian, China

Histological diagnosis	Vitamins		Placebo		Total no.
	No.	%	No.	%	
Normal	17	13.2	21	17.2	38
Superficial gastritis	3	2.3	5	4.1	8
Diffuse interstitial gastritis	9	7.0	10	8.2	19
Chronic atrophic gastritis (CAG)					
Mild	49	38.0	51	41.8	100
Moderate	22	17.0	20	16.4	42
Severe	1	0.8	0	0.0	1
CAG with intestinal metaplasia (IM)					
IM mild	16	12.4	4	3.3	20
IM moderate	6	4.6	6	4.9	12
IM severe	0		0		0
IM with dysplasia					
Hyperplastic dysplasia	5	3.9	3	2.5	8
Adenomatous dysplasia	1	0.8	2	1.6	3
Total	129	100.0	122	100.0	251

Table 28. Combined effects of alcohol and tobacco; relative risk and number of cases

Alcohol per day (g)	Cigarettes per day				Total (RR for alcohol)
	0–7	8–15	16–25	26+	
Endolarynx					
0–40	1.0 (13)	6.68 (44)	12.72 (95)	11.47 (37)	1.0 (189)
41–80	1.65 (18)	5.94 (39)	12.23 (94)	18.51 (40)	1.10 (191)
81–120	2.31 (9)	10.70 (38)	21.01 (82)	23.55 (35)	1.78 (164)
121+	3.78 (10)	12.20 (26)	31.55 (86)	43.21 (61)	2.66 (183)
Total (RR for tobacco)	1.0 (50)	4.51 (147)	9.26 (357)	11.14 (173)	(727)
Hypopharynx					
0–40	1.0 (4)	4.65 (9)	13.91 (27)	4.90 (5)	1.0 (45)
41–80	2.99 (10)	14.58 (32)	19.54 (42)	18.43 (15)	2.18 (99)
81–120	5.52 (7)	27.47 (28)	48.25 (52)	37.62 (22)	4.63 (109)
121+	14.67 (11)	71.59 (39)	67.81 (56)	135.46 (50)	10.18 (156)
Total (RR for tobacco)	1.0 (32)	4.89 (108)	7.20 (177)	7.32 (92)	(409)

A multinational population-based case-control study of cancer of the larynx and hypopharynx was carried out in France, Italy, Spain and Switzerland in order to study the role of alcohol and tobacco and their combined effect on the risk of these cancers. Additional hypotheses concerned the possible role of occupation, diet, kind of tobacco and kind of alcoholic beverages. Special attention was also given to the study of the risk at various subsites of the larynx and hypopharnx. The effect of cigarette smoking is similar for all subsites and the risk for 'ever' smoking is of the order of 10. The risks for alcohol drinking depend on subsite: they are similar for the epilarynx and hypopharynx (10.6 and 12.5, respectively, for drinking more than 120 g of ethanol per day relative to less than 20 g per day) and smaller for the endolarynx (2.6 for the same categorization). The multiplicative model provides a good description of the simultaneous effects of alcohol and tobacco for the endolarynx and for the hypopharynx/epilarynx combined (Table 28). The effect of alcohol is at least as strong for very light smokers as for heavy smokers. It was also demonstrated that, both for the endolarynx and for the hypopharnx/epilarynx, people who smoke only black tobacco have twice the risk of those who smoke only blond tobacco[61]. Important information on the distribution of risk factors in the six populations of the study was

[61] Tuyns, A.J., Estève, J., Raymond, L., Berrino, F., Benhamou, E., Blanchet, F., Boffetta, P., Crosignani, P., Del Moral, A., Lehmann, W., Merletti, F., Péquignot, G., Riboli, E., Sancho-Garnier, H., Terracini, B., Zubiri, A. & Zubiri, L. (submitted for publication)

gathered and analysed. Quantitative information on dietary habits showed in particular a wide variation in the polyunsaturated:saturated fatty acid ratio, ranging from 0.27 in Calvados to 0.54–0.65 in the two populations of Spain. It confirms also the high prevalence of heavy drinkers in these populations, in which up to 36% of men drink more than 80 g ethanol a day. Detailed information on the smoking history of each individual allowed a comparison of smoking habits in successive generations of smokers. It confirms that the tobacco epidemic is still increasing in those areas where it began more recently, especially in Spain[62–64].

(*d*) *Nasopharyngeal cancer and foodstuffs* (Dr S. Poirier, Dr Y.M. Shao, Dr H. Ohshima, Dr C. Malaveille and Dr H. Bartsch; in collaboration with Dr G. de-Thé and Dr A. Hubert, Faculty of Medicine A. Carrel, Lyon, France; and Professor Y. Zeng, Institute of Virology, Chinese Academy of Preventive Medicine, Beijing)

Nasopharyngeal cancer (NPC) shows a marked world-wide geographical and ethnic distribution and is particularly prevalent among three widely different populations: Chinese in South-east Asia, Arabs in North Africa and Eskimos in the Arctic. Etiological factors that have been associated with NPC include infection by Epstein–Barr virus and genetic and environmental factors. Among the latter, consumption of salted fish was found in epidemiological case-control studies to be a significant risk factor for NPC in southern China[65]. Therefore, representative food items were collected from Tunisia, Greenland and south China (Macao) and analysed for volatile nitrosamines[66].

Relatively high levels of *N*-nitrosodimethylamine were found in Tunisian dried mutton preserved in olive or soya oil and in stewing base. Similar levels were found in Eskimo dried, but not salted, fish samples. High levels were also observed in Chinese hard and soft salted fish samples as well as in fermented vegetables in brine. *N*-Nitrosopyrrolidine and *N*-nitrosopiperidine were also detected at various levels in Chinese vegetables and in various samples from Tunisia. From the present results, it is difficult to conclude whether volatile nitrosamines are important factors in the etiology of NPC, because exposure to nitrosamines formed endogenously has not been appropriately evaluated. In addition, daily intake by high-risk populations of preformed nitrosamines through ingestion of foods has not been estimated in case-control studies.

Experiments are in progress to measure the mutagenicity of aqueous, hexane and ethylacetate extracts of the selected preserved foods in the *Salmonella* assay, the SOS-function inducing activity in *E. coli* and the EBV early antigen inducing activity in Raji cells. To reveal the presence in food of aqueous extracts of precursors of nitroso compounds with genotoxic and EBV reactivating activity, assays are also performed after chemical nitrosation.

(*e*) *Cervical cancer*

(i) *Male sexual behavour and human papilloma virus in high- and low-risk areas for cervical cancer* (Dr N. Muñoz, Dr F.X. Bosch, Dr J. Kaldor, Ms N. Charnay and Ms D. Magnin; in collaboration with Dr L. Tafur and Dr N. Aristizabal,

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[62] Riboli, E., Péquignot, G., Repetto, F., Axerio, M., Raymond, L., Boffetta, P., Zubiri, A., Del Moral, A., Estève, J. & Tuyns, A.J. (submitted for publication)

[63] Berrino, F., Merletti, F., Zubiri, A., Del Moral, A., Raymond, L., Estève, J. & Tuyns, A.J. (submitted for publication)

[64] Péquignot, G., Crosignani, P., Terracini, B., Ascunce, N., Zubiri, A., Raymond, L., Esteve, J. & Tuyns, A.J. (submitted for publication)

[65] Armstrong, W., Armstrong, M.J., Yu, M.C. & Henderson, B.E. (1983) *Cancer Res., 43,* 2967–2970

[66] Poirier, S., Ohshima, H., Hubert, A., Bourgade, M.C. & Bartsch, H. (1987) *Int. J. Cancer, 39,* 293–296

Department of Medicine, University of Valle, Cali, Colombia; Dr N. Ascunce, Institute of Public Health, Pamplona, Spain; Dr M. Gili, Faculty of Medicine, Seville, Spain; Dr L.C. Gonzalez, Office of Social Welfare, Salamanca, Spain; Dr I. Izarzugaza, Department of Health and Social Security, Vitoria, Spain; Dr C. Martos, Department of Health, Zaragoza, Spain; Dr C. Navarro, Department of Health, Murcia, Spain; and Dr P. Viladiu, St Catarina Hospital, Gerona, Spain)

A pilot study carried out in three cities of Spain (Gerona, Sevilla and Zaragoza) and in Cali, Colombia, between May 1985 and January 1986 showed that it was possible to set up a case-finding network to identify virtually all incident cases of invasive cervical cancer before they were treated, that the questionnaire and collection of specimens (blood and exfoliated cells from the uterine cervix of cases and controls and from the penis of their partners) was in general well accepted (although the randomly identified population controls in Spain were not as responsive as the other study groups), and that the exfoliated cells collected were suitable for human papilloma virus (HPV)-DNA hybridization tests.

On the basis of the results obtained in the pilot phase, the questionnaire was slightly modified, strategies to improve the collaboration of the population controls were considered and the sample size was increased to include at least 300 cervical cancer cases in each country (150 in-situ and 150 invasive cancers), 300 age-matched controls and the male partners of cases and controls.

Table 29. Females expected, interviewed and in whom specimens have been collected in Cali, Colombia, July 1985–June 1987

Group	Number of cases		Number interviewed		Number with cells		Number with sera	
	Expected	Observed	Cases	Controls	Cases	Controls	Cases	Controls
In-situ cancer	240	256	207	187	178	177	202	184
Invasive cancer	260	159	127	133	103	129	123	131
Total	500	415	334	320	281	306	325	315

A full-scale study involving nine centres in Spain (Alava, Gerona, Guipuzcoa, Murcia, Navarra, Salamanca, Sevilla, Vizcaya and Zaragoza) and Cali, Colombia, was initiated in June 1986. A training course for interviewers was held in Gerona in June 1986, and case ascertainment began in June 1986 in the new centres. In April 1987, a mid-term meeting of coordinators was held in Lyon to review progress. Tables 29 and 30 summarize the degree of coverage achieved in Cali and the number of subjects identified, interviewed and from whom specimens have been collected up to June 1987. Similar data are shown for Spain in Tables 31 and 32. The

Table 30. Male partners expected, interviewed and from whom specimens have been collected in Cali, Colombia, July 1985–June 1987

Group	Number of cases		Number interviewed		Number with cells		Number with sera	
	Expected	Observed	Cases	Controls	Cases	Controls	Cases	Controls
In-situ cancer	256	136 (53%)	96	99	79	85	87	92
Invasive cancer	159	73 (46%)	39	62	28	46	35	54
Total	415	209 (50%)	135	161	107	131	122	146

Table 31. Females expected, interviewed and from whom specimens have been collected in Spain; June 1986–June 1987

Group	Number of cases		Number interviewed		Number with cells		Number with sera	
	Expected	Observed	Cases	Controls	Cases	Controls	Cases	Controls
In-situ cancer	153	183	157	78	144	73	156	74
Invasive cancer	254	183	147	95	135	76	145	87
Total	407	366	304	173	279	149	301	161

Table 32. Male partners expected, interviewed and from whom specimens have been collected in Spain; July 1985–June 1987

Group	Number of cases		Number interviewed		Number with cells		Number with sera	
	Expected	Observed	Cases	Controls	Cases	Controls	Cases	Controls
In-situ cancer	175	132	118	64	106	58	111	59
Invasive cancer	174	114	92	62	73	46	81	54
Total	349	246	210	126	179	104	192	113

low ratios of observed:expected are probably due to errors in the estimation of expected numbers. One considerable difficulty that has emerged is the low proportion of women in Colombia who had a husband or sexual partner at the moment of the interview.

Since the group of subjects who will be the most informative for evaluation of the male role will be couples in which the woman has been monogamous throughout her lifetime; these preliminary findings might imply an extension of the study period further than December 1987.

The laboratory results obtained during the pilot phase suggest that the sensitivity of the two hybridization techniques used to determine the presence of HPV-DNA in cervical and urethral scrapings might differ according to differences in the physical status of the virus or viral products. There have been suggestions that for the same small amount of HPV-DNA, the methods that involve DNA extraction (Southern blot and dot blot) are more sensitive when there is a low copy number of viral DNA per cell, as appears to be the case in most cervical cancers, while the filter in-situ method is more sensitive when the copy number is high in relatively few cells, as is found in condyloma and normal cervix. It was therefore suggested that a validation study of the two hybridization tests (filter in-situ and dot) be considered before deciding which technique to use on the biological specimens collected in the study.

(ii) *Cervical cancer and human papilloma virus: case-control studies using prospectively collected smears* (Dr N. Muñoz, Dr M. Casas-Cordero, Dr M. Hollstein and Dr F.X. Bosch; in collaboration with Dr J.E. MacGregor, Department of Pathology, University of Aberdeen, Scotland, UK; and Dr E. Lynge, Danish Cancer Registry, Copenhagen, FI/92/3)

The in-situ hybridization test for HPV-DNA in fixed tissue sections and smears is being set up at the Agency to be used in two case-control studies nested within a cohort. In the Grampian region of Scotland, the 50 most recent cases of cervical cancer, with at least one smear taken five years or more before the diagnosis of cancer, were selected from 115 cases diagnosed during 1968 to 1982. Four controls were selected per case, matched by age and date of the first smear of

the case. In Denmark, 60 women who had developed invasive cervical cancer up to December 1985, and who had had at least one negative smear taken between 1966 and 1978, were selected from a screening cohort of 27 811 women from the old Maribo county. Five control women, matched for age, were selected for each case, who had had the same number of negative smears, had been followed up for at least the same interval since the last negative smear as the case and had not developed cervical intraepithelial neoplasia during the follow-up period.

Tissue sections from the 110 cervical cancers and about 1100 smears (500 from the 50 women with cervical cancer and the 200 control women in Scotland, and about 600 smears from 60 women with cervical cancer and 300 control women in Denmark) will be tested for HPV-DNA using tritium-labelled probes.

(*f*) *Breast cancer*

(i) *Breast cancer and reproductive and endocrinal factors in premenopausal Chinese, Chinese–American and Caucasian–American women* (Dr A.J. Sasco, Dr E. Riboli and Dr R. Saracci; in collaboration with Professor M.X. Hu and Dr B.H. Zhen, Sun Yat Sen University of Medical Sciences, Guangzhou, China, DEC/86/11; Dr D.P. Rose and Dr N.J. Haley, American Health Foundation, Valhalla, NY, USA, DEC/86/10; and Dr P. Toniolo, New York University, New York, NY, USA)

The aim of the study is to evaluate the relationship between hormonal profiles and breast cancer incidence in females. A main focus is on testing the 'free-oestrogen' hypothesis of breast cancer etiology as a unifying way of explaining epidemiological evidence of increased risk for nulliparous women, women with a late first birth, with anovulatory cycles, early menarche and late menopause, as well as the observed association of breast cancer with other hormone-dependent cancers, such as cancers of the ovary and endometrium.

The study uses a case-control approach and also permits comparisons of control women among population groups with contrasting incidence of the disease. Study groups will be selected from the population of Guangdong province in China, the Chinese population of New York City in the USA, and the Caucasian population of New York City.

Incident cases, all of them premenopausal, will be selected from each population group. Control women are pair-matched to the cases on the basis of age ($\pm$ two years), race and residence. Women taking contraceptive pills or any other hormonal treatment, reserpine or tranquillizers are excluded, as are women having or having had a pregnancy in the preceding 12 months, whether carried to full term or ending in a spontaneous or induced abortion, women having lactated in the preceding six months, and women having documented hormonal disease, gynaecological conditions or chronic debilitating conditions.

A detailed questionnaire is administered to cases and controls, including the following items: personal identification data, details of diagnosis, reproductive life history, age at menarche, personal history of diseases, family history of cancer, diet history and other factors. Saliva and blood specimens are collected between day 20 and day 24 of the menstrual cycle. Laboratory determinations are to be carried out at the American Health Foundation in New York City of testosterone, oestradiol, progesterone, prolactin and growth hormone.

(ii) *European case-control study of male breast cancer* (Dr A.J. Sasco and Dr R. Saracci; in collaboration with Professor D. Trichopoulos, University of Athens Medical School, Athens; and Dr D.P. Rose and Dr N.J. Haley, American Health Foundation, Valhalla, NY, USA, DEB/86/14)

Given the rarity of the disease and the exploratory nature of the present study, which is aimed at testing several hypotheses regarding the etiology of breast cancer in males, an

international case-control approach has been chosen. The study will evaluate the role of reproductive life, personal history of disease and drug use, family history of cancer, tobacco and alcohol consumption, nutritional habits, body build and hepatic function. Of specific interest will be the evaluation of the etiological role of hormones.

Several case-control groups will be assembled in participating European centres. The aim will be to enrol in each population group 15 breast cancer cases, at least ten of which are incident cases. The matching ratio will be three controls per case, leading to an enrollment of 45 control subjects per study centre. Cases and controls will be matched for age (age of the case ± two years).

A detailed questionnaire will be administered to cases and controls, including the following items: personal identification data, data on diagnosis, reproductive and sexual life history, life-long history of hormonal use, personal history of diseases, family history of cancer, nutritional and personal habits, body build. Blood samples will be collected for determination of hormonal profiles (androgens and oestrogens) and hepatic function. The logistical aspects of the study are currently being worked out.

(iii) *Survey of breast cancer in the Département du Rhône* Dr A.J. Sasco; in collaboration with Dr B. Fontanière, Léon Bérard Centre, Lyon, France; Professor J. Fabry, Claude Bernard University, Lyon, France; and Dr V. Sciortino, Social Security, Lyon, France)

No population cancer registry exists for the Département du Rhône. The present survey is aimed at estimating the incidence of breast cancer, the distribution of stage at diagnosis, and the histology of all cases diagnosed in 1985 to gain preliminary information useful to the establishment of a registration scheme as an instrument to evaluate a screening programme now under development.

(*g*) *Bladder cancer*

(i) *Temporal aspects* (Dr J. Estève; in collaboration with Dr P. Vineis, Turin, Italy)

The influence of various aspects of smoking on the risk of bladder cancer was examined by re-analysing a case-control study carried out in Turin. It was demonstrated that duration of smoking was a more important determinant of risk than number of cigarettes; the effect of age at start and quitting was also considered both for smoking and occupational exposure in relation to models of multistage carcinogenesis[67].

(ii) *Bladder cancer in Argentina* (Dr J. Estève, Dr N. Muñoz and Ms A. Arslan; in collaboration with Dr J. Iscovich, La Plata, Argentina)

The analysis of a case-control study carried out in La Plata, Argentina, confirmed that smoking of black cigarettes (air-cured) has a greater impact on risk of bladder cancer than smoking of blond cigarettes (flue-cured). In this study, we also investigated the effect of consumption of various beverages. Coffee drinking had an effect which persisted after adjustment for smoking, but no relationship was found between bladder cancer risk and *maté* drinking. A significant association was also found for truck and railway drivers[68].

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[67] Vineis, P. & Estève, J. (1987) *Toxicol. Pathol., 15* (in press)

[68] Iscovich, J., Castelleto, R., Estève, J., Muñoz, N., Colanzi, R., Coronel, A., Deamezola, I., Tassi, V. & Arslan, A. (1987) *Int. J. Cancer* (in press)

(iii) *Excretion of mutagens thioethers and N-nitroso compounds in the urine of nonsmokers and smokers of blond tobacco and of black tobacco* (Dr M.C. Malaveille, Dr H. Oshima, Mrs G. Brun, Mrs A. Hautefeuille, Dr. H. Bartsch and Dr J. Estève; in collaboration with Dr P. Vineis, Dr G. Ronco and Dr B. Terracini, Institute of Anatomical Pathology, Turin, Italy)

Previous epidemiological studies suggested that the risk of cigarette smoking is greater among users of cigarettes made of black tobacco than among user of cigarettes made of blond tobacco. These findings led to experiments aimed at measuring the level of mutagens, thioethers and nitrosamines excreted in the urine of smokers. Two 24-h urine samples were collected over a three-day period and analysed for nicotine/cotinine, thioethers, *N*-nitroso compounds and creatinine. Urinary extracts for measurement of mutagenicity and SOS-inducing activity were prepared based on procedures described by Kriebel *et al.*[69] and Yamasaki and Ames[70]. The mutagenicity of the urine, as measured by number of revertants per mmol of creatinine, increased with nicotine/cotinine (Table 33) and there was a significant difference between black tobacco smokers and blond tobacco smokers after adjustment for the nicotine/cotinine content of urine.

Table 33. Mutagenicity of urine according to dose and type of tobacco[a]; geometric mean of number of revertants per mmol creatinine

	Nicotine/cotinine[b]				
	0	0–0.5	0.5–1.5	1.5–2.5	>2.6
Nonsmoker	127 (16)	11 (5)	—	—	—
Black tobacco smoker	—	—	5 820 (3)	8 169 (4)	12 301 (2)
Blond tobacco smoker	739 (2)	2452 (3)	2 040 (8)	5 739 (8)	9 114 (5)
Mixed smoker	—	—	3 392 (4)	—	3 193 (1)

[a] No. of subjects in parentheses

[b] μmol/mmol creatinine; intervals include upper limits

There is also a clear dose-effect relationship between dose of tobacco, as measured by nicotine/cotinine, and amount of thioethers excreted in the urine, but no difference between smokers of black cigarettes and smokers of blond cigarettes. There are less clear relations between either SOS activity or nitrosamine excretion and nicotine/cotinine content. The results of this study have been submitted for publication.

(iv) *Reappraisal of the role of tobacco smoke in bladder carcinogenesis* (Dr E. Cardis, Mr X. Nguyen-Dinh, Dr J. Estève and Dr N. Day; in collaboration with Dr R.A. Cartwright, Yorkshire Regional Cancer Organization, Leeds, UK; Dr S. Cordier, Unit of Statistical Research, National Institute for Health and Medical Research, Villejuif, France; Dr C. Gonzalez, Hospital Sant Jaume i Santa Magdalena, Barcelona, Spain; Dr D. Hémon, Unit of Statistical Research, National Institute

[69] Kriebel, D., Commoner, B., Bronsdon, A., Gold, J. & Henry, J. (1983) *Mutat. Res., 108*, 67–79

[70] Yamasaki, E. & Ames, B. (1977) *Proc. natl Acad. Sci. USA, 79*, 3555–3559

for Health and Medical Research, Villejuif, France; Dr J.R. Marshall, State University of New York at Buffalo, NY, USA; Dr O. Møller-Jensen, Danish Cancer Registry, Copenhagen; Dr S. Mommsen, Institute of Cancer Research, Aarhus, Denmark; Dr A.S. Morrison, Brown University, Providence, RI, USA; Dr J. Siemiatycki, Armand Frappier Institute, Laval, Quebec, Canada; Dr D. Silverman, National Cancer Institute, Bethesda, MD, USA; and Dr P. Vineis, Institute of Anatomical Pathology, Turin, Italy)

A meeting was held in 1984 at the Agency among researchers who had carried out case-control studies on bladder cancer, to re-evaluate the role of smoking in bladder carcinogenesis by re-analysing the data in a standard way. This analysis showed that the differences between the studies in the magnitude of the estimated risk is real: the risk is highest in Italy and Spain and lowest in the USA and UK. This finding is in agreement with the observed risk difference between black and blond tobacco. The effect of amount of tobacco smoked on the relative risk of bladder cancer is well described by the product of duration of smoking and logarithm of average consumption. Total lifetime consumption is not a good predictor of risk, and duration of smoking is the strongest explanatory variable for the relative risk for bladder cancer. The effect of stopping smoking is very strong, even when duration and average consumption are taken into account; the benefit does not, however, increase significantly with time since quitting. This suggests possible differences in the carcinogenic mechanisms of tobacco smoke at the level of the lung and of the bladder.

(v) *Bladder cancer and multiple risk factors in Spain* (Dr E. Riboli; in collaboration with Dr C. Gonzalez, Unit of Epidemiology and Biostatistics, Hospital Sant Jaume i Santa Magdalena, Barcelona, Spain; Dr G. Lopez-Abente, Unit of Preventive Medicine, Ramon y Cajal Centre, Madrid; Dr A. Escolar Pujolar, Public Health Service, Cadiz, Spain; Dr M. Errezola, State Public Health Service, Basque Government, Vitoria, Spain; Dr A. Companys, Municipal Health Service, Barcelona, Spain)

A preliminary study of bladder cancer was conducted in Mataro county in 1981–1982. Although the sample size was relatively small, it suggested that there were strong associations between bladder cancer and tobacco smoking and occupational exposures[71,72]. No association was found with coffee drinking. These results led to the setting up of larger study, adding other Spanish regions and a greater number of cases. A multicentre case-control study was started in 1985 in four centres (Barcelona, Cadiz, Madrid and the Basque country), with the participation of 13 hospitals. The following potential risk factors were studied: occupational exposures, tobacco smoking, passive smoking exposure, medical history of diabetes, urinary-tract infections, diet, coffee drinking and artificial sweetener use.

Data collection was completed in December 1986 with the inclusion of 497 cases and 1114 controls. Controls were matched to cases for sex, age and residence. Half of the controls were patients in hospitals and half were healthy subjects living near the cases. Statistical analysis is in progress, and the final results should be available by the end of 1987.

(*h*) *Descriptive epidemiology of selected sites of cancer*

(i) *International incidence of childhood cancer* (Dr D.M. Parkin and Mr A. Bieber; in collaboration with Dr G. Draper and Mr C. Stiller, Childhood Cancer Research

[71] Gonzalez, C.A., Lopez-Abente, G., Errezola, M., Castejon, J. Estrada, A., Garcia-Mila, M., Gili, P., Huguet, M., Serrat, M., Soler, F. & Rodriguez C. (1985) *Cancer, 55*, 2031–2034

[72] Gonzalez, C.A., Lopez-Abente, G. & Riboli, E. (1987) *Am. J. ind. Med.* (submitted for publication)

Group, University of Oxford, UK; Professor B. Terracini, University of Turin, Italy; and Dr J. Young, National Cancer Institute (SEER), Bethesda, MD, USA)

The *Cancer Incidence in Five Continents* series presents data derived from registries in many parts of the world by tumour site in five-year age groups. This format is a handicap to study of the important group of cancers occurring during childhood, for which categorization by histological type is much more relevant. For this project, therefore, participating registries were asked to send data on individual cases of childhood cancer (aged 0–14 years), including the site and histology of the tumour (with a verbal description of the diagnosis as well as ICD-O or other histology code), and basis of diagnosis. All centres provided a description of the area from which the registered cases were drawn, including, for population-based cancer registries, data on the size of the child population for the years in which the cases were registered.

Data for 81 populations from 72 registries (or groups of registries) were finally accepted for inclusion; the time period requested was 1970–1979, although several registries supplied more recent data. For most of these centres, incidence rates (per million children) could be calculated, although for 17 centres (mainly in Africa and Asia) the absence of a defined population at risk meant that only the relative frequency of the different tumour types could be calculated. Tumours were classified into 12 major groups and 40 subgroups, primarily according to their histology, based upon a modification of the scheme of the Manchester Childrens Tumour Registry, UK[73].

The results will be published as a monograph in 1988. The entry for each centre comprises a pair of tables (male, female) showing incidence (or relative frequency) by age, with summary age-standardized indices, accompanied by a description of the registry, a commentary on the results and references to previously published work. A set of summary tables compares the age-standardized rates for all the principal childhood cancers among the different participating centres. An example is given in Table 34.

Table 34. Wilms' tumour, cumulative incidence per million (0–14), both sexes

Registry	Incidence/10^9
USA — SEER registries: black	153
: white	121
Federal Republic of Germany: Childrens Tumour Registry	107
Denmark	102
Australia: New South Wales	101
Italy: Turin	98
England and Wales	97
Hungary	89
Costa Rica	77
India: Bombay	51
Singapore: Chinese	44
Japan: Miyagi	38

[73] Birch, J.M. & Marsden, H.B. (1987) *Int. J. Cancer* (in press)

(ii) *Malignant melanoma* (Mrs J. Nectoux and Dr C.S. Muir; in collaboration with Dr E. van der Esch, The Netherlands Cancer Institute, Amsterdam)

Two studies were completed in 1986–1987. The subsite distribution of cutaneous, ocular and internal malignant melanomas was studied in 39 population- and hospital-based cancer registries. Fairly large differences emerged between different geographical areas for cutaneous malignant melanomas and ocular malignant melanomas, whereas melanomas of the buccal cavity and genital organs are the most frequent mucosal sites in all areas. A report is in preparation.

In order to evaluate whether changes in diagnostic criteria for pigmented skin lesions could be responsible for the increased incidence of malignant melanoma, 13 laboratories were invited to contribute histological material for 1930, 1955 and 1980. Slides from approximately 2500 cases were received. The results in terms of benign, dubious benign, dubious malignant, in-situ and malignant lesions are undergoing evaluation.

(iii) *Laryngeal cancer* (Mrs J. Nectoux and Dr D.M. Parkin; in collaboration with Dr S. Wilson, Birmingham and West Midlands Regional Cancer Registry, UK; Dr M. Cotter, Cancer Registry of Wales, Cardiff, UK; Dr A.P. Mirra, São Paulo Cancer Registry, Brazil; Dr Y.T. Omar, Kuwait Cancer Control Centre; Dr D.J. Jussawalla, Bombay Cancer Registry, India; Professor S. Schraub, Doubs Cancer Registry, Besançon, France; Dr P. Schaffer, Bas-Rhin Cancer Registry, Strasbourg, France; and Professor J. Gaillard, Croix Rousse Hospital, Lyon, France)

There are interesting differences in the subsite distribution of cancers of the larynx and hypopharynx, as recorded in routine information from cancer registries. A review of available data is under way; however, because of difficulties in determining the exact point of origin of such tumours, and probable lack of comparability in reporting, a project is being planned to delineate more accurately the descriptive epidemiology of these tumours. This will involve up to ten registries in different parts of the world, with varying ratios of laryngeal : hypopharyngeal and glottic : subglottic cancers, as suggested by the review. Otorhinolaryngologists in each centre will assess the point of origin of each case registered.

(iv) *Colon cancer* (Mrs J. Nectoux and Dr D.M. Parkin)

Cancers from the caecum to the rectum are frequently considered together, but in fact tumours of different parts of the large bowel probably have rather distinct etiologies. A review of studies that have produced information on the changing subsite distribution of large-bowel cancer suggests that the patterns are quite different in western and in Japanese populations, and there may also be differences according to sex.

A review of the geographic variation in subsite distribution of colon cancer in the data submitted for *Cancer Incidence in Five Continents Volume V* is proposed. It is intended to follow up time trends in colon cancer incidence by subsite for selected registries, particularly those in which the site within the colon is not well specified for only a small proportion of cases.

(i) *Case-control studies network (the SEARCH programme)* (Dr P. Boyle and Dr R. Saracci)

The SEARCH (Surveillance of Environmental Aspects Related to Cancer in Humans) programme of the Agency emerged in its present form as a collaborative multicentre case-control study network from a meeting of experts held in Lyon in July 1982. The principal objective of SEARCH remains the identification and international epidemiological study of populations with disparate risks of cancer, of hypotheses on environmental hazards, 'life-style'

and host factors possibly related to cancer by a group of collaborative centres capable of conducting population-based case-control studies.

Through the SEARCH case-control study network, the Agency promotes the conduct of studies of cancers of current interest to the Agency and its collaborators. The most important goal of SEARCH is to permit replication of research protocols in dispersed and dissimilar populations, so that important findings are subjected to the test of reproducibility at an early stage. The programme offers collaborators an opportunity to obtain advice from peers at all phases of study design, implementation and analysis, and makes available both in-house and external technical experts who assist collaborators in conducting their studies.

The SEARCH collaborating centres have access to data on incident cases of cancer from populations of at least one million persons; they also have the possibility of identifying random population controls and interviewing both cases and controls; they have data processing facilities and local operating funds. The Agency provides funds and facilities for regular meetings of collaborating investigators, central administrative and scientific coordination, common data processing and ad-hoc technical expertise.

The choice of cancer sites to be investigated is determined by the interest of current active collaborators and by Agency staff in consultation with outside experts; these choices are further reviewed by the Scientific Council at its annual meetings. At present, four studies are in various stage of development: on cancers of the pancreas, bile duct and gall bladder; on brain tumours in children; on brain tumours in adults; and on breast and intestinal cancers in women.

(i) *Cancers of the pancreas, bile duct and gall bladder* (Dr P. Boyle and Dr R. Saracci; in collaboration with Dr H.B. Bueno de Mesquita, National Institute for Public Health, Bilthoven, The Netherlands; Professor N.W. Choi, Manitoba Cancer Treatment and Research Foundation, Winnipeg, Manitoba, Canada; Dr P. Baghurst, Commonwealth Scientific and Industrial Research Organization, Adelaide, Australia; Professor G. Howe, Epidemiology Unit, National Cancer Institute of Canada, Toronto, Ontario, Canada; Dr P. Ghadirian, Montreal Cancer Institute, Quebec, Canada; and Dr W. Zatonski, Institute of Oncology, Warsaw, DEB/84/12. Professor A.J. McMichael, Professor A.B. Miller and Dr A.M. Walker continue to participate in this study group.)

The project was initiated in 1983, with pilot studies to ascertain the feasibility of obtaining data relating to life-style — particularly dietary and personal habits. As described in the 1985 *Annual Report*[74], the principal hypotheses under consideration are that:

(1) regular exposure to stimulators of cholecystokinin release predisposes to pancreatic cancer;

(2) fats from animal sources are associated with risks that are identifiably different from those associated with fats from vegetable sources;

(3) the timing and variability of food and alcohol consumption are risk factors for disease, over and above the simple effects of total quantity of exposure; and

(4) the diabetes associated with pancreatic cancer is associated with age at onset and insulin-dependence patterns that reflect chronic islet-cell toxicity.

In addition, data on a variety of known or possible risk factors are also being collected, both to control for confounding in the analysis and to evaluate older observations.

[74] IARC (1985) *Annual Report 1985*, Lyon, p. 25

The phase of active data collection ended in June 1987, 700 cases and 850 controls having been entered into the study. A schema of data analysis has been prepared; and, after editing of the data base, preliminary results are expected from January 1988.

(ii) *Brain tumours in children* (Dr S. Preston-Martin, Dr P. Boyle, and Dr R. Saracci; in collaboration with Dr R. Peris Bonet, National Register of Childhood Tumours, Valencia, Spain; Dr N.W. Choi, Manitoba Cancer Treatment and Research Foundation, Winnipeg, Manitoba, Canada; Dr S. Cordier, National Institute for Health and Medical Research, Paris; Dr G. Filippini, D. Besta Neurological Institute, Milan, Italy; Dr D. Forman, Imperial Cancer Research Fund, Oxford, UK; Professor G. Howe, Epidemiology Unit, National Cancer Institute of Canada, Toronto, Ontario, Canada; and Professor B. Modan, Chaim Sheba Medical Center, Tel-Hashomer, Israel; DEB/84/12)

A detailed description of this study was given in the 1985 *Annual Report*[75]. In summary, the purposes of the study are:

(1) to evaluate the etiological role of exposure to *N*-nitroso compounds both *in utero* and during childhood, as well as to their precursors and modulators of their metabolism. Classes of exposure to be assessed include diet, drinking-water, drugs, supplementary vitamins, cosmetics, tobacco, alcoholic beverages and parental occupation;

(2) to quantify the impact of certain already known etiological factors (e.g., radiation and specific genetic syndromes); and

(3) to explore the relation between brain tumour occurrence and a variety of other personal characteristics and exogenous exposures of both the child and the child's parents (for example, head trauma, birth order, use of barbiturates, use of hair dyes, urban *versus* rural residence, ethnicity, exposures in certain industries and occupations, certain diseases in subjects and their relatives).

After definition and development of the protocol, including preparation of interviewer's and coder's manuals, data collection is now under way. It is expected that in order to achieve sample size sufficient to guarantee adequate power to the main statistical analyses, the enrolment of subjects will continue until the end of 1990.

(iii) *Brain tumours in adults* (Dr P. Boyle and Dr R. Saracci; in collaboration with Dr A. Ahlbom, National Institute of Environmental Medicine, Stockholm; Dr N. Choi, Manitoba Cancer Treatment and Research Foundation, Winnipeg, Manitoba, Canada; Professor G. Howe, Epidemiology Unit, National Cancer Institute of Canada, Toronto, Ontario, Canada; Dr R. Gurevicius, Institute of Epidemiology, Microbiology and Hygiene, Vilnius, Lithuanian SSR, USSR; Professor A.J. McMichael, Department of Community Medicine, University of Adelaide, Australia; Dr F. Menegoz, Cancer Registry of the Département de l'Isère, Meylan, France; Professor D. Trichopoulos, Department of Hygiene and Epidemiology, University of Athens Medical School, Athens; and Professor J. Wahrendorf, German Cancer Research Centre, Heidelberg, Federal Republic of Germany)

Etiological hypotheses about brain tumours in children, as outlined above, are closely related to those about brain tumours in adults. Most aspects of study design, including case and control acquisition, questionnaire formulation and time periods of interest, are, however, different.

[75] IARC (1985) *Annual Report 1985*, Lyon, p. 26

Accordingly, a second protocol has been formulated for brain tumours in adults. Potential collaborators have been identified, and a first organizational meeting was held in January 1986. At the most recent meeting held in Lyon in June 1987, minor modifications were made to the final core questionnaire, and it was reported that data collection was under way in most of the eight participating centres. Subject enrolment is expected to extend until 1989.

(iv) *Breast and intestinal cancers in women*

Present SEARCH collaborators, in conjunction with Agency staff, identified several cancer sites as being of priority for incorporation into SEARCH, primarily to replace the study of pancreatic cancer, in which data collection is due to end during 1987. These proposals, with others submitted from individuals not actively involved at present in SEARCH, were considered by the SEARCH Advisory Group in Lyon in May 1987. There emerged consensus from this meeting that priority should be given to an international study of breast cancer and colorectal cancer in women. It was also felt that such a study provided a suitable opportunity to perform an international study of adenocarcinoma of the small intestine, the etiology of which is virtually unknown, in view of its relative rarity.

Specific epidemiological issues to be addressed in this collaborative study include:

(1) the role of alcohol intake, in whatever form, in increasing the risk of both breast cancer and colorectal cancer;

(2) the association between total fat intake, saturated fat intake, cholesterol intake, unsaturated fat intake and total caloric intake on the risk of both forms of cancer;

(3) the role of reproductive factors, such as age at first birth and parity, on the risk of breast cancer and (right-sided) colon cancer;

(4) the possible protective effect of frequent consumption of salad vegetables on both forms of cancer;

(5) the role of 'ever' use of oral contraceptives in colon cancer and, particularly, the role of long duration of use of oral contraceptives prior to a first full-term pregnancy or at an early age on influencing breast cancer risk.

In addition, data on a variety of known or possible risk factors will be collected, both to control for confounding in the analysis and to evaluate older observations.

The first exploratory meeting of collaborators in this study will take place in Lyon in December 1987. A large number of international centres has expressed interest in participating in this study, and special attention will be given to incorporating centres in areas of the world where women receive less than 30%, and even less than 25%, of their total caloric intake from fat sources.

(v) *Technical support for collaborators*

In conjunction with the SEARCH programme, a number of projects has been and is being carried out with the goal of providing needed technical or informational support to collaborators. Many of these were listed in the *Annual Report* 1985[76]; new activities in this direction include:

(1) preparation of critical literature reviews covering the relation of breast cancer to a variety of putative risk factors;

(2) use of incomplete data to construct a list of food sources of nitrate and nitrite in Spain;

[76] IARC (1985) *Annual Report 1985,* Lyon, p. 27

(3) provision of funds and resources for the training of interviewers in certain collaborative centres; and

(4) provision of specific nutritional assistance to collaborating centres.

4. NUTRITION AND CANCER

(*a*) *Methodological study on dietary assessment methods and related biochemical parameters* (Dr E. Riboli, Dr R. Saracci, Ms B. Charnay and Ms G. Burnod; in collaboration with Dr E. Callmer, Department of Medical Nutrition, Huddinge University Hospital, Huddinge, Sweden; Dr F. Lindgarde, Department of Medicine and Dr B. Akesson, Department of Clinical Chemistry, University of Lund, Malmö, Sweden, DEB/85/05)

In relation to the planning of a prospective sudy in Malmö (Sweden) a methodological study was set up in 1984. The main aims of the study are:

(1) to provide information helpful in selecting those dietary assessment methods most appropriate to the accurate characterization of an individual's usual diet and at the same time simple enough to be used in a very large population study;

(2) to study usual diet and seasonal variations in a random sample of middle-aged men and women in Malmö;

(3) to explore, by repeated measurements, the relation between dietary assessment and biochemical indices of nutritional intake; and

(4) to test adherence and drop-out rates and the feasibility of dietary and biochemical investigations in terms of time, manpower and logistic organization.

The study was carried out in Malmö in the section of Preventive Medicine of the Department of Medicine. A random sample of the Malmö population, 450 men and 450 women in the age group 50–69, were invited to attend the Malmö Health Screening Programme and to participate in a one-year dietary study. About 60% of those invited attended, and almost 50% agreed to participate in the dietary study. The study design allows comparison of three different self-administered dietary questionnaires to a reference method. The reference method is based on weighed recording of foods consumed for three consecutive days over six periods of one year, from September 1984 until October 1985. During these 18 days, the subjects were asked to weight (using an electronic digital scale) or to measure all food and drink consumed. Food consumed outside the home was also measured and, when possible, weighed.

Biochemical measurements were included in the study to validate the reference method. In a subsample of 69 subject, 24-h urine samples were collected eight times during the study for measurement of urinary nitrogen as a marker of protein intake. *para*-Aminobenzoic acid was given to the subjects during the last urine collection period, and measurements of its recovery in urine will provide an estimate of completeness of the samples. Adipose tissue biopsies were taken from about 100 subjects at the end of the study period. Fatty acid composition of adipose tissue is a good indicator of the type of fat consumed during the preceding one to two years and will be used to validate self-reported fat intake. Blood samples were taken at the beginning and at the end of the study for all subjects and during each of the six diet recording periods. Analyses were performed for retinol, α-tocopherol, lecithin fatty acids, ascorbic acid, selenium and carotenoids.

The dietary methods to be tested in the study are:

(1) a short food frequency questionnaire mailed with the invitation letter (Method F);

Table 35. Study design: dietary assessment methods followed by each subgroup

Subgroup	Validity study				Repeatability study	
	1	2	3	4	5	6
At invitation	F_1	F_1	F_1	F_1	F_1	F_1
After health screening, 1984	A_1	A_1	B_1	B_1	A_1	B_1
Two months later	—	—	—	—	F_2	B_2
For 12 months	Reference method				—	—
Second invitation	F_3	F_3	F_3	F_3	F_3	F_3
After health screening, 1985	A_3	B_3	B_3	A_3	A_3	B_3
Total number of subjects in each subgroup at the end of the study	59	53	58	50	66	69
Total number of subjects per substudy	220				135	

(2) a self-administered dietary history questionnaire (280 questions) asking for frequency of consumption and serving size for each item. A book containing visual examples of three to four portion sizes for several foods and beverages was used by the subjects to estimate their usual intake (Method A); and

(3) a new method constructed for this study which includes both a retrospective (dietary history) and prospective (14-day diet diary) measurement of diet (Method B).

Subjects were first randomized into two sections — one to estimate validity, the other for reproducibility. They were then randomized into subgroups with different sequences and repetitions of Methods F, A and B, according to the schema shown in Table 35.

Data analysis is in progress, and results will be available by the end of 1987.

(*b*) *Cancer of the large bowel in southern Europe*

(i) *Rome* (Dr E. Riboli and Dr D.G. Zaridze; in collaboration with Dr M. Crespi, Dr M. Caperle, Dr M. Tarquini and Dr V. Ramazzotti, Regina Elena Institute, Rome, DEB/81/040; and Dr M. Hill, Public Health Laboratory Services, Centre for Applied Research, Salisbury, Wilts, UK, DEB/81/041)

A pilot study on 50 subjects has been carried out to evaluate and compare the feasibility and reliability of a food frequency questionnaire and a seven-day diet diary. The results of the pilot study indicate that the questionnaire is much better accepted than the diary by the study subjects: none refused to answer the questionnaire, whereas 40% did not fill out the diary. Among those who complied with both methods, there was a fair to good correlation between the two with regard to consumption of major foods. On this basis, the questionnaire, with the addition of questions on cooking style, seasonal consumption and changes in dietary habits during the last 10–15 years, was chosen for the main study[77].

[77] Riboli, E., Caperle, M., Sabatino, C. & Crespi, M. (1986) *Ital. J. Gastroenterol., 18,* 245–248

The case-control study was started in 1985. To date, 100 cases and 120 controls have been interviewed, and samples of faeces and blood have been collected from all participating subjects. In a subgroup of subjects, biopsies of colon mucosa have also been taken for investigation of cell kinetics. Cases are subjects in whom polyps of the colon or rectum had been diagnosed endoscopically. Two sets of controls are enrolled: subjects found free of polyps at colonoscopy and subjects attending local screening programmes who did not undergo endoscopy. Data collection will be completed by the end of 1987, and results should be available in 1988.

(ii) *Marseilles* (Dr E. Riboli and Mr R. Kaaks; in collaboration with Dr G. Macquart-Moulin and Dr J. Cornée, Unit of Research on Digestive Pathology, National Institute for Health and Medical Research U31, Marseille, France)

Two case-control studies were started in parallel during 1979–1980 in the Marseille metropolitan area — the first on cancers and the second on adenomatous polyps of the colon and rectum. The study on cancers was completed and analysed in 1985[78]. It included 399 matched controls. Results indicated an increased risk associated with low intake of vegetable fibres, potassium, vitamin C, oil and several vegetables.

Data collection for the study on adenomatous polyps was completed in 1986[79]. Differences in usual past diet were investigated between 252 subjects with newly diagnosed adenomatous or villous polyps of the colon and rectum and a group of 238 hospital controls. Cases and controls were interviewed in hospital by three nutritionists, using a dietary history questionnaire focused on the diet during the preceding year. Nutrient intake was estimated by means of ad-hoc food tables adapted from French and British tables. Values for the fibre content of bread and other cereal products were based on ad-hoc laboratory analyses of local foods performed by Dr D.A.T. Southgate and his colleagues (MRC Dunn Nutritional Unit, Cambridge, UK). Out of 16 food groups considered in the analyses, the cases reported lower consumption of oil and potatoes and higher consumption of sugar added to food and drink. Among nutrients, cases had a lower consumption of carbohydrates (not taking into account added sugar), potassium, magnesium and vitamin B_6. Cases had a slightly lower intake of total fibre and a slightly higher intake of saturated fat, although neither was statistically significant. Intake values for fibre and for carbohydrates were highly intercorrelated, and, due to measurement errors, the effect of one may be masked by the other and *vice versa*.

The relation with fibre intake was further investigated by breaking down total fibre into four subgroups: from vegetables, from fruit, from potatoes and from cereals and flour. While there is no association of fibres from cereals and flour with the risk of colorectal polyps, the three other groups of fibre showed a consistent tendency towards a reduction in risk with increasing levels of consumption (Table 36). The results of this study point to the fact that some component of carbohydrates (starches, natural sugars and fibres) plays a protective role in relation to the biology of tumours of the intestinal tract. Analyses of the association between consumption of alcohol and tobacco and cancers and polyps of the colon and rectum is now in progress, and final results will be available by the end of 1987.

(c) *Diet-related case-control studies in Singapore* (Dr N. Day and Dr J. Estève; in collaboration with Dr H.P. Lee, Ms L. Gourney and Dr S. Duffy, Singapore Cancer Registry)

The changes in life style that have occurred in recent years in Singapore and the concurrent changes in cancer incidence make this country ideally suited for studying the relationship

[78] Macquart-Moulin, G., Riboli, E., Cornée, J., Charnay, B., Berthezène, P. & Day, N. (1986) *Int. J. Cancer, 38*, 183–191

[79] Macquart-Moulin, G., Riboli, E., Cornée, J., Kaaks, R. & Berthezène, P. (1987) *Int. J. Cancer, 40* (in press)

Table 36. Relative risk of colorectal adenomatous polyps associated with consumption of selected foods and nutrients. The four levels correspond to the quartile distribution. RR are adjusted for age, sex, calories and weight

	RR_1	RR_2	RR_3	RR_4	χ^2 trend	p
Oil	1.0	1.50	0.83	0.64	4.1	0.043
Sugar and sweets	1.0	0.94	1.32	1.98	6.7	0.010
Potatoes	1.0	0.85	0.51	0.39	12.2	<0.001
Carbohydrates	1.0	0.76	0.52	0.33	7.0	0.008
Sugar added to food and drink	1.0	1.20	1.01	2.17	4.8	0.028
Fibre						
from vegetables	1.0	0.63	0.58	0.61	3.3	0.07
from fruit	1.0	1.66	0.86	0.69	3.9	0.048
from potatoes	1.0	0.79	0.46	0.38	13.8	<0.001
from cereals and flour	1.0	0.62	1.13	1.38	0.1	Not significant

between diet and cancer. A dietary research team was set up in Singapore in 1984, and a survey on dietary habits and food availability permitted the design of a semiquantitative food frequency questionnaire adapted to the style of eating among Singaporeans and specifically conceived to measure fat and fibre consumption. A case-control study of colorectal cancer was carried out, and data on 150 cases and 300 controls will be analysed at the end of 1987. A case-control study on breast cancer using a slightly different version of this questionnaire is under way and should be completed during 1988. The feasibility of a case-control study on nasopharyngeal cancer is also being investigated.

(*d*) *Aflatoxins*

(i) *Detection of aflatoxins in human breast milk by immunoassay* (Dr C. Wild, Miss B. Chapot, Dr F. Pionneau and Dr R. Montesano; in collaboration with Dr C.J. Chetsanga and Mr C.F. Mutiro, Biochemistry Department, University of Zimbabwe, Harare; Dr A. Hall, Medical Research Council Laboratories, Fajara, Gambia)

Several epidemiological studies have shown a positive association between dietary exposure to aflatoxins and an increased incidence of HCC. One area in which little information is available is exposure of newborn children to aflatoxins in mothers' milk. An enzyme-linked immunosorbent assay (ELISA) to detect aflatoxins in human breast milk has been developed which allows the quantification of 2 pg aflatoxin M_1 per ml of milk, using less than 10 ml of sample. A good correlation was observed between ELISA and a high-performance liquid chromatography-fluorescence technique using naturally contaminated milk at levels up to 40 pg aflatoxin per ml. Of 54 samples collected from women in rural villages in Zimbabwe, six were found to be positive (11%) in ELISA, with levels up to 50 pg aflatoxin per ml (Fig. 4). No positive sample was detected out of 42 milks obtained from women in France[80]. This sensitive and rapid methodology will be useful in examining the importance of an interaction between exposure to aflatoxin and infection with HBV early in life.

[80] Wild, C.P., Pionneau, F.A., Montesano, R., Mutiro, C.F. & Chetsanga, C.J. (1987) *Int. J. Cancer, 40,* 328–333

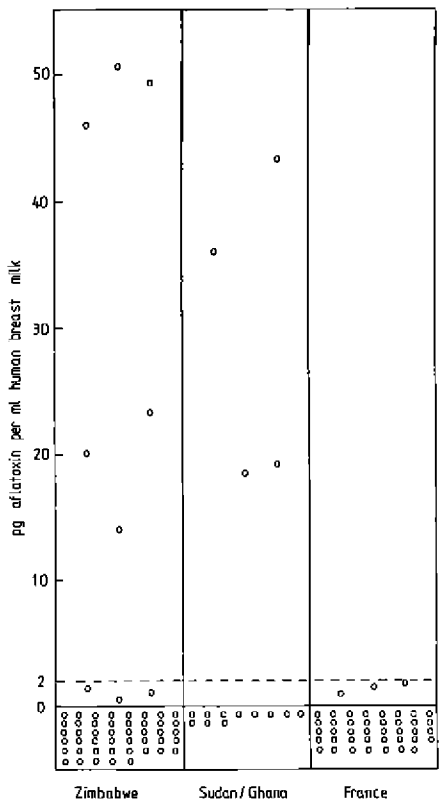

Fig. 4. Analysis of human breast milk by ELISA.

Samples of human breast milk from Zimbabwe are compared with samples from Lyon, France, and the Sudan or Ghana and analysed by ELISA. Samples giving no inhibition are presented in the boxes at the bottom. The practical limit of sensitivity of 2 pg aflatoxin/ml is indicated by the broken line. A total of 54 samples from Zimbabwe, 18 from the Sudan or Ghana and 42 from France were assayed.

Analysis of 22 milk samples collected in Gambia during the month of February 1987 gave negative results. Parallel experiments are under way in lactating rats to assess the relevance of the presence of aflatoxins in dams' milk to cancer induction in the progeny.

(ii) *Comparative studies on the effects of aflatoxin M_1 (AFM_1) and aflatoxin B_1 (AFB_1) in newborn rats* (Dr C.P. Wild, Dr J.R.P Cabral, Mrs M.P. Desvaux and Mrs D. Galendo; in collaboration with Dr G.E. Neal, Medical Research Council Toxicology Unit, Carshalton, Surrey, UK)

The objective of these studies is to assess the carcinogenicity of aflatoxins in newborn rats. The first part of the experiment was designed to compare the effects of AFM_1 when given *via* dams' milk and when given directly by a single intraperitoneal administration. In the second part of the experiment, groups of one-week-old rats receive a single administration of AFM_1 or AFB_1; adequate groups of controls are available. All animals will be kept for life to assess the development of liver tumours.

(iii) *Quantification of aflatoxins in human body fluids* (Dr C.P. Wild, Miss B. Chapot, Dr N. Muñoz and Dr R. Montesano; in collaboration with Dr H.C. Whittle and Dr A. Hall, Medical Research Council Laboratories, Fajara, Gambia; Dr R. Ryder, Section of Epidemiology, Schoold of Public Health, Boston University, Boston, MA, USA; Dr G.N. Wogan, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA, USA; Dr J.D. Groopman, Environmental Health, School of Public Health, Boston University, Boston, MA, USA; Dr R.C. Garner, Cancer Research Unit, Department of Chemistry, University of York, York, UK; and Professor C.J. Oon, Department of Medicine, Singapore General Hospital, Singapore)

An assay procedure for quantifying aflatoxins in human urine using antibody affinity column purification followed by ELISA was described previously[80]. This method has been slightly modified so that aflatoxins are eluted from the affinity column with 60% methanol in phosphate-buffered saline at pH 3.0. The assay has been used to measure aflatoxins in urine from four populations: in the Gambia, the Philippines, Singapore and Lyon, France, the last of which was used as a control population. The results, shown in Figure 5, demonstrate that the method permits detection of significant differences between high- and low-exposure populations[81]. The highest proportion of positive samples were from the Gambia and the Philippines, the majority of samples containing 0.1–1.0 ng AFB_1 equivalent per ml urine.

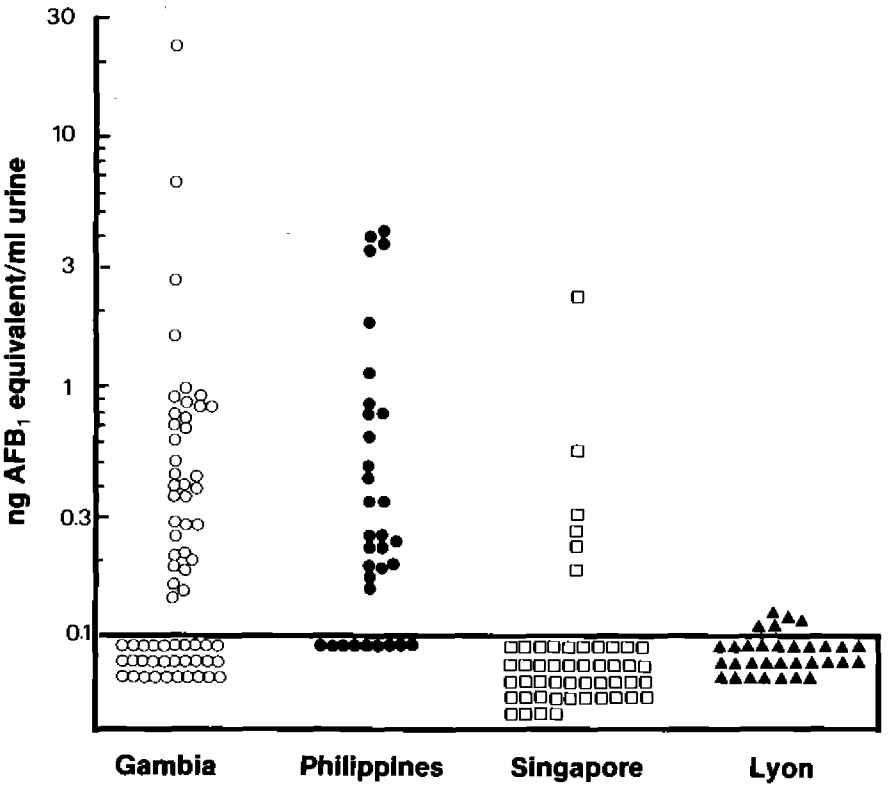

Fig. 5. Aflatoxin levels in human urine

Assuming that 10% of ingested aflatoxins is excreted in the urine in humans, the levels we found would represent daily exposures of 15–150 ng AFB_1/kg body weight. These levels are similar to those found in areas of high risk for HCC[81].

Further interlaboratory studies are under way to validate this approach to assessing short-term human exposure to aflatoxin. In particular, investigations are planned to examine the relationship between dietary intake of AFB_1 and urinary excretion of aflatoxins in the same individual.

In addition, the possibility of establishing an assay system to quantify the more stable aflatoxin-albumin adduct is being investigated. Experimental data obtained during chronic treatment with AFB_1 suggest a constant relationship between aflatoxin-albumin adducts and DNA adduct levels in the liver, the target organ for AFB_1 carcinogenesis in rats[82].

(iv) *Role of aflatoxin B_1 in the induction of hepatocellular carcinoma in Peking ducks infected with duck hepatitis B virus* (Dr C. Trepo and Mrs E. Cova, Research Unit on Hepatitis and the Role of Hepatotropic Viruses in Oncogenesis, National Institute for Health and Medical Research, Lyon, France, DEC/83/12; Dr J.R.P Cabral, Dr R. Montesano and Dr L. Tomatis)

The scope of the present studies is to examine the relationship between duck HBV (DHBV) and AFB_1 in the induction of HCC in Peking ducks. Preliminary studies were undertaken to establish the doses of AFB_1 necessary for long-term studies. Long-term administration of AFB_1 is now being carried out: (1) groups of 15 ducks infected with DHBV were administered 0, 0.02 or 0.08 mg/kg AFB_1 intraperitoneally once a week; (2) groups of 15 uninfected ducks also received 0, 0.02 or 0.08 mg/kg AFB_1 intraperitoneally once a week; (3) one group of 15 DHBV-negative ducks received no AFB_1; and (4) one group of 15 DHBV-positive/naturally infected ducks also received no AFB_1. A few of the infected ducks treated with the high dose of AFB_1 died. Histopathological examination of the liver showed marked bile-duct proliferation, cirrhotic changes and nodular hyperplasia. The experiment is scheduled to last at least two years.

5. GENETICS AND CANCER

Variations in cancer incidence are considered to result mainly from variation in exposure to environmental factors; however, it is also clear that there are individual variations in susceptibility to the carcinogenic effect of these agents. Such individual genetic differences are still poorly understood, and the aim of this programme is to evaluate, using a molecular approach, the importance of genetic predisposing conditions in the etiology of human cancer. Until recently, only a few cancer types had been shown to have a clear genetic component, and the identification of 'genetic risk factors' for other cancers occurring in the general population was almost impossible. However, recombinant DNA technology has provided new sources of molecular markers (cloned genes or oncogenes, DNA polymorphic markers) which may be used to identify the genetic factors that contribute to the development of cancer. This approach could also permit better identification of the environmental factors associated with a given cancer. Studies in our laboratory have so far addressed a rare immunodeficiency syndrome, a familial form of thyroid cancer, breast cancer and genetic variations at the *c-myc* oncogene locus.

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[81] Wild, C.P., Chapot, B., Scherer, E., Den Englese, L. & Montesano, R. (1988) In: Bartsch, H., Hemminki, K. & O'Neill, I.K., eds, *DNA Damging Agents in Man: Applications in Cancer Epidemiology (IARC Scientific Publications No. 89)*, Lyon, International Agency for Research on Cancer (in press)

[82] Wild, C.P., Garner, R.C., Montesano, R. & Tursi, F. (1986) *Carcinogenesis, 7*, 853–858

(*a*) *Studies of the X-linked lymphoproliferative syndrome* (Dr B.S. Sylla, Ms Q. Wang, Ms S. Pauly and Dr G. Lenoir; in collaboration with Dr D. Hayoz, Friburg, Switzerland; and Dr N. Philippe, Debrousse Hospital, Lyon, France)

The X-linked lymphoproliferative syndrome (XLP) is a recessive genetic disorder linked to the X chromosome which affects boys carrying the susceptible gene. It is a very rare disease, characterized by a fatal chronic infectious mononucleosis, acquired hypogammaglobulinaemia, or malignant lymphoma, following Epstein-Barr virus (EBV) infection[83]. XLP represents a very interesting model in humans, in which an infectious environmental agent (EBV) and a strong genetic predisposing condition lead to development of malignant lymphomas. A genetic linkage study is being carried out, identifying cosegregation between the susceptible gene(s) of XLP and a marker(s) displaying restriction fragment length polymorphism (RFLP)[84].

We have identified four families affected by XLP. The largest kindred, which was obtained from Switzerland, is presented in Figure 6[85]. We have established lymphoblastoid cell lines from all members of these families, and analysis of DNAs from informative members with the X-specific RFLP markers is in progress. The RFLP approach will make it possible to identify DNA marker(s) closely linked to susceptibility to XLP. These markers will be valuable for future genetic counselling of affected members in these families. They will also represent the ultimate step in isolating the XLP gene and studying its function.

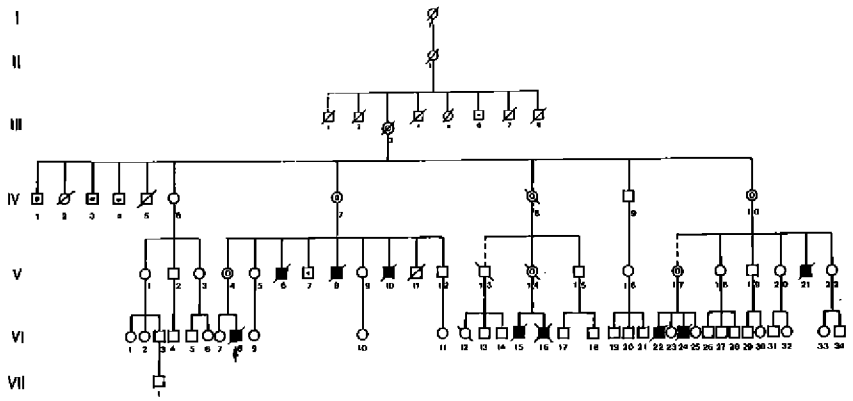

Fig. 6. Family affected by X-linked lymphoproliferative syndrome

A meeting of investigators studying this syndrome, including Professor D.T. Purtilo who initially described the disease, is planned for December 1987. This will permit better collaboration between the various groups working on conditions predisposing to this cancer.

(*b*) *Studies on multiple endocrine neoplasia type IIa* (*MEN IIa*) (Dr H. Sobol, Ms M.F. Lavoué and Dr G. Lenoir; in collaboration with the Group for the Study of Calcitonin Tumours: Secretariat, Dr C. Calmettes, Saint Antoine Hospital, Paris; and Dr B. Ponder, The Royal Cancer Hospital, Sutton, UK)

MEN IIa is an autosomal dominant inherited cancer syndrome characterized by medullary carcinoma of the thyroid, phaeochromocytoma and hyperparathyroidism. It cannot be

[83] Purtilo, D.T., Sakamoto, K., Barnabei, V., Seeley, J., Bechtold, T., Rogers, E., Yetz, J., Harada, S. & the XLP collaborators (1982) *Am. J. Med., 73,* 49–56

[84] White, R., Leppert, M., Bishop, D.T., Barker, D., Berkowitz, J., Brown, C., Callahan, P., Holm, T. & Jerominski, L. (1985) *Nature, 313,* 101–105

[85] Hayoz, D., Lenoir, G.M., Nicole, A., Pugin, P. & Regamey, C. (1987) *Am. J. Med.* (in press)

considered a rare disease since at least 30% of medullary thyroid cancers occur in genetically predisposed families. Almost all gene carriers will develop the disease (a very high penetrance of the gene), but their identification still relies on a screening test that detects an early stage of the malignancy. The nature and location of the predisposing gene are unknown.

Through the Groupement d'Etude des Tumeurs à Calcitonine (GETC) in France and contacts with various European institutions, over 80 families have been identified, and blood has already been collected from more than 20 of them. The recent identification by two groups (B. Ponder, personal communication) of a linkage between the MEN IIa locus and DNA polymorphic markers located on chromosome 10 has led to the suggestion that MEN IIa may represent one of the first situations in humans in which screening and genetic counselling of affected families could be carried out. Genetic heterogeneity for the defect is being evaluated in the material being collected at the Agency, and we are attempting to identify and clone the predisposing gene.

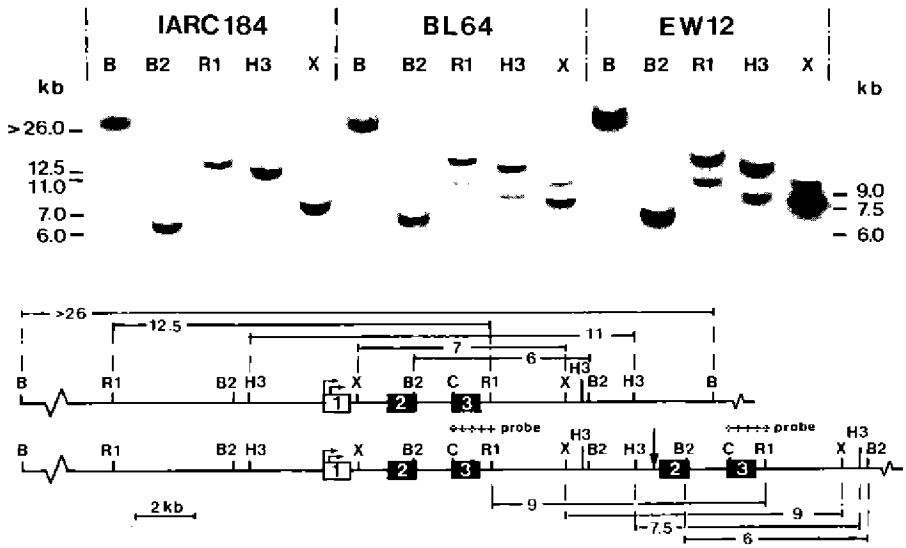

Fig. 7. Hybridization of genomic DNA from IARC 184, BL64 and EW12 cell lines digested with different restriction endonucleases, fractionated in a 0.6% agarose gel, and transferred onto nitrocellulose with a 3'-c-*myc*-specific probe.

On the left-hand side of the autoradiogram, shown in the upper part of the figure, the sizes of c-myc-specific fragments are indicated; these are present and identical in the DNA of all three lines. On the right-hand side, the sizes of newly arising submolar, single-copy fragments are shown, which were detected only in the DNA of patients 1 and 2. The lower part of the figure is a diagrammatic representation of the structure of both c-myc alleles in the BL64 cell line deduced from detailed restriction enzyme analysis of overlapping λ-clones isolated from a genomic library. Blocks designate possible transcribed regions; filled blocks indicate the coding sequences of the expressed gene, and arrows show the normal transcriptional start sites. The length and the location of normal (above) and of additional single-copy fragments (below) are indicated. The vertical arrow shows the breakpoint of the duplication. Abbreviations for restriction enzymes: B, BamHI; B2, BglII; C, ClaI; H3, HindIII; R1, EcoRI; and X, XbaI

(*c*) *Breast cancer: collection of biological samples from multiple-case families* (Dr G. Lenoir and Ms M. Vuillaume; in collaboration with Dr H. Tulinius, The Icelandic Cancer Registry, Reykjavik; and Dr H. Muller, Humangenetik, Basel, Switzerland)

Study of genetic predisposing conditions for breast cancer remains difficult. A collaborative effort is being made to identify large multiple-case families that could be investigated for linkage using molecular probes (RFLP). Such families are being identified, and blood samples are being collected from all family members. Molecular studies are likely to be successful only if a relatively large number of informative families (around 20) is accessible for investigation.

(*d*) *Study of a constitutional* c-myc *gene duplication in two cancer patients and their families* (Dr G. Lenoir and Dr C. Drevon; in collaboration with Dr M. Lipp, Institute for Biochemistry, Munich, Federal Republic of Germany; Dr G. Bornkamm, Institute for Virology, Freiburg, Federal Republic of Germany; and Dr Y. Ladjadj and Professor M. Aboulola, Mustapha University Hospital Centre, Algiers)

We have identified a major structural rearrangment of the c-*myc* oncogene in two Algerian paediatric cancer patients. The anomaly, which was detected in both malignant and nonmalignant tissue, is constitutional. Its molecular structure has been identified and corresponds to a duplication of the entire coding region of the gene (Fig. 7). A large collection of blood samples from family members and from other populations indicates that the genetic anomaly was transmitted from the parents, does not occur in non-Algerian individuals, and occurs in Algerians at a low frequency (4%) as seen in the population of a town from which one of the cancer cases originated[86]. A study is being performed to evaluate whether this abnormal gene is a cancer risk marker in the population and whether other rare c-*myc* alleles occur in other populations.

6. ROLE OF VIRUSES AND CYTOGENETIC ANOMALIES IN THE ETIOLOGY OF HUMAN CANCER

The main objective of this programme is to evaluate, using laboratory investigations linked to epidemiological studies, the role of viruses in the etiology of human cancer and to identify each of the molecular steps leading to the development of a given cancer. The model of cancer chosen is Burkitt's lymphoma (BL), a cancer known to show great geographic variation in its incidence, to be associated with the Epstein–Barr virus (EBV) and to carry specific cytogenetic anomalies.

(*a*) *Collection of biological material related to Epstein–Barr virus/Burkitt's lymphoma* (Dr G. Lenoir, Ms C. Bonnardel, Ms M. Vuillaume and Ms S. Pauly)

As part of various Agency projects, we have constituted a very large collection of sera, human material and cell lines, which are used widely by the scientific community for studies of EBV, BL, NPC and B-cell neoplasia. Sera, biopsies and cell lines (over 120 BL cell lines have been established in cultures at the Agency, representing by far one of the largest collections of human tumour cell lines for a given cancer) are shipped free of charge on request to institutions all over the world. During the period under review, 720 lymphoid cell lines were sent to 63 institutions in 16 countries in the form of live cells, frozen cells or DNA, as well as various other materials, such as sera, biopsies and probes.

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[86] Lenoir, G.M., Lipp, M., Bechet, J.M., Hartl, P., Bornkamm, G.W., Lavoué, M.F., Ladjadj, Y., Aboulola, M. & Philip, T. (submitted for publication)

(*b*) *Publication of* Burkitt's Lymphoma: A Human Cancer Model

The proceedings of the international symposium on Burkitt's lymphoma, organized by Dr G. Lenoir, Dr G.T. O'Conor and Dr C. Olweny (WHO Consultant) and supported in part by the Association pour le Développement de la Recherche sur le Cancer (France) and the General Motors Cancer Research Foundation (USA), have now been published[87]. They represent a comprehensive 'state of the art' reference source for this tumour, which has indeed proved to be one of the best models for the investigation of human cancer.

(*c*) *Studies on Burkitt-type lymphoma in France* (Dr G. Lenoir; in collaboration with Dr T. Philip, Léon Bérard Centre, Lyon, France)

Identification of BL cases in France is continuing in order to obtain large numbers of EBV-associated and non-EBV-associated cases for comparative analysis. In more than 40 EBV-positive cases now identified (representing only 15% of BL cases), our first analysis indicates that some occurred following infectious mononucleosis or Hodgkin's disease. This finding, together with the development of EBV-associated BL in patients with acquired immunodeficiency syndrome, suggests that, in low-incidence areas, alteration of some cellular immune dysfunction could represent risk factors for this cancer in its EBV-associated form. Attempts to identify risk factors for the non-EBV-associated BL are being made retrospectively.

(*d*) *Laboratory studies on Epstein–Barr virus* (Dr G. Lenoir and Ms M.F. Lavoué)

These studies are aimed at evaluating the role of EBV in inducing cell proliferation. Serological investigations are being made to evaluate the importance of the immune response to EBV as a risk factor in the development of EBV-associated lymphomas.

(i) *EBV genes involved in cell immortalization and tranformation* (Mr A. Calender, Mr M. Billaud, Ms M. Cordier and Dr G. Lenoir; in collaboration with Dr G. Bornkamm, Institute for Virology, Freiburg, Federal Republic of Germany)

Various attempts have been made to identify the region of the EBV genome involved in the process of cellular immortalization. Transfection of cloned DNA fragments of the EBV genome into primary rat fibroblasts does not permit the establishment of permanent cell lines such as those obtained with cloned oncogenes like c-*myc*, SV40 large-T gene and adenovirus E1A gene. We have been able, using DNA-mediated gene transfer, to express the EB nuclear antigen-2 (EBNA2) protein[88]. The gene that codes for this molecule is deleted in the nonimmortalizing strain of EBV, P3HR-1, suggesting that it plays an important role in B-cell immortalization.

We demonstrated recently that a set of B-cell activation markers, the EBV/C3d receptor (CR2-CD21), Blast2 antigen (CD23) and Bac1 antigen (which was recently identified as a potential B-cell growth factor receptor), can be turned on by infecting EBV-genome-negative lymphoma cells with the B95-8 immortalizing strain (B95-8) of the virus. The non-immortalizing EBV variant, P3HR1, does not induce expression of these markers (Fig. 8). These results suggest that the immortalizing potential of EBV is correlated with its ability to induce expression of B-cell activation markers, which are suspected to play a major role in the physiological pathway leading to lymphoid cell proliferation. Our experiments suggest that EBNA2 gene

[87] Lenoir, G., O'Connor, G.T. & Olweny, C. eds (1985) *Burkitt's Lymphoma: A Human Cancer Model* (*IARC Scientific Publications No. 60*), Lyon, International Agency for Research on Cancer

[88] Mueller-Lantzsch, N., Lenoir, G.M., Sauter, M., Takaki, K., Béchet, J.M., Kuklik-Roos, C., Wunderlich, D. & Bornkamm, G.W. (1985) *EMBO J.*, *4*, 1805–1811

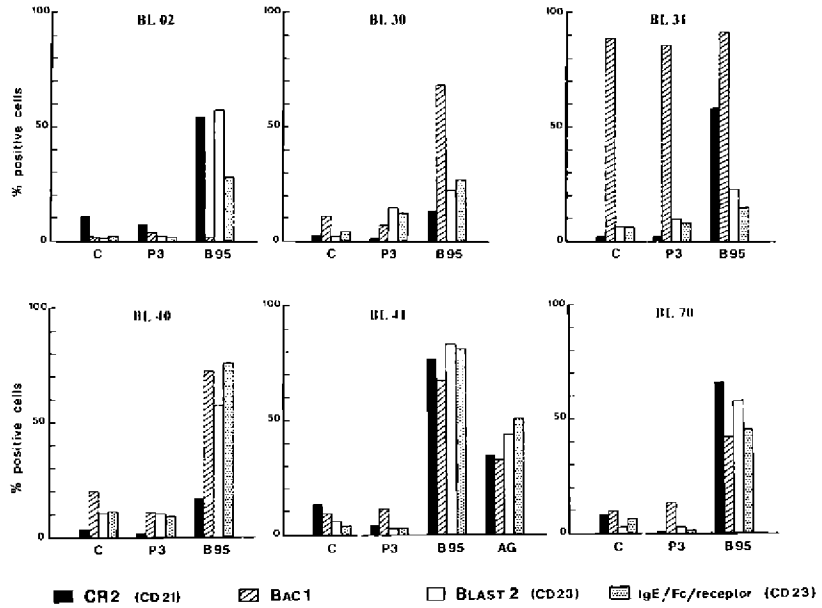

Fig. 8. Expression of four B-cell activation markers in six EBV-negative lymphoma cell lines and their EBV-genome-positive convertant

Phenotypic analyses were performed on the original cell line and on clones in which 100% of cells expressed EBNA after conversion with the B95-8 (B95) or P3HR-1 (P3) strains of EBV. The results presented are based on the analysis of a single cell clone, but similar results were always obtained with other independent clones and with the initial converted cell population before cloning. Results for each cell line are grouped as follows: EBV-negative control (C), P3HR-1-converted (P3), B95-8-converted (B95) and AG876-converted (AG).

might act as a transactivator of some cellular genes, similar to the way in which the gene of the HTLV1 retrovirus can turn on expression of the I12 receptor gene in T-lymphoid cells[89].

Attempts are now being made to introduce specific EBV latent genes into these cells, in order to analyse their effect directly.

(ii) *Immunological response to EBV and EBV-infected cells*

New serological tests have been developed, using cells transfected by small regions of the EBV genome and thus expressing specific EBV antigens[90]. Antibody responses have been evaluated in various populations. The anti-EBNA2 immunological response was found not to be a good serological marker for EBV-associated malignancies such as BL and NPC, but a better

[89] Calender, A., Billaud, M., Aubry, J.P., Banchereau, J., Vuillaume, M. & Lenoir, G.M. (1987) *Proc. natl Acad. Sci. USA* (in press)

[90] Miller, G., Grogan, E., Fischer, D.K., Niederman, J.C., Schooley, R.T., Henle, W., Lenoir, G. & Liu, C.-R. (1985) *New Engl. J. Med., 312,* 750

marker of some cellular immune disfunctions[91]. Preliminary data suggest that it might be used to follow up patients with acquired immune deficiency syndrome.

Our study on EBNA2 has also led to the identification of two types of EBV virus, characterized by different EBNA2 alleles (A and B) and showing different geographical prevalence[92]. The restricted geographical localization of EBV type 2 in parts of the southern hemisphere and its similarity to herpesvirus papio could suggest that such viruses evolved by recombination of EBV with a related old-world-monkey virus.

(*e*) *Laboratory studies on the genesis of Burkitt's lymphoma*

Studies of BL indicate the the EBV cannot be considered the sole cause of the disease (see I.6.*b*). At the cellular level, chromosomal rearrangements — also detected in EBV-free BL tumours — are suspected to play a critical role in the malignant process. Over the last few years, a large part of our activity has been devoted to studying the biological significance of these chromosomal anomalies and to identifying the steps in the development of the malignant BL clone.

(i) *BL tumorigenicity assays* (Dr V. Gurtsevitch, Dr G.T. O'Conor, Dr G. Lenoir and Dr V. Turusov)

A quantitative in-vivo assay for evaluating the tumorigenicity of BL cell lines in nude mice is being developed. It is based on the dose-response kinetics of BL cell lines in pre-irradiated (480 rad) nude mice following subcutaneous injection of four different cell doses. This model system was used to estimate the xenografting potential of 26 BL cell lines derived from BL patients of different geographic and ethnic origins, and of lymphoblastoid cell lines (LCLs) established by EBV immortalization of normal B lymphocytes. The results indicate that most BL cell lines are tumorigenic but LCLs fail to produce progressively growing tumours in nude mice. However, the BL cell lines have different degrees of tumorigenicity and can thus be divided into four groups, with high, moderate, low or no tumorigenicity. Preliminary attempts to correlate the xenografting phenotype of BL lines with other characteristics demonstrate that: (1) aberrations of chromosome 1 are more often encountered in cell lines with high and moderate tumorigenicity; (2) EBV-positive BL lines do not have a higher tumorigenic phenotype than EBV-negative lines; (3) cell lines that carry translocation t(8;22) and t(2;8) appear to fall more frequently into the groups of lines with high and moderate tumorigenicity; and (4) when LCLs grow in nu/nu mice, rejection always occurs and is associated with massive tumour necrosis. These findings suggest that the tumorigenicity of BL cell lines in immunosuppressed animals is not related to EBV, but to particular chromosomal abnormalities (BL-specific and non-specific), indicating that this in-vivo model system can be used for identifying other factors or stages involved in the development of BL[93].

The metastasizing potential of BL cells can also be estimated by intravenous injection of BL cells. The pattern of tumours obtained suggests that BL cells retain specific homing properties that might explain the tissue distribution of this tumour *in vivo*.

(ii) *Cytogenetic investigations of malignant lymphoid cell lines* (in collaboration with Dr J. Fraisse and Dr M.F. Bertheas, Blood Transfusion Centre, St Etienne, France)

All new cell lines established at the Agency are karyotyped. All BL cell lines so far analysed (over 120) show one of the specific BL translocations, t(8;14), t(2;8) or t(8;22). This strongly

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[91] Seigneurin, J.M., Lavoué, M.-F., Genoulaz, O., Bornkamm, G.W. & Lenoir, G.M. (1987) *Int. J. Cancer* (in press)

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suggests that the cytogenetic anomaly represents a crucial, necessary step in the development of BL.

(iii) *Molecular studies* (Dr G. Lenoir; in collaboration with Dr P. Leder, Harvard Medical School, Boston, MA, USA,; Dr G. Bornkamm, Institute for Virology, Freiburg, Federal Republic of Germany; and Dr J.C. Kaplan, Institute of Pathology and Cellular and Molecular Biology, Paris)

The Agency's BL cell lines have been investigated in collaboration with national institutions to study the molecular consequences of the chromosomal translocation at the c-*myc* oncogene locus[94-97].

Two interesting observations were made: (1) that the translocation leads to alterations in the *myc* locus, resulting in the creation of aberrant c-*myc* RNA with longer half-lives; and (2) that, in BL with the t(8;22) variant translocation, the c-*myc* locus is not rearranged by chromosomal translocation but by somatic mutations clustered in and around the first c-*myc* exon. This could be the gene alteration that leads to deregulation of c-*myc* expression in this subgroup of BL.

(*f*) *Human T-cell leukaemia virus type 1 (HTLV1) prevalence in healthy individuals and in patients with lymphoproliferative malignancies in the USSR* (Dr V. Gurtsevitch and Dr G. Lenoir; in collaboration with the Cancer Research Center, Academy of Medical Sciences of the USSR, Moscow; and Dr Y. Hinuma, The Institute for Virus Research, Kyoto University, Japan)

Recently, it has been demonstrated the HTLV1 is causally associated with adult T-cell lymphoma/leukaemia (ATL). Both HTLV1 and ATL have been shown to be endemic in northern and south-western islands of Japan, in countries of the Caribbean basin and in sub-Saharan Arfrica. Antibodies to HTLV1 were found in the sera of almost all patients with ATL and in a considerable number of healthy adults in endemic regions. The aim of the proposed study is to investigate the prevalence of HTLV1 in healthy populations and in ATL patients of different geographic regions of the USSR, with special attention to the eastern border of the country (Kamchatka, Sakhalin), which is in immediate proximity to an endemic region (Japan). No such investigation has yet been performed in these regions.

7. BIOCHEMICAL AND METABOLIC PARAMETERS AS INDICATORS OF INDIVIDUAL SUCEPTIBILITY TO CANCER

(*a*) *Drug-metabolizing enzyme activities in lung and mucosa specimens from lung-cancer and cancer-free patients: effect of cigarette smoking* (Miss A.-M. Camus, Dr M. Ahotupa, Dr A. Aitio, Dr E. Hietanen, Dr R. Saracci and Dr H. Bartsch; in collaboration with Dr S. Petruzzeli and Professor C. Giuntini, National Research Council, University of Pisa, Italy)

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Lung tissue specimens were taken during lung surgery from 74 middle-aged men with either lung cancer (LC, n = 54) or a nonneoplastic lung disease (NLC, n = 20)[98,99]. Information concerning the patients' smoking habits, occupation, disease history and other data was collected by a 140-variable questionnaire. The overall exposure of patients to tobacco smoke was evaluated as number of pack-years. Patients were divided into smokers (S) and nonsmokers (NS) according to their smoking status at six months from surgery. For S who gave up smoking, the number of days was recorded.

Supernatant fractions (12 000 × *g*) were prepared from parenchymal and bronchial lung tissue and used for determination of arylhydrocarbon hydroxylase (AHH), 7-ethoxycoumarin *O*-deethylase (ECDE), epoxide hydrolase (EH), glutathione *S*-transferase (GST) and UPD-glucuronyltransferase (UDPG-T) activities, as well as for the estimation of glutathione (GSH) and malondialdehyde (MDA) contents. In parenchymal lung tissue, large interindividual variations were found for GSH content and for all the enzyme activities that showed unimodal distribution. All the enzyme activities were significantly correlated to each other, with positive correlations among AHH, ECDE, EH and UPDGT ($p < 0.05$) and a negative correlation between GST and the other enzymes ($p < 0.05$). No relationship was found between enzyme activities and pack-years, except for GSH content ($r = 0.22$; $p < 0.05$); no difference was found between LC and NLC patients, either for enzyme activity or GSH content.

When enzyme activites were plotted against the number of days of refraining from smoking, S showed significant relationships for AHH ($r = -0.312$) (Fig. 9), EH ($r = -0.371$), UDPGT ($r = -0.251$) and GST ($r = 0.282$). The activities of these enzymes returned to the level found in NS only after 59, 108, 67 and 40 days, respectively. This long-lasting effect can best be explained by tar deposition in the lungs. Our results demonstrate for the first time that human pulmonary drug metabolism can be induced by smoking. Moreover, in recent smokers (< seven days before surgery), the proportion of lung cancer cases with induced drug metabolizing enzymes was higher than in smoking controls: 14/21 *vs* 1/5 (AHH, ECDE); 22/24 *vs* 5/7 (EH); 10/22 *vs* 2/6 (UDPGT). Although the differences were not statistically different, the data suggest a genetic determinant of suceptibility to lung cancer in tobacco smokers. This notion, which needs further investigation, is also supported by our results, which show striking similarities with two rodent models: lung cancer and metabolism in rats, after intratracheal instillation of 3-methyl-cholanthrene-crystals[100] and *Ah*-locus controlled responsiveness to enzyme- and cancer-induction in inbred mice strains.

(*b*) *Sister chromatid exchange (SCE) as an indicator of individual sensitivity to N-ethyl-N-nitrosourea (ENU)-induced carcinogenesis in rats* (Dr A. Aitio, Dr J.R. Cabral, Miss A.-M. Camus, Mrs D. Galendo and Dr H. Bartsch; in collaboration with Dr M.-L. Aitio, Dr H. Norppa, Dr S. Salomaa, Dr M. Sorsa, Dr K. Husgafvel-Pursiainen and Dr M. Nurminen, Institute of Occupation Health, Helsinki)

In order to study the relationship between induced SCE response and tumorigenesis at the individual level, SCE rates were determined in cultured blood lymphocytes before, at 24 h and

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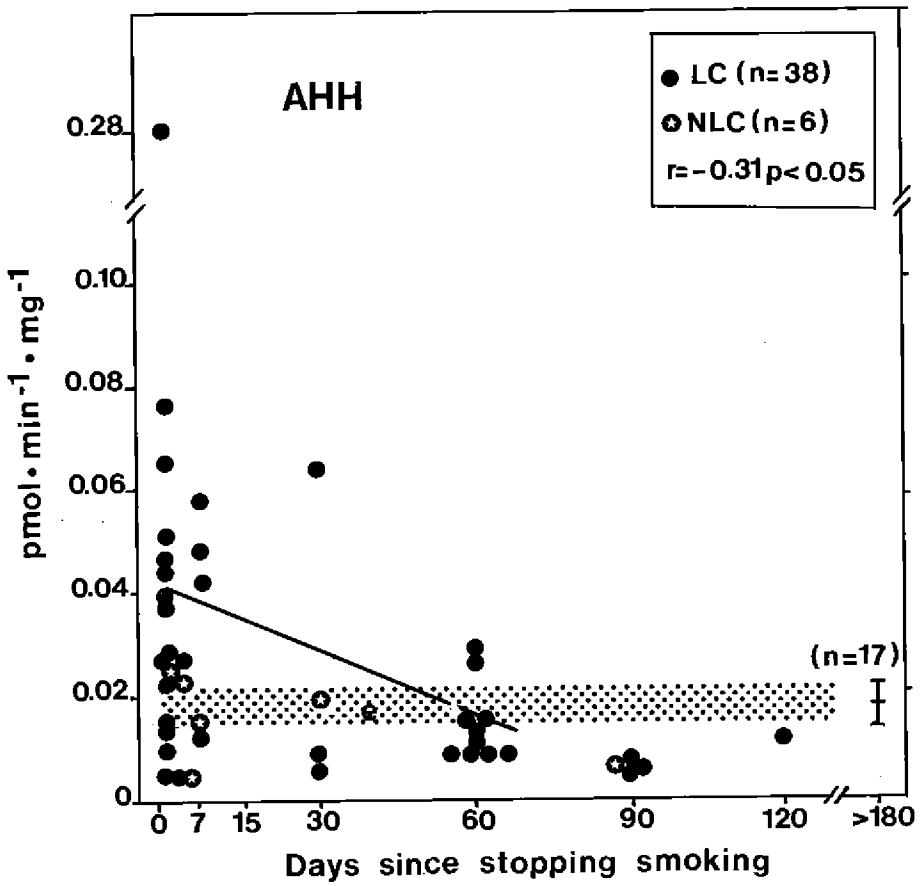

Fig. 9. Studies on drug-metabolizing enzyme activities

seven days after a single intraperitoneal administration of ENU to Wistar rats. Proliferation index and mitotic index were also determined. The rats were followed until death, and the interrelationships between the observed parameters and the presence/absence of tumours, as well as their latency were analysed using various statistical approaches.

SCE rates after exposure to ENU did not indicate individual susceptibility in outbred laboratory rats; on a group basis, however, animals with high SCE rates were at increased cancer risk. Using the same study material, chromosomal aberrations are now being analysed to see whether they can serve as predictors of ENU-induced cancers[101].

(*c*) *Interaction of partial hepatectomy and carcinogen administration in liver carcinogenesis in rats*[102] (Dr H. Bartsch, Dr A. Aitio and Dr J.R.P. Cabral; in collaboration with Ms V. Préat and Professor M. Roberfroid, Unit of Toxicological and Oncological Biochemistry, Catholic University of Louvain, Brussels)

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[102] Bartsch, H., Préat, V., Aitio, A., Cabral, J.R.P. & Roberfroid, M. (submitted for publication)

Partial hepatectomy (PH) is known to enhance liver carcinogenesis when it is performed several hours before or after administration of a hepatocarcinogen. This effect has been attributed to the cell proliferation that is induced by PH and which is a necessary step for fixation of DNA damage leading to initiation of cancer cells. We now report for the first time that PH can also strongly increase tumour incidence and decrease latency when performed eight to ten weeks before treating rats with a hepatocarcinogen, *N*-nitrosodiethylamine. This animal model offers a valuable tool for investigating the underlying mechanism of the 'memory effect' and increased tumour susceptibility of liver cells after PH. Additionally, it should be explored as a more sensitive rodent bioassay for testing putative carcinogens.

Our results raise the question of whether cell necrosis and regeneration of the liver in response to injuries by hepatotoxins, such as aflatoxins, alcohol and viral infections, exert a similar long-term 'memory effect'. A recent case-control study on liver cancer in Greece confired that in the presence of cirrhosis the relative risk for HBsAg-positive subjects with HCC was considerably higher than among HBsAg-positive subjects without cirrhosis (i.e., 31 *versus* 7)[103].

(*d*) *Monoclonal antibody (MAb)-directed analysis of cytochrome P450-dependent monooxygenases and mutagen activation in the liver of rodents and humans* (Dr C. Malaveille, Mrs G. Brun and Dr. H. Bartsch; in collaboration with Dr H.V. Gelboin, National Cancer Institute, Bethesda, MD, USA; and Professor U. Mohr, School of Medicine, Hanover, Federal Republic of Germany)

MAbs that completely inhibit the enzyme activity of the epitope containing cytochrome P450, to which they bind, proved to be useful for the identification and quantification of the contribution of individual or classes of P450 to the total metabolism of chemicals in tissue preparations[104].

We have recently studied the contribution of two MAb-specific P450s, i.e., the major rat liver phenobarbital (PB)- and 3-methylcholanthrene (MC)-induced P450s, to metabolic activation of various carcinogens and to the metabolism of drugs, hormones and carcinogens in mouse liver preparations[105]. As a follow-up to that study, work is in progress using MAbs to ethanol-induced P450, to examine the contribution of ethanol-inducible P450 in the metabolism and activation of various nitrosamines by rodent and human liver preparations. We shall assess the usefulness of these MAbs for the tissue and reaction phenotyping required to identify hypersusceptibility to alcohol and to *N*-nitroso compounds.

(*e*) *Monoclonal antibody (MAb) characterization of hepatic and extrahepatic cytochrome P450 activities in rats treated with phenobarbital (PB) or 3-methylcholanthrene (MC) and fed various cholesterol diets* (Dr M. Ahotupa, Dr H. Bartsch, Mr J.-C. Béréziat and Dr H. Hietanen; in collaboration with Dr H.V. Gelboin, National Institutes of Health, Bethesda, MD, USA)

Cytochrome P450-mediated monooxygenases (P450) play a central role in the metabolic conversion of numerous chemicals to ultimate carcinogens. P450s are known to exist in multiple molecular forms with distinct, but overlapping substrate specificities. Several forms of P450 have been purified, and MAbs against different isozymes have been prepared. We are currently using

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MAbs to study the contributions of P450s in the functioning of carcinogen-metabolizing enzymes.

MAbs against MC- and PB-inducible forms of P450s[106] were used to characterize changes in aryl hydrocarbon hydroxylase (AHH) and ethoxycoumarin *O*-deethylase (ECDE) activities modulated by dietary cholesterol[105]. Rats were induced with MC or PB, and immunochemical inhibition of AHH and ECDE activities was studied as an indication of changes in P450 patterns. Feeding of a cholesterol-free diet markedly decreased both enzyme activities in liver and in small intestinal mucosa; and the highest activities were observed after feeding rats a high (2%)-cholesterol diet for one month. As a control, a normal pelleted diet (0.1% cholesterol) was used; in rats fed this diet, intermediate levels of monooxygenase activites were present. Although no diet-dependent change in total AHH and ECDE activities was observed in kidneys and lungs, diet apparently modulated isozyme composition in the lungs, as indicated by a change in the immunochemical inhibition pattern with MAb; no such shift was observed in the kidneys. In liver and intestine, in addition to changes in total activity, isozyme composition was also altered, as indicated by inhibition of the catalytic activities of P450 by MAb.

Our data[107] infer that dietary cholesterol can (1) modulate total monooxygenase activities, especially in the intestine; (2) change the P450 composition in liver and intestine; (3) change isozyme composition without changing overall enzyme activity, e.g., in lungs; and (4) have no effect in a tissue (e.g., kidney) that lacks constitutionally the P450 isozyme responsive to cholesterol.

(*f*) *Effect of dietary constituents on lipid peroxidation and foreign compound metabolism and its role in tumour initiation and progression* (Dr M. Ahotupa, Mr J.-C. Béréziat, Mrs V. Bussachini-Griot, Miss A.-M. Camus, Dr E. Hietanen and Dr H. Bartsch)

A high level of dietary fat is known to be associated with an increased risk for certain types of cancer, especially that of the breast and colon[108–110], although this relationship has been recently questioned for breast cancer[111]. In experimental animals, increasing the fat content of the diet enhances the development of spontaneous tumours[112–114], as well as the development of tumours induced by ultra-violet radiation[115,116] or carcinogenic chemicals[117–120]. Thus far, no unifying mechansim has been presented to explain the modulation of tumour development by dietary fat, but, rather, several possibilities seem to exist. In order to identify possible links between lipid

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peroxidation, drug metabolism and the development of tumours, we have started long-term studies in which we follow changes caused in numerous metabolic parameters by dietary lipids alone or in combination with a chemical carcinogen, together with the appearance of tumours.

Groups of rats dosed chronically with *N*-nitrosodiethylamine (NDEA) and controls were fed an equicaloric diet containing either 2% (LF) or 30% (HF) polyunsaturated fat. Biochemical parameters, including indicators of lipid peroxidation, antioxidant defence, drug-metabolizing enzymes, membrane composition and blood chemistry, were measured during and at the end of the experiment in different organs, blood, urine and exhaled air. The presence and absence of tumours was related to the biochemical changes measured.

Out of 22 rats that received HF/NDEA, 13 have liver tumours, whereas LF/NDEA produced liver tumours in nine out of 23 rats; the latter group also had extrahepatic tumours (of the kidneys, olfactory neuroblastoma and colon), with a tumour incidence for all sites of 18/23 rats. In the LF/NDEA group, hepatocellular carcinomas were found only in the left and caudate lobes of liver. In the HF/NDEA group, tumours were equally distributed among the four liver lobes.

Total, free cholesterol and triglyceride concentrations in plasma were lower in the LF/NDEA group than in the LF/O group. No difference due to NDEA treatment or between tumour-bearing and tumour-free animals was found for blood enzymes (glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, γ-glutamine transaminase). Only minor or no change was detected in blood for catalase activity, concentration of malondialdehyde, glutathione (GSH) and GSH-metabolizing enzymes or excretion of thioethers in the urine due to dietary modulation or NDEA exposure.

Ethane exhalation by rats *in vivo* was increased by the HF and by NDEA treatment. Lipid peroxidation, as measured in liver *in vitro*, was elevated by the HF. Liver cytosolic GSH contents and superoxide dismutase activity showed changes that could be attributed to increased oxidative stress. Thus, these results demonstrate that a high level of dietary fat, and the alkylating carcinogen NDEA can induce oxidative stress in rodents[121].

In the case of drug-metabolizing enzymes, HF increased hepatic cytochrome P450 contents, ECDE, NDMA-demethylase, testosterone 6β-hydroxylase, epoxide hydrolase and UDP-glucuronyltransferase activities, while it decreased testosterone 7α- and 16α-hydroxylase activities. The most dramatic diet-related changes were found in the hexose monophosphate shunt (HMS) activity: NADPH production by the HMS was 20-fold higher in the LF group than in the HF group after four or 12 weeks on diet; after 40 weeks on diet, the difference was 2.5 to three fold.

In the HF group, NDEA treatment increased P450 content, ethoxyresorufin *O*-deethylase, aminopyrine demethylase and epoxide hydrolase activities, but decreased AHH activity in young rats. In the LF group, NDEA induced NDMA-demethylase and epoxide hydrolase activities, and decreased AHH and testosterone 16α-hydroxylase activities. NADPH production by the HMS was not affected by NDEA treatment.

The presence of tumours, either hepatic or extrahepatic, did not affect the levels of enzyme activities measured in the histologically normal part of the liver. However, the P450 content and all monooxygenase activities were lower, and the activities of epoxide hydrolase, glucuronoyltransferase and HMS were higher in the tumorous than in the nontumorous liver tissue of similarly treated rats. No major diet- or NDEA-related change was seen in drug-metabolizing enzyme activities in lung and kidney.

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The results demonstrate that a large amount of highly polyunsaturated fat alters the activities of a number of drug-metabolizing enzymes in the liver; and these changes appear to be, at least in part, responsible for the altered organ distribution of tumours. Diet-related changes in NADPH production by HMS may reflect defence against oxidative stress.

(*g*) *Studies on the role of prooxidant states in carcinogenesis* (Dr M. Ahotupa, Mr J.-C. Béréziat, Mrs V. Bussacchini-Gruiot, Miss A.M. Camus and Dr H. Bartsch)

The cellular prooxidant states, defined as increased concentration of active oxygen, organic peroxides and radicals[122], are suggested to be involed in pathological processes leading to the development of cancer (for reviews, see[122–124]). Most studies thus far have dealt with prooxidant states and tumour promotion, and few attempts have been made to describe the effects of initiating chemical carcinogens.

We have shown previously that chronic treatment of rats with NDEA[125] or *N*-nitrosodimethylamine (NDMA)[126] increases lipid peroxidation *in vivo*, as estimated by the amount of ethane in exhaled air. In the present study[127], we have investigated the generation of prooxidant state shortly after a single administration of various *N*-nitrosamines to rats. The level of oxidative stress *in vivo* was estimated by determining the amount of resulting lipid peroxidation in whole animals and in liver tissue, and the antioxidant capacity of liver tissue.

As an indication of enhanced peroxidative processes *in vivo*, NDMA treatment rapidly produced an increase in the rate of ethane exhalation in dose-dependent fashion, even after a single dose of 0.5 mg/kg. Ethane exhalation remained elevated for several days after single doses. Similarly, lipid peroxidation in the liver of NDMA-treated rats (measured by diene conjugation, chemiluminescence and the production of fluorescent and thiobarbituric acid-reactive material) was found to be increased rapidly, showing a peak 20 min after dosing. The increase in lipid peroxidation was preceded by a decrease in liver retinol concentration. Moreover, NDMA treatment slightly decreased antioxidant enzyme activities and GSH contents in the liver. In isolated rat hepatocytes, lucigenin-dependent chemiluminescence as well as peroxide release were increased by micromolar concentrations of NDMA. Finally the rate of NADPH-stimulated ethane production by hepatic microsomes prepared from untreated rats was increased in the presence of NDMA. In all, these results show that low doses of NDMA rapidly produce oxidative stress both in rats and in isolated rat hepatocytes after exposure to NDMA.

Two additional carcinogenic *N*-nitrosamines were tested for their ability to induce oxidative stress. *N*-Nitrosodiethanolamine, which is hepatocarcinogenic to rats[128], enhanced ethane exhalation and lipid peroxidation in the liver, although, when compared to NDMA, a higher dose was required. *N*-Nitrosomethylbenzylamine, which is an oesophageal but not a liver carcinogen in rats[129] had, on the contrary, no effect on lipid peroxidation in rats. These data, obtained on a limited number of compounds, suggest that those *N*-nitrosamines that initiate liver carcinogenesis may also cause oxidative stress in the target tissue. Whether the same is true for other initiators of carcinogenesis and in tissues other than the liver remains to be investigated.

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II. STUDIES ON MECHANISMS OF CARCINOGENESIS

1. STUDIES ON DNA DAMAGE AND REPAIR IN HUMAN AND RODENT CELLS

(*a*) *Repair of* O^6*-methyldeoxyguanosine* (O^6*-medG*) *and* O^4*-methylthymidin* (O^4*-meT*) *in rat and hamster cells* (Dr M. Serres, Dr. P. Degan, Miss H. Brésil, Mrs G. Planche-Martel, Mrs C. Piccoli and Dr R. Montesano)

The mutagenic and carcinogenic effects of methylating agents like *N*-methyl-*N*-nitrosourea (MNU) and *N*-nitrosodimethylamine (NDMA) are associated with the presence and persistence of two DNA adducts: O^6-medG and O^4-meT, which possess miscoding properties during DNA replication[1]. In *Escherichia coli,* repair of both these DNA adducts is carried out by the same O^6-methylguanine DNA alkytransferase[2]; the data for mammalian cells are contradictory and limited.

The aim of the experiments reported was to compare the rate of disappearance of O^4-meT from DNA extracted from hamster and rat cells after treatment *in vitro* and *in vivo,* respectively, with MNU and NDMA. It is known that cells from these two species differ significantly in their capacity to repair O^6-medG. The level of the DNA adducts was determined using specific antibodies against the alkylated nucleosides following their separation by high-performance liquid chromatography.

Rat liver epithelial cells (IAR 27) and V79 hamster cells were treated with MNU (1 mM), and the DNA adducts were then measured at various times. The mutagenic responses to MNU in these two cell types (high mutagenic effect in V79 and low in IAR 27) parallels the repair of O^6-medG (low in V79 and high in IAR 27 cells); no such correlation is observed for repair of O^4-meT.

(*b*) *Development and characterization of antibodies against* N^7*-methyldeoxyguanosine* (N^7*-medG*) *and* O^4*-methylthymidine* (O^4*-meT*) (Dr C.P. Wild, Dr P. Degan, and Dr R. Montesano)

The exposure of organisms to alkylating agents results in alkylation at 12 sites on the DNA bases, and these are associated with the toxic, mutagenic and carcinogenic effects of these chemicals. The main target of methylating agents is the N^7 position of guanine, which accounts for 70–80% of total DNA methylation. This DNA adduct persists for a long time in cellular DNA since it is repaired enzymatically with low efficiency. Determination of this adduct in human tissue DNA with a sensitivity and specificity applicable to epidemiological studies would thus be informative in assessing past exposure to alkylating agents. O^6-medG, which is formed at a ten times lower level than N^7-medG, has been detected in human tissues[3].

Polyclonal antibodies against N^7-medG were raised in rabbits by immunization with two antigens: N^7-medG imidazole ring open form (i.r.o), conjugated either to bovine serum albumin

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Table 37. Specificity, sensitivity and affinity of polyclonal antibody to O^4-methylthymidine

Inhibitor	Amount required to give 50% inhibition [^{3}H]O^4-MeThy[a]-antibody binding in radioimmunessay (pmol)
O^4-Methylthymidine	0.6
O^4-Ethylthymidine	7.8
Deoxythymidine	3.2×10^5
Deoxycytidine	6.7×10^5
7-Methyldeoxyguanosine[b]	$>5 \times 10^5$
Deoxyguanine[c], deoxyadenine[d]	$>10^6$

[a] Specific activity, 16 Ci/mmol; prepared by Dr R. Saffhill, Paterson Laboratories, Manchester, UK

[b] 24% inhibition with 5×10^5 pmol

[c] 27.6% inhibition with 10^6 pmol

[d] 18.7% inhibition with 10^6 pmol

Affinity constant, 0.8×10^9 L/mol

or haemocyanin. The sera obtained were purified by affinity chromatography; ELISA permits the detection of 1.25 pmol N^7-medG i.r.o for 50% inhibition. With 1 mg DNA, this allows detection of one N^7-medG adduct in 10^7 deoxyguanosines. The antibodies recognized both the i.r.o and the closed form of N^7-medG, but with a sensitivity 200 times lower for the closed form. The amounts of other DNA adducts and normal nucleosides required to produce 50% inhibition were: N^7-methylguanine, 1.25×10^4 pmol; deoxyguanine, 1.1×10^5 pmol; deoxyadenine, -thymidine and -cytosine, $>1 \times 10^6$ pmol.

Chromatography procedures were also developed that permit use of a single hydrolysed DNA sample for the separation of N^7-medG, O^6-medG and O^4-meT, with subsequent detection by ELISA or radioimmunoassays (RIA). Immunoassay measurements were validated with radiochemical determinations. Recent experiments *in vivo* show that N^7-medG and O^6-medG can be detected in peripheral blood cell DNA after treatment of rats with NDMA. This is a promising indication for application of these techniques to human peripheral blood cell samples.

Polyclonal antibodies were also raised against O^4-meT using O^4-meT-bovine serum albumin as the immunogen[4]; its properties are shown in Table 37.

(*c*) *Detection of alkylated DNA bases in human tissues* (Dr C.P. Wild, Dr P. Degan, Dr N. Mironov, Dr M. Serres, Mrs G. Martel-Planche, Mrs H. Brésil and Dr R. Montesano; in collaboration with Dr R. Saffhill, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, UK; Dr M.F. Rajewsky, Institute for Cell Biology, University of Essen, Federal Republic of Germany; Dr A.M. Mandard, Francois Baclesse Centre, Caen, France; Dr Lu Shih-Hsin, Cancer Institute, Chinese Academy of Medical Sciences, Beijing; Dr A.J. Likhachev, N.N. Petrov Research Institute of

[4] Wild, C.P., Lu, S.H. & Montesano, R. (1987) In: Bartsch, H., O'Neill, I. & Schulte-Hermann, R., eds, *Relevance of N-Nitroso Compounds to Human Cancer: Exposure and Mechanisms* (*IARC Scientific Publications No. 84*), Lyon, International Agency for Research on Cancer, pp. 534–537

Oncology, Leningrad, USSR; Dr P. Swann, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, UK; and Dr J. Boulez and Dr C. Partensky, Edouard Herriot Hospital, Lyon, France)

Polyclonal and monoclonal antibodies are available against three different DNA alkylation adducts: O^6-medG, N^7-medG and O^4-meT. These antibodies are highly specific, and separation by high-performance liquid chromatography followed by immunoassay results in a high sensitivity (see Table 38) that permits application of these methods to human tissue samples.

Table 38. Detection of DNA alkylated bases by high-performance liquid chromatography separation and immunoassays using 1 mg DNA

DNA adduct	Antibodies	Immunoassay	Molar ratio of DNA adduct: parent nucleoside
O^6-MedGua	Monoclonal	RIA	$4.0:10^{-8}$
N^7-MedGua i.r.o.	Polyclonal	ELISA	$3.2:10^{-7}$
O^4-MeThy	Polyclonal	RIA	$1.0:10^{-7}$

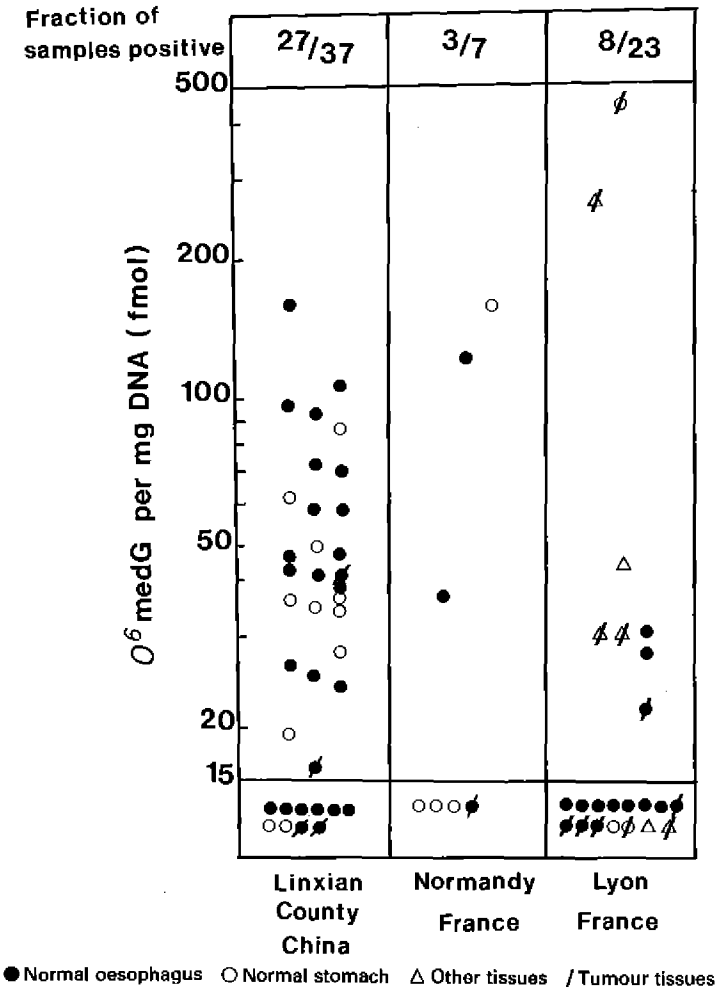
The original studies[5] on the presence of O^6-medG in oesophageal surgical specimens from Linxian County, China, have been extended to a new series of samples from the same region and to samples from Normandy, France. Table 39 shows the levels of O^6-medG; other samples are being examined for the presence of N^7-medG and O^4-meT. More data must be acquired on the level of DNA alkylation adducts in humans in order to ascertain the 'background' levels. It is thus important to find means to detect these adducts in DNA from peripheral blood cells, which are easily obtained and not subject to the biases that condition the selection of surgical tissue specimens. It is equally important to determine that a good correlation exists between the levels of DNA adducts found in tissues and the degree of exposure to alkylating agents, such as nitrosamines.

Preliminary results show that O^6-medG, N^7-medG and O^4-meT can be detected in DNA in blood samples from cancer patients who had been treated with MNU and other chemotherapeutic agents (in collaboration with Dr A. Likhachew, N.N Petrov Research Institute of Oncology, Leningrad, USSR; DEC/85/06). These studies, still in progress, should permit a proper quantitative evaluation of this methodolgy for their suitability in epidemiological studies aimed at elucidating the role of nitrosamines in human cancer. Preliminary studies also show that these antibodies can be used for the immunocytochemical detection of DNA adducts in intact cells in tissue sections, using an immunoperoxidase staining technique.

The DNA repair capacity of tissues from various individuals is also being assessed, using double-stranded oligonucleotides containing either O^6-medG or O^4-meT as substrates.

[5] Umbenhauer, D., Wild, C.P., Montesano, R., Saffhill, R., Boyle, J.M., Huh, H., Kirstein, U., Thomale, J., Rajewsky, M.F. & Lu, S.H. (1985) *Int. J., Cancer, 36,* 661–665

Table 39. O^6-Methyldeoxyguanosine in human tissues



2. ROLE OF ONCOGENES IN THE DEVELOPMENT OF TUMOURS IN HUMANS AND IN EXPERIMENTAL ANIMALS

Transforming cellular oncogenes have been detected in tumours from humans and animals, lending support to the hypothesis that cellular oncogene activation is one of the genetic bases of carcinogenesis[6]. Further support is provided by studies showing that carcinogen-specific mutation of a proto-oncogene, Ha-*ras*, occur consistently in tumours induced in experimental animals by certain chemical carcinogens[7]. Analysis of human and animal tumours has also

[6] Bishop, M. (1987) *Science, 235,* 305–316
[7] Barbacid, M. (1986) *Carcinogenesis, 7,* 1037–1042

shown that activation of cellular oncogenes can result not only from point mutation but also from chromosomal rearrangement or amplification[6]. On the basis of this information, we consider that identification of etiological risk factors may be facilitated by examining oncogene activation in human tumours found in a population whose exposure to a specific risk factor(s) is well characterized. Several animal models have also been developed to help our understanding of molecular mechanisms of multistage carcinogenesis. Such models can also be used to study the interaction of environmental carcinogens and cellular oncogenes.

(*a*) *Transplacental* ras *activation in mouse skin tumours* (Dr H. Yamasaki, Dr M. Hollstein, Miss N. Martel, Mrs D. Galendo, Dr J.R.P Cabral and Dr L. Tomatis)

In order to develop experimental animal models of tumour promotion by 12-*O*-tetradecanylphorbol 13-acetate (TPA) within the context of initiation of carcinogenesis by *ras* mutation, we modified the classical mouse-skin two-stage carcinogenesis assay so that initiation occurs during fetal development and promotion occurs after birth[8]. Pregnant CD-1 mice were injected intraperitoneally on gestation day 15 with 10 or 100 mg per kg body 7,12-dimethylbenz[*a*]anthracene (DMBA). When the offspring were two weeks old, their backs were painted with either TPA (twice a week, 3 μg/application) or acetone. Transplacental initiation by DMBA alone at 10 mg/kg is insufficient to produce papillomas or carcinomas; it is necessary to apply TPA susequently in order to obtain tumours[9]. This was also the case when we used benzo[*a*]pyrene at 3 mg/mouse as an initiator[8]. Therefore, we can clearly separate the transplacental initiation process from postnatal promotion in skin carcinogenesis.

Table 40. Summary of analysis of Xba I RFLP in mouse skin tumours

Sample	Treatment		Xba I RFLP (positive/total number)
	Transplacental	Postnatal	
Carcinoma	DMBA, 100 mg/kg bw	TPA	2/2
	DMBA, 10 mg/kg bw	TPA	7/7
	—	Benzo[*a*]pyrene + TPA	0/4[a]
	Benzo[*a*]pyrene	TPA	0/1
Papilloma	DMBA, 100 mg/kg bw	TPA	5/9
	DMBA, 10 mg/kg bw	TPA	1/3
	—	TPA	0/4
Normal tissues (skin and liver from treated and untreated animals)			0/14

[a] From C57BL/6 mice

We analysed DNA from tumours for possible activation of cHa-*ras* gene, taking advantage of the information that DMBA can activate mouse cHa-*ras* by a specific mutation that is detectable by XbaI restriction fragment length polymorphism (RFLP)[10]. Table 40 lists the DNA samples from mouse papillomas and carcinomas tested by XbaI RFLP analysis for the cHa-*ras* codon 61 A to T mutation. Representative results from autoradiographed Southern blots of these DNA restriction endonuclease analyses are shown in Figure 10. The mutation is identified by the

[8] IARC (1984) *Annual Report 1984, Lyon, p.* 85

[9] Yamasaki, H., Hollstein, M., Martel, N., Cabral, J.R.P., Galendo, D. & Tomatis, L. (1987) *Int. J. Cancer* (in press)

[10] Quintanilla, M., Brown, K., Ramsden, M. & Balmain, A. (1986) *Nature, 322,* 78–80

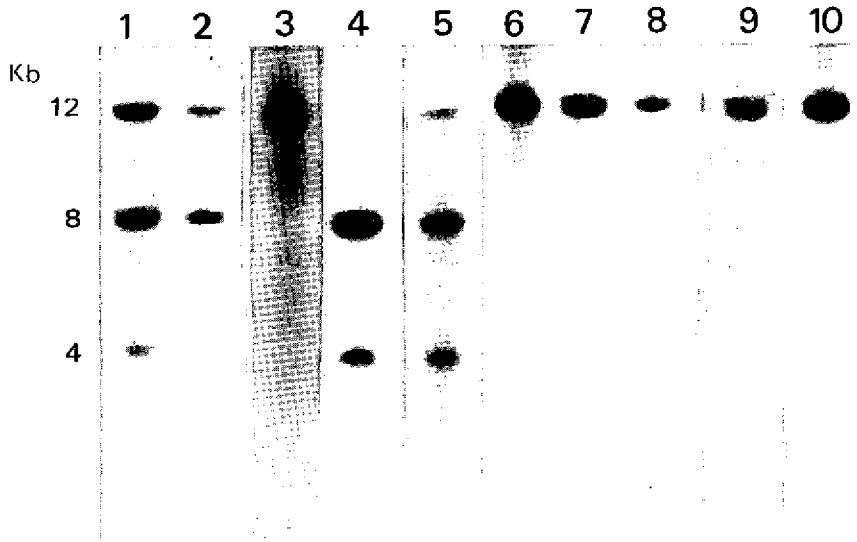


Fig. 10. Xba I restriction endonuclease analysis of the HA-*ras* locus from DNA of primary, carcinomas, papillomas and normal mouse tissue

DNA (10 mg) was digested with restriction endonuclease Xba I, electrophoresed on 0.7% agarose gels and hybridized, after transfer to nylon membranes, with ^{32}P-labelled p-ras 1. Lanes: 1 and 2, DNA from skin carcinomas of CD-1 mice exposed transplacentally to DMBA and postnatally to topical application of TPA; 3, DNA from carcinoma of CD-1 mouse treated transplacentally with benzo[a]pyrene and postnatally with TPA; 4, reference standard: DNA (gift of A. Balmain) from NIH 3T3 mouse carcinoma induced by DMBA and TPA, homozygous for Xba I Ha-ras RFLP; 5 and 6, DNA from papillomas of a tumour-bearing mouse treated transplacentally with DMBA and postnatally with TPA; 7 and 8, normal skin and liver DNA of a CD-1 mouse exposed to DMBA and TPA; 9, normal liver DNA of untreated CD-1 mouse; 10, DNA from papilloma of mouse treated postnatally with topical application of TPA; approximate size of Xba I DNA fragments containing the mouse c-Ha-ras gene are indicated in the left-hand margin.

appearance of two characteristic Ha-*ras* hybridizing Xba I DNA fragments of 8 kb and 4 kb, whereas the normal cHa-*ras* allele yields a single 12-kb band. All nine carcinomas tested showed the presence of the mutated allele, whereas only 50% of the papillomas tested did so[9]. When several papillomas from the same multiple tumour-bearing mouse were tested, both RFLP-positive and RFLP-negative papilloma DNAs were found (Fig. 10, lanes 5 and 6).

All the RFLP-positive samples that we tested are apparently heterozygous for the mutated allele. We also tested five skin carcinomas from mice initiated with benzo[*a*]pyrene and promoted with TPA, and in none of these tumours did we detect the Ha-*ras* 61st codon A to T transversion (Table 35), confirming the specificity of this mutation with respect to initiating agents, as has been shown by others[10,11]. No sample of papillomas produced from TPA treatment alone was positive for the RFLP, nor were samples of normal tissues taken from exposed and unexposed mice (Table 40).

[11] Bizub, D., Wood, A.W. & Shalka, A.M. (1986) *Proc. natl Acad. Sci. USA, 83,* 6048–6052

Since all the carcinomas produced by transplacental exposure to DMBA showed the A to T transversion at codon 61 of cHa-*ras*, this specific mutation is probably a critical, essential event in the production of these tumours. Since we know that all carcinomas tested were derived from pre-existing papillomas, and since we used individual primary carcinomas and papillomas for RFLP analysis, the activated cHa-*ras* must have been present at the papilloma stage. Since only 50% of papillomas were positive in the XbaI RFLP analysis, our data suggest that those papillomas with an A to T transversion in cHa-*ras* progress preferentially to carcinomas.

(*b*) ras *activation as a mechanism of in-vitro cell transformation* (Dr M. Hollstein, Miss N. Martel, Mrs C. Piccoli and Dr H. Yamasaki; in collaboration with Dr T.J. Slaga and Dr R. Klann, University of Texas System Cancer Center, Smithville, TX, USA)

Our in-vivo experiment showed that all mouse skin carcinomas arising from transplacental exposure to DMBA and neonatal TPA treatment arose from cells with an Ha-*ras* activating mutation at precisely the same location. In order to see whether the same mutation is responsible for cell transformation *in vitro*, we transformed BALB/c 3T3 cells by DMBA and analysed the DNA of transformed cells for the presence of an A to T transversion at the 61st codon of H-*ras*. In parallel, mouse epidermal cell lines derived from a carcinoma, a papilloma and presumptive 'initiated' mouse skin cells were also analysed.

Many morphologically transformed foci were produced when subconfluent BALB/c 3T3 clone 1–1 cells were exposed to medium containing DMBA. The DNA of cells from 19 separate foci was analysed, using the restriction enzyme XbaI, for the presence of the Ha-*ras* 8-kb and 4-kb fragments, indicative of an A to T codon 61 transversion. The results showed no evidence of this mutation: all the clones appear to contain only the normal 12-kb allele.

We also analysed the DNA from a cell line established from a mouse skin carcinoma produced by a DMBA-TPA protocol, and the DNA from a cell line derived from mouse skin epidermal cells initiated (DMBA) *in vivo*. As for DNA taken directly from carcinomas, that from the carcinoma cell line also showed RFLP indicative of an A to T transversion at codon 61 of Ha-*ras*. However, a cell line selected for high resistance to calcium after DMBA treatment *in vivo* did not show this RFLP. Since calcium-resistant cells are considered to be initiated[12], this finding is consistent with our in-vivo data with papillomas that DMBA initiation can be attained by molecular mechanisms different from A to T transversion at the 61st codon of Ha-*ras*.

(*c*) *Analysis of tumour DNA for oncogene mutations* (Dr M. Hollstein, Dr K. Enomoto, Dr R. Montesano, Miss N. Martel, Mrs A.M. Aguelon and Dr H. Yamasaki; in collaboration with Dr S.H. Lu, Cancer Institute, Chinese Academy of Medical Sciences, Beijing; Dr M. Barbacid, Frederick Cancer Research Center, Frederick, MD, USA; Dr D. Huang, Chinese University of Hong Kong; and Dr J. Bos, University of Leiden, Netherlands)

c-*ras* mutations in human oesophageal tumour DNA from Linxian Country, China, and Normandy, France, (two high-risk areas) and Lyon, France, are being tested directly for codon 12, 13 and 61 activating mutations in the cHa-*ras*, cKi-*ras* and N-*ras* genes, using differential synthetic oligonucleotide hybridization[13]. This analysis was perfomed in collaboration with Dr J. Bos at the University of Leiden, where a bank of 100 probes specific for these mutation is available. Screening of 23 DNA samples for Ki- and N-*ras* mutations has recently been completed; there was no positive sample. The Ha-*ras* locus has not yet been analysed. In this analysis, it is possible that the tumour mass from which the DNA is extracted contains a

[12] Yuspa, S.H. & Morgan, D.L. (1981) *Nature, 293*, 72–74

[13] Bos, J.L., Verlaande Vries, M., Jansen, A.M., Veeneman, G.H., Van Boom, J.H. & Van der Eb, A.J. (1984) *Nucleic Acids Res., 12*, 9155–9163

significant fraction of nontumour cells from surrounding tissues, which could yield false-negative results by diluting out the mutated allele with the normal allele. Particular attention will be given in future testing to clarification of this problem.

In order to detect activated oncogenes other than c-*ras* genes, we used the transfection assay and initiated a search for transforming activity in oesophageal tumour DNA. Of five oesophageal tumour DNAs from China, one was found by assay with NIH 3T3 cells to contain a novel transforming sequence, tentatively designated oncF, which is in the process of being cloned and sequenced in the laboratory of Dr Barbacid. Transforming sequences were also detected in the human oesophageal tumour-cell line CuII. Transformed foci in BALB/c 3T3 cells were expanded in nude mice, and DNAs from the resulting tumours were shown to have human *alu* sequences, confirming the presence of foreign human tumour DNA in the transformed BALB/c 3T3 cells. It is not known yet whether these sequences correspond to a previously characterized oncogene.

(*d*) *Analysis of normal and tumour DNA from cancer patients for oncogene polymorphisms* (Dr M. Hollstein, Dr K. Enomoto, Dr R. Montesano, Miss N. Martel, Mrs A.M. Aguelon and Dr H. Yamasaki)

In 1985, Dr J. Lidereau and coworkers showed that a rare form of the c-*mos* proto-oncogene was found more frequently in breast cancer patients (6/75) than in individuals without cancer

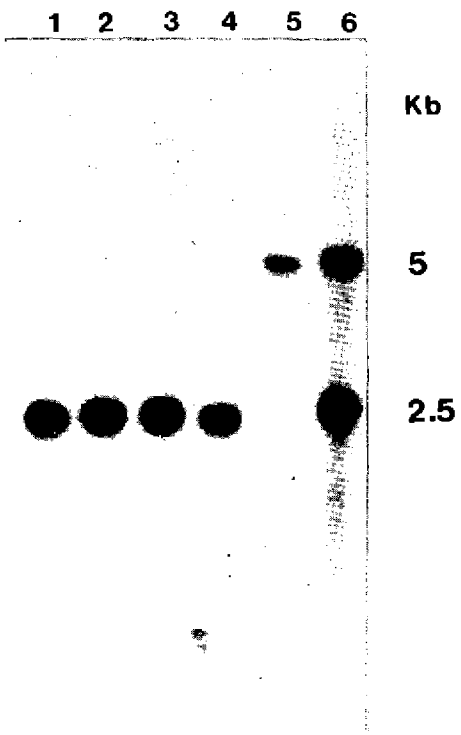


Fig. 11. c-*mos* Eco RI polymorphism in oesophageal cancer patients

Lanes 1–4: DNA from four cancer patients in Normandy, France, showing the normal 2.5-kb c-mos *allele; lanes 5,6: DNA from two patients in Lyon, France, with the rare 5-kb allele, one of which (lane 5) is homozygous for the polymorphism.*

(0/100)[14]. We wished to learn whether the presence of this rare allele was also associated with oesophageal cancer.

A pilot study of 12 oesophageal tumour DNAs from Caen and Lyon, France, revealed the presence of the rare allele in two cases (Fig. 11); the mutation is constitutive since DNA from both normal and tumour tissues of affected individuals contains the altered c-*mos* form. Since our report of this preliminary RFLP analysis[15], an additional 24 samples from Normandy have been tested, one of which was positive for the rare allele.

Further studies are in progress to ascertain if this association is a spurious one or is indicative of predisposition to oesophageal cancer.

3. CHEMICAL CARCINOGENESIS AND MUTAGENESIS IN CULTURED CELLS

(*a*) *Selective killing of transformed cells through intercellular communication: a possible therapeutic method* (Dr H. Yamasaki and Mr F. Katoh)

We demonstrated previously that when transformed foci are induced on BALB/c 3T3 monolayer cells by a carcinogen, the cells have selective communication capacity: transformed cells have the usual level of intercellular communication among themselves but do not communicate with surrounding normal cells[16]. Selective communication was also confirmed in transformed rat liver epithelial cells[17]. These results indicate that transformed cells have their own gap-junctional communication compartment, which is independent from that of surrounding normal cells. They also provide the theoretical basis for supposing that when a toxic substance that cannot diffuse through the cell membrane but which can be transferred *via* gap junctions is micro-injected into a transformed cell, it would spread only among transformed cells but not to surrounding normal cells, resulting in selective killing of transformed cells. On the basis of this idea, we developed a method for killing transformed cells selectively by microinjecting Lucifer Yellow CH dye followed by blue-light irradiation at about 430 nm, a treatment that can kill cells[18].

We produced transformed foci on BALB/c 3T3 cells by a chemical carcinogen, 3-methylcholanthrene, or by an activated oncogene, pEJ-*ras*-H-1[19]. Figure 12 shows EJ-*ras*-transformed foci before and after killing; only transformed cells were killed by blue-light irradiation.

We have extended our method to epithelial cells, using transformed and nontransformed rat-liver epithelial cells. Since there is no method for inducing transformed epithelial foci by carcinogens *in situ*, we artificially cocultured transformed and nontransformed rat liver epithelial cells so that they were in contact. When transformed cells (IAR 27 or IAR 6-1) were cocultured with nontransformed cells (IAR 20), the border between the two types was clearly visible, because of the difference in their morphological appearance (Fig. 13). Microinjected Lucifer Yellow CH again spread selectively, and, following exposure to near ultraviolet light the targeted cells were killed.

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[14] Lidereau, J., Mathieu-Mahul, D., Theillet, C., Renaud, M., Mauchauffé, M., Gest, J. & Larsen, C.J. (1985) *Proc. natl Acad. Sci. USA, 82,* 7068–7070; and personal communication

[15] Hollstein, M., Montesano, R. & Yamasaki, H. (1986) *Nucleic Acids Res., 14,* 8695

[16] Enomoto, T. & Yamasaki, H. (1984) *Cancer Res., 44,* 5200–5203

[17] Mesnil, M. & Yamasaki, H. (in preparation)

[18] Miller, J.P. & Selverston, A.I. (1979) *Science, 206,* 702–704

[19] Yamasaki, H., Hollstein, M., Mesnil, M., Martel, A. & Aguelon, A.M. *Cancer Res.* (in press)

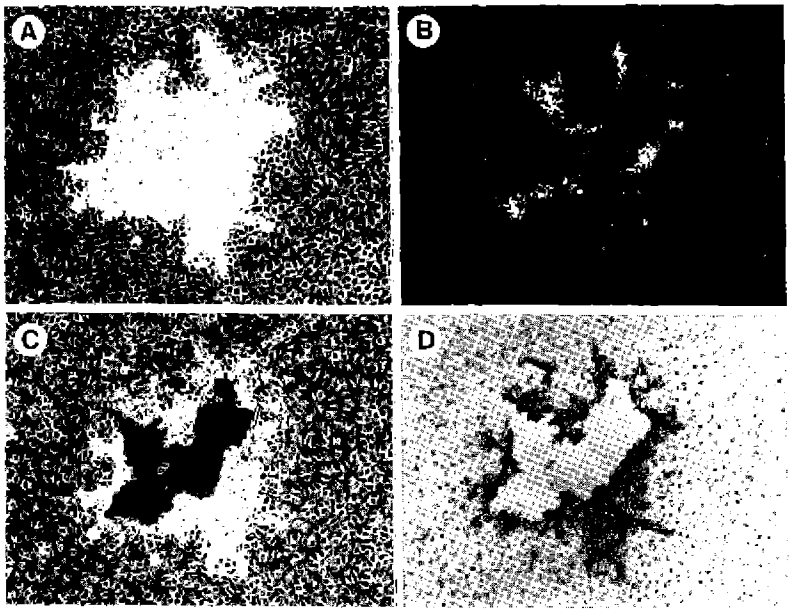

Fig. 12. Selective elimination of a transformed focus

*BALB/c 3T3 cells were transfected with purified plasmid EJ-*ras *DNA (0.3 μg/plate); then, Lucifer Yellow CH solution (10%) was microinjected into several cells in a transformed focus and the dish was radiated with blue light. A, pEJ-*ras*-H-1 transformed BALB/c 3T3 focus; B, fluorescent micrograph of the same focus after microinjection of Lucifer Yellow CH; C, phase-contrast micrograph of the transformed focus after blue-light irradiation (Note that most of the focus is raised above the culture dish, probably due to death of cells at the surface.); D, trypan blue staining of C (Note that the remaining transformed cells, and not the surrounding cells, are clearly stained, suggesting that the transformed cells have been killed selectively.).*

The efficiency of such selective killing is directly dependent on the communication capacity of the target cells. Thus, the greater the number of cells into which Lucifer Yellow CH spreads, the more cells are killed by blue-light irradiation. We also increased killing efficiency by stimulating gap-junctional communication with an exogenous compound, dibutyrl cAMP.

These results were obtained *in vitro*. A number of problems must be resolved before the method can be extended to killing of tumorigenic cells *in vivo*. Although possible applications to chemotherapy are still at the hypothetical stage, the method is effective for killing tumour cells. We believe this to be an important new approach.

(*b*) *Inhibition of cell transformation and reversion of transformed phenotypes by restoration of selective intercellular communication with normal cells* (Dr H. Yamasaki and Mr F. Katoh)

Since our previous data suggested that blocked intercellular communication is involved in the promotion phase of cell transformation[20], we thought it possible to inhibit cell transformation by

[20] Yamasaki, H. (1986) *Toxicol. Pathol.*, *14*, 363–369

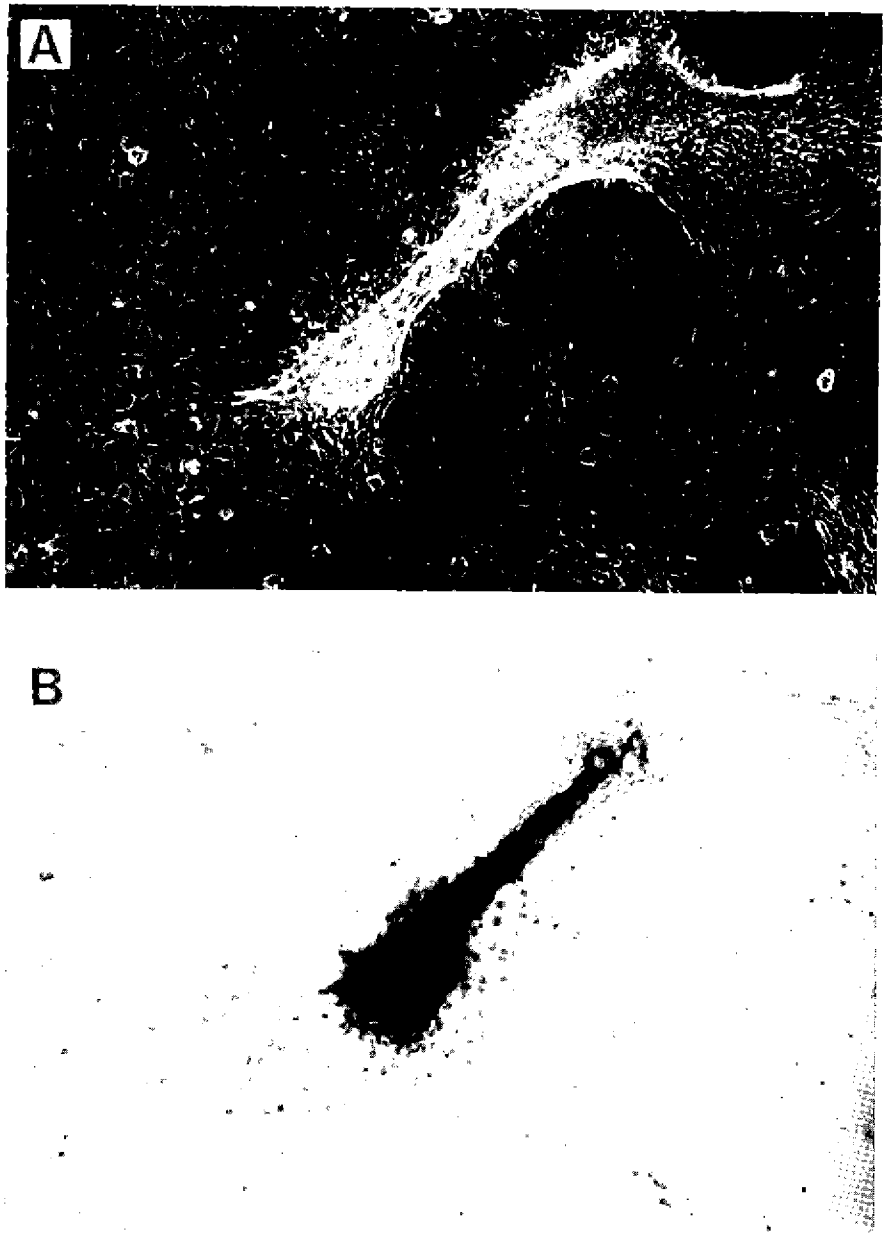

Fig. 13. Selective killing of transformed rat-liver epithelial cells

Tumorigenic (IAR6-1) and nontumorigenic (IAR20) rat-liver cells were cocultured so that there was a clear boundary between them. The two types of cell are readily distinguishable by their different morphology. Lucifer Yellow solution was microinjected into IAR 6-1 cells, and they were irradiated with blue light, as described in the legend to Figure 12. A, phase-contrast micrograph of a coculture of IAR 6-1 and IAR 20 cells. The IAR 6-1 cells are piled up and look brighter, whereas the surrounding IAR 20 cells form a regular layer. B, trypan blue staining of the same field as in A. Note that only IAR 6-1 cells (and possibly a few IAR 20 cells) are stained, suggestive massive killing of IAR 6-1 cells.

reversing the communication capacity of target cells. We used retinoic acid, dexamethasone, fluocinolone acetonide and dibutyryl cyclic AMP — which have been reported to inhibit TPA-mediated mouse skin tumour promotion[21] — to antagonize TPA inhibition of intercellular communication. When these chemicals were added to the BALB/c 3T3 cell transformation system, subsequent to a high dose of methylcholanthrene alone or a low dose of methylcholanthrene plus phorbol 12,13-didecanoate, there was a significant decrease in the production of transformed foci. These results suggest that these chemicals inhibit cell transformation by stimulating intercellular communication, further confirming the hypothesis that communication blockage is involved in cell transformation.

We demonstrated previously that when BALB/c 3T3 cells are transformed by a variety of different chemicals or by an activated oncogene, selective intercellular communication evolves, whereby transformed cells communicate among themselves but not with surrounding monolayer cells[16,19]. We postulated that it would be possible to reverse the transformed phenotype if communication could be re-established between transformed and normal cells. When dibutyryl cAMP, dexamethasone, fluocinolone acetonide or retinoic acid was added to a culture of BALB/c 3T3 cells in which a number of transformed foci had been produced by methylcholanthrene, many, although not all, transformed foci began to disappear; some foci completely disappeared after three weeks of treatment (Fig. 14). Transformed cells started to communicate with surrounding normal cells after one week of treatment. Since reversion did not occur when these chemicals were added to cultures in which only transformed foci were present, these results indirectly support our hypothesis that transformed cells must have their own intercellular communication compartment in order to maintain autonomous cell growth and transformed phenotypes.

(c) *Use of cultured rat-liver cells to study molecular and cellular mechanisms of carcinogenesis* (Dr K. Enomoto, Mr M. Mesnil, Dr R. Montesano, Mrs C. Piccoli and Dr H. Yamasaki; in collaboration with Dr C. Guguen-Guillouzo and Dr J.M. Fraslin, National Institute for Health and Medical Research U49, Rennes, France)

Since most human cancers originate from epithelial cells, it is important to establish epithelial cell transformation systems and to study molecular and cellular mechanisms therein. A series of rat-liver epithelial cell lines with different malignant phenotypes, ranging from nontumorigenic to metastastic, has been developed in our laboratory. They have proved to be an excellent model system for studying the process of epithelial cell transformation and for identifying markers associated with the transformation[22,23]. So far, we have characterized these cell lines at the cellular level and found that their tumorigenicity generally correlates well with their gap-junctional communication capacity, as well as with classical markers such as ability to grow in soft agar[24]. In order to study molecular mechanisms of expression of transformed phenotypes, DNA samples from four chemically transformed, two spontaneously transformed and two nontransformed rat-liver epithelial cell lines were examined by Southern blot hybridization analysis. DNA extracted from each cell line and from normal rat liver was digested with restriction enzymes and hybridized with labelled v-K-*ras*, v-H-*ras*, v-*myc* and v-*raf* oncogenes. After hybridization with v-H-*ras* gene, we found amplification of the EcoRI-digested 13-kb

[21] Yamasaki, H. & Enomoto, T. (1985) In: Barrett, J.C. & Tennant, R.W., eds, *Carcinogenesis — A Comprehensive Survey*, Vol. 9, New York, Raven Press, pp. 179–194
[22] Montesano, R., St Vincent, L., Drevon, C. & Tomatis, L. (1975) *Int. J. Cancer, 16,* 550–558
[23] Montesano, R., St Vincent, L. & Tomatis, L. (1973) *Br. J. Cancer, 28,* 215–220
[24] Mesnil, M., Montesano, R. & Yamasaki, H. (1986) *Exp. Cell Res., 165,* 391–402

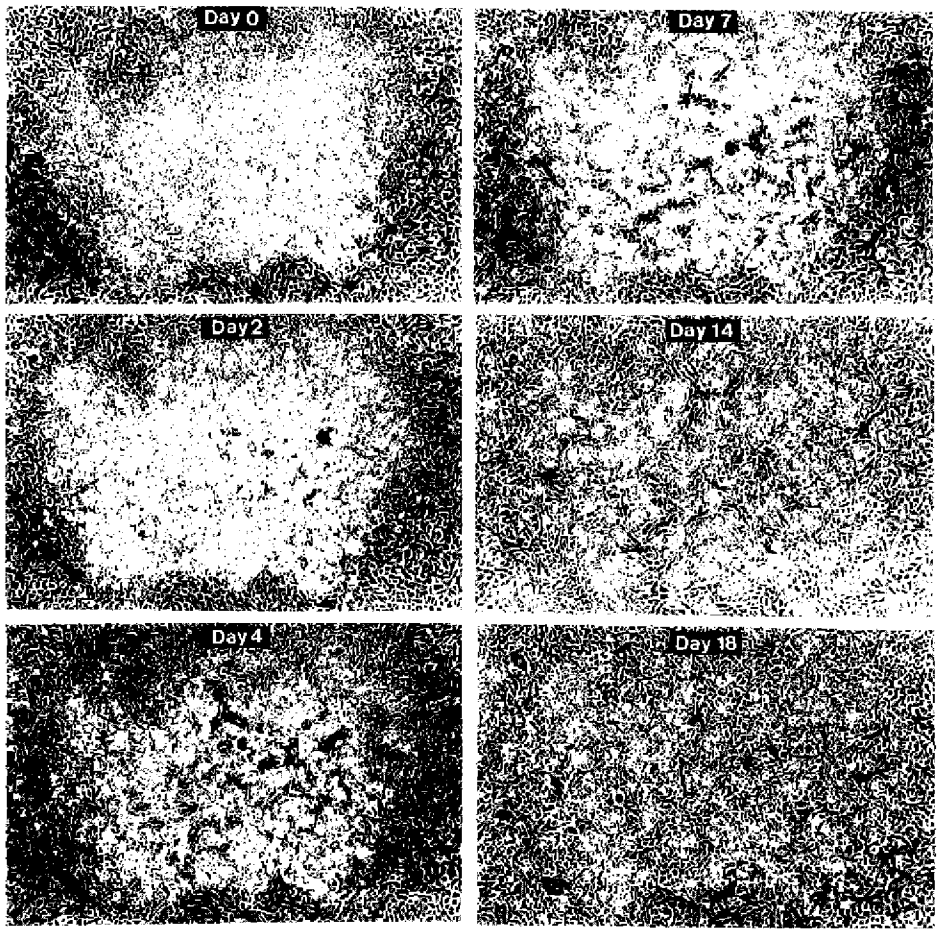

Fig. 14. Disappearance of a transformed focus after prolonged treatment with dexamethasone

BALB/c 3T3 A31-1-1 cells were transformed by methylcholanthrene, and, when foci appeared, dexamethasone (1 mg/ml) was added to the culture medium (day 0). Culture medium was changed twice weekly, with addition of dexamethasone. Note that the morphologically transformed focus started to disappear on day 4 and can barely be seen on day 18.

band, which corresponds to the rat H-*ras* 2 gene in a chemically (*N*-nitrosodimethylamine) transformed IAR 6-1 cell line, whereas no such amplification was seen in other cell lines or in normal rat liver. Alteration of the molecular size of the *myc* gene were also observed in IAR 6-1, but there was no change in K-*ras* and *raf* oncogenes. When these cells were grown in soft agar, the number of IAR 6-1 colonies was five to ten times greater than that of other transformed cell lines. In addition, IAR 6-1 showed the most potent tumorigenicity in nude mice and metastatic ability in newborn rats. It thus appears that H-*ras* 2 and *myc* gene amplification seen in IAR 6-1

cells might be related to expression of their transformed phenotypes. Further analysis, including DNA transfection, is under way.

The specific differentiated gene expression and morphology of adult rat hepatocytes can be maintained for as long as eight weeks *in vitro* only when the cells are cultured in the presence of biliary epithelial cells; when primary hepatocytes are cultured alone, they lose these functions within two to three days[25]. We obtained evidence suggesting that contact between hepatocytes and biliary epithelial cells is necceary for maintaining hepatocyte function and examined whether junctional communication between and among hepatocytes and biliary epithelial cells is required for long-term maintenance of hepatocyte functions, using a dye-transfer method, in different cocultures. The established epithelial cell line (IAR 20) and early-passage cultures of biliary epithelial cells maintained hepatocyte-specific functions in culture for 40 and 70 days, respectively.

When the hepatocytes were cultured alone, they lost their characteristic morphology within five to eight days, and almost no dye transfer was observed. In coculture, the capacity of biliary epithelial cells to communicate among themselves remained relatively high throughout the culture period, whereas hepatocytes showed almost no junctional communication at an early phase of culture and first began to communicate after two weeks; communication capacity increased for at least the next ten day of culture. The most notable finding was that there was no dye transfer between hepatocytes and biliary epithelial cells in any coculture system[26]. These results suggest that maintenance of hepatocyte-specific functions requires intercellular contact but probably not gap-junctional communication between hepatocytes and biliary epithelial cells. Our preliminary results suggest that BALB/c 3T3 cells can also be used as a coculture partner to maintain rat hepatocytes. This system is useful for studying heterotypic cell–cell interactions and the control of gene expression.

(*d*) *Simultaneous assay of cytotoxicity, mutagenicity and cell transformation by environmental chemicals in BALB/c 3T3 cells* (Dr H. Yamasaki and Mrs C. Piccoli; in collaboration with Dr T. Kakunaga, Osaka University, Osaka, Japan)

Attempts to develop a system in which cytotoxicity, mutability and transformability of chemicals can be tested simultaneously are being continued. We have tested three chemicals: benzene, DDT and diethylstilboestrol in this assay, using *N*-nitrosodimethylamine, *N*-methyl-*N*-nitrosourea and methylcholanthene as control carcinogens. None of the test chemicals induced ouabain resistance in the presence or absence of a primary rat-liver hepatocyte metabolic activation system, but they gave borderline induction of morphological transformation. In the presence of rat-liver hepatocytes, the frequency of induction decreased. These results suggest that BALB/c 3T3 cells can be used to demonstrate transforming activity of nonmutagenic carcinogens but that rat-liver hepatocytes are not the ideal metabolic activation system.

(*e*) *International symposium on cell differention and carcinogenesis — critical gene expression during carcinogenesis* (Dr H. Yamasaki; in collaboration with Dr T. Kakunaga, Osaka University, Osaka, Japan)

An international symposium to discuss molecular mechanism of carcinogenesis was held in Osaka on 24–26 February 1987. Twenty-five invited scientists from Japan, Europe and the USA presented papers on various aspects of the molecular mechanisms of multistage carcinogenesis,

[25] Guguen-Guillouzo, C., Clement, B., Baffet, G., Beaumont, C., Morel-Chany, E., Glaise, D. & Guillouzo, A. (1983) *Exp. Cell Res., 143,* 47–54

[26] Mesnil, M., Fraslin, J.M., Piccoli, C., Yamasaki, H. & Guguen-Guillouzo, C. (1987) *Exp. Cell Res.* (in press)

including oncogene activation, suppression genes, perinatal carcinogenesis, tumour promotion and cell-cell interaction. About 100 scientists participated in the symposium; the proceedings are scheduled to be published as an *IARC Scientific Publication.*

(*f*) *Overexpression of the epidermal growth factor (EGF) receptor and amplification of its genes in human squamous-cell carcinomas (SCC) in culture* (Dr T. Kuroki, Institute of Medical Science, University of Tokyo, Tokyo, DEC/79/006)

EGF is a polypeptide hormone with a molecular weight of 6000 which is mitogenic for a variety of cells of both epithelial and mesenchymal origin. The action of EGF is mediated by a cell surface receptor which shows tyrosine-specific protein kinase activity and the gene of which is related to the *erb* B oncogene of avian erythroblastosis virus.

The level of EGF receptor was examined in 24 human cell cultures, including 13 of SCC of the skin, oral cavity and oesophagus[27]. The amounts of EGF receptor on these SCC cells were measured by a binding assay of membrane preparations using ^{125}I-EGF. Of the 13 SCC cell cultures tested, all except three of oesophageal SCC showed higher levels of EGF receptor than normal epidermal keratinocytes. Amplification of the EGF receptor gene was also found in SCC cells[28]. In HSC-1 cells, which contain about 53-fold more EGF receptors than epidermal keratinocytes, the *erb* B gene is amplified about 50-fold relative to that in keratinocytes. Thus, overexpression of EGF receptor in SCC cells probably results from amplification of the c-*erb* B proto-oncogene. In SCC cells, EGF was found to inhibit growth and colony formation at doses that are mitogenic in many other cells. Sensitivity to the inhibitory effect of EGF correlated well with the elevated level of EGF receptor. The study suggests that amplification of the EGF receptor gene may be causally related to carcinogenesis in epidermal cells.

(*g*) *Cellular and biochemical markers of neoplastic transformation of epithelial cells in culture* (Professor J.M. Vasiliev, All-Union Cancer Research Centre of the Academy of Medical Sciences of the USSR, Moscow, DEC/79/010)

Monoclonal antibodies (MAb) that selectively recognize simple human epithelial keratin were developed, which have made it possible to extend studies on keratin expression in various human tumours.

TPA has been shown to induce alterations in the interrelationship between actin and tubulin cytoskeletal systems in a fibroblastoid cell line. The molecular mechanisms of these changes are being investigated. The study has been continued on the influence of the extracellular matrix on the behaviour of cultured epithelial cells. It has been shown that the time course and morphology of cell spread on the matrix differ from that on glass. Epithelial cells and their islets orientated themselves along matrix fibres composed mainly of fibronectin. Experiments with native complex matrix (basement membrane, connective tissue films) are under way.

4. MECHANISMS OF TUMOUR PROMOTION

(*a*) *Diacylglycerols as endogenous functional analogues of phorbol esters in the enhancement of cell transformation* (Dr U. Frixen and Dr H. Yamasaki)

Tumour-promoting phorbol esters and diacylglycerols have a variety of biochemical and biological effects in common in a number of cell culture systems; thus, it has been suggested that

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[27] Kamata, N., Chida, K., Rikimaru, K., Horikoshi, M., Enomoto, S. & Kuroki, T. (1986) *Cancer Res., 46,* 1648–1653

[28] Yamamoto, T., Kamata, N., Kawano, H., Shimizu, S., Kuroki, T., Toyoshima, K., Rikimaru, K., Nomura, N., Ishizaki, R., Pastan, I., Gamou, S. & Shimizu, N. (1986) *Cancer Res., 46,* 414–416

diacylglycerols are endogenous functional analogues of phorbol esters[29]. We have studied the promoting effect of 1-oleoyl-2-acetyl glycerol (OAG) in the two-stage transformation model with BALB/c 3T3 cells. When cells were treated once with a low dose of methylcholanthrene, almost no increase in cell transformation occurred; however, repeated application of OAG after initiating treatment enhanced cell transformation. It also continuously inhibited junctional intercellular communication among BALB/c 3T3 cells treated daily at confluence. These results indicate that diacylglycerols mimic phorbol esters in promoting cell transformation and that the enhancement may be related to an inhibitory effect on intercellular communication[30]. Together with our previous findings that OAG inhibits murine erythroleukaemia cell differentiation[31], the present results indicate that diacylglycerols are general endogenous functional analogues of phorbol ester tumour promoters.

(*b*) *Tumour-promoting activity of transforming growth factor β (TGFβ)* in vitro (Dr E. Hamel, Mr F. Katoh and Dr H. Yamasaki; in collaboration with Dr G. Müller and Dr W. Birchmeier, Friedrich Miescher Laboratory, Max Planck Institute, Tübingen, Federal Republic of Germany)

TGFβ is a physiological factor secreted by numerous transformed cells as well as by normal cells. It has been characterized initially as an inducer of growth in soft agar of normal cells in the presence of EGF[32]. TGFβ shares several common biological effects with a class of tumour-promoting agents, phorbol esters. We therefore tested whether it has tumour-promoting activity.

We used the in-vitro, two-stage BALB/c 3T3 cell transformation system. Cells were treated with a low dose (1 μg/ml) of methylcholanthrene, followed by treatment with promoting agents. At this dose, methylcholanthrene alone has only weak transforming ability, but promoters such as phorbol-12,13-didecanoate greatly enhanced the number of transformed foci of methylcholanthrene-initiated cells. TGFβ is even more potent than the phorbol ester in tumour promotion in this assay (Fig. 15). The promoting activity was dose-dependent with 0.01–1 mg/ml TGFβ[33]. To our knowledge, this is the first direct demonstration that TGFβ exhibits tumour-promoting activity *in vitro*. Since phorbol-12,13-didecanoate is a potent inhibitor of intercellular communication, we measured the effect of TGFβ on BALB/c 3T3 cells by a dye transfer assay. TGFβ had no effect[33], however, BALB/c cells transformed by methylcholanthrene and TGFβ showed selective inhibition of intercellular communication, i.e., transformed cells communicated among themselves but not with surrounding normal cells[33].

(*c*) *Control of cellular oncogene expression during cell differentiation and its modulation by tumour-promoting agents* (Miss L. Giroldi, Dr M. Hollstein and Dr H. Yamasaki)

We have studied the interactions of TPA with proto-oncogenes in the Friend erythroleukaemia cell system (FELC). Upon treatment with hexamethylene bisacetamide (HMBA), these cells differentiate into cells resembling erythrocytes[34], and TPA reversibly inhibits this

[29] Nishizuka, Y. (1984) *Nature, 308,* 693–698
[30] Frixen, U. & Yamasaki, J. (1987) *Carcinogenesis* (in press)
[31] Giroldi, L., Hamel, E. & Yamasaki, H. (1986) *Carcinogenesis, 7,* 1183–1186
[32] Massague, J. (1987) *Cell, 49,* 437–438
[33] Hamel, E., Katoh, F., Müller, G., Birchmeier, W. & Yamasaki, H. (1987) In: *European Association of Cancer Research Meeting, Helsinki, 1–3 June 1987,* Abstracts, p. 85
[34] Marks, P.A. & Rifkind, R.A. (1987) *Ann. Rev. Biochem., 47,* 419–448

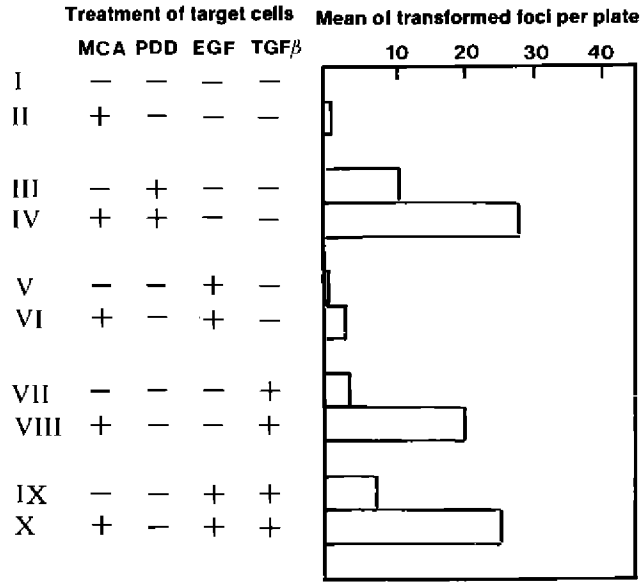

Fig. 15. Two-stage in-vitro BALB/c 3T3 cell transformation

Cells are seeded at 10^4 cells per dish and treated 24 h later with 1 μg/ml MCA for 96 h; phorbol-12,13-didecanoate (100 ng/ml), EGF (2 ng/ml) or TGFβ (1 ng/ml) was added at each medium change, twice a week, for four weeks. The plates were fixed with methanol and stained with a 2% Giemsa solution. Average of two experiments.

differentiation process[35]. FELC variants resistant to TPA-mediated inhibition of differentiation have been isolated and characterized[36]. In addition, long-term treatment with HMBA plus TPA of TPA-sensitive clones has permitted us to isolate a clone committed to differentiate (HT cells): when TPA and HMBA are removed, these cells differentiate spontaneously[37]. With our comparative studies of TPA interaction with proto-oncogenes (primarily the effects of TPA on c-onc expression), we hope to identify critical events relevant to inhibition of differentiation.

(i) *c*-myc

When the differentiation inducer HMBA is added to a parent FELC line, c-*myc* mRNA levels decrease sharply within 1 h but return to control levels within the next 24 h. It has been proposed[38] that this drop in c-*myc* mRNA is an essential event in FELC differentiation; however, our data show that TPA does not influence FELC differentaiation by this route, since the pattern of c-*myc* expression is the same in resistant clones, in which differentiation is not blocked by TPA.

(ii) *c*-myb

c-*myb* mRNA levels decrease during FELC differentiation, full extinction occurring after 1 h of treatment with HMBA. c-*myb* is thought to play a role in cellular proliferation, and it has

[35] Yamasaki, H., Fibach, E., Nudel, U., Weinstein, I.B., Rifkind, R.A. & Marks, P.A. (1977) *Proc. natl Acad. Sci. USA, 74,* 3451–3455

[36] Fibach, E., Yamasaki, H., Weinstein, I.B., Marks, P.A. & Rifkind, R.A. (1978) *Cancer Res., 38,* 3685–3688

[37] Yamasaki, H., Martel, N., Fusco, A. & Ostertag, W. (1984) *Proc. natl Acad. Sci. USA, 81,* 2075–2079

[38] Coppola, J.A. & Cole, M. (1986) *Nature, 320,* 760

been suggested[39] that the observed decline in *myb* mRNA levels that accompanies cell differentiation could be related simply to growth arrest. However, since c-*myb* mRNA is barely detectable in the continuously growing committed clone HT-CL2, we think that the decline in c-*myb* mRNA levels may indeed be a part of the commitment process.

(iii) *c*-fos

In contrast to mRNA of c-*myb* and c-*myc*, c-*fos* mRNA is barely detectable in FELC. It is induced transiently within 1 h after exposure to HMBA and then returns to control levels within the next 9 h. It is doubtful that TPA could inhibit FELC differentiation by interfering with c-*fos* expression, since TPA strongly induces c-*fos* mRNA in both TPA-sensitive and TPA-resistant clones, and this effect is transient.

(*d*) *Control of oncogene expression through intercellular communication and its modulation by tumour-promoting agents* (Dr H. Yamasaki and Mr F. Katoh; in collaboration with Dr M. Bignami, National Institute of Health, Rome; and Dr F. Tato, La Sapieza University, Rome)

In order to examine the relationship between oncogene expression and intercellular communication, we investigated the role of adjacent normal cells in the modulation of focal outgrowth of mammalian fibroblasts transformed by the viral oncogenes, v-*myc*, v-*src* and v-*ras*. NIH 3T3 cells transformed by these three oncogenes were derived by transfection or infection; they showed comparable efficiencies for cloning in semisolid medium. However, when a small number of clonogeneic transformed cells were replated together with a vast excess of normal C3H 10T1/2 mouse embryo fibroblasts, v-*ras*- and v-*src*-transformed cells could overgrow the monolayer and form distinct foci, whereas v-*myc*-transformed cells lacked this ability. Addition of tumour promoting agents (TPA, phorbol-12,13-didecanoate or mezerein) efficiently restored focus formation by v-*myc*-transformed cells.

In order to see whether normal cells suppress the outgrowth of transformed cells because of junctional intercellular communication between v-*myc*-transformed cells and surrounding nontransformed cells, we measured communication by the microinjection-dye transfer assay. There was indeed communication between *myc*-transformed cells and normal cells, whereas v-*src*- and v-*ras*-transformed cells did not communicate with surrounding normal cells.

Taken together with the fact that phorbol ester tumour promoting agents are potent inhibitors of intracellular communication, these results suggest that the outgrowth of v-*myc*-transformed cells is inhibited by communication with normal cells and the tumour promoting agents restore their outgrowth by blocking such communication[40].

(*e*) *Role of selective intercellular communication in tumour promotion* (Dr D.J. Fitzgerald and Dr H. Yamasaki; in collaboration with Dr R. Klann and Dr T.J. Slaga, University of Texas System Cancer Center, Smithville, TX, USA; and Dr N.E. Fusenig, German Cancer Research Center, Heidelberg, Federal Republic of Germany)

As discussed above, we showed earlier in our 3T3 cell transformation assay that gap-junctional communication exists among cells in transformed foci and among surrounding normal cells but not between focus cells and normal cells. Such selective communication may provide a

[39] Gonda, T.J. & Metcalf, D. (1984) *Nature, 310,* 249

[40] Bignami, M., La Rocca, A., Falcone, G., Tato, F., Katoh, F. & Yamasaki, H. (1987) *Proc. Am. Assoc. Cancer Res., 28,* 120

means whereby clonally proliferating transformed cells are insulated from growth regulatory factors that they might otherwise receive *via* junctional communication with normal cells. It is important to determine the generality of such selective communication, and we have therefore studied a system involving coculture of SENCAR mouse primary epidermal cells with SENCAR mouse cell lines at different stages of neoplastic transformation. Selective communication was not observed in this system. However, in homologous cultures, cell–cell communication decreased in a step-wise fashion from 68 ± 5 recipients per injection in initiated cells, to 21 ± 2 in late-stage papilloma-derived cells, to 6 ± 1 in cells derived from a squamous-cell carcinoma. Thus, diminished capacity for communication may be a hallmark of tumour progression in the mouse skin system. We are also studying selective communication using a range of human keratinocyte lines. Initial studies have shown that, in homologous culture, tumorigenic cells are in general relatively poor communicators (average, 5.3 recipients/injection, compared with 17.3 for nontumorigenic cells). However, a highly tumorigenic line, HaCaT *ras* II/3, was communication-competent (20.9), while a nontumorigenic line, HaSV, was communication incompetent (0.5). Future work will examine the selective communication capacity of these cells in coculture with normal human epidermal keratinocytes.

Of much interest is whether or not selective communication exists *in vivo*, and we have undertaken a study in rat liver during the evolution of carcinogen-induced preneoplastic foci.

Further to our studies with SENCAR mouse epidermal cell lines, we compared the effect of TPA on cell–cell communication in 3PC (a 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated cell line) and primary epidermal cells. In a dose-dependent and time-dependent manner, TPA inhibited communication in primary cell cultures — 80% inhibition with 100 ng/ml TPA over 7 h — but did not affect communication in 3PC cells over the same period. TPA resistance of 3PC cells was observed previously in colony-forming efficiency assays. Thus, concerning phorbol ester tumour promoter action *in vivo*, we may speculate that (1) phorbol esters decrease communication between normal cells and initiated cells, and (2) initiated cells, if promoter-resistant, can proliferate selectively, while promoter-sensitive normal cells succumb to the influences of promoters, possibly including enhanced differentiation.

(*f*) *Modulation of cellular phorbol ester binding and protein kinase C activity by human placental fractions* (Dr E. Hamel and Dr H. Yamasaki; in collaboration with Dr J.L. Tayot, Mérieux Institute, Marcy-l'Etoile, France; and Dr Y. Nishizuka, Kobe University, Kobe, Japan)

We have been searching for factors in human placenta that can mimic or modulate the membrane interaction of phorbol ester tumour promoters. We have identified two such factors: (1) PEBIF, which can inhibit the binding of ^{3}H-phorbol-12,13-dibutyrate (^{3}H-PDBu) on cultured cells or a membrane fraction[41] but has no inhibitory action on the binding of ^{3}H-PDBu to a homogenous C kinase preparation (presumed phorbol esters binding sites); and (2) CKAF, which stimulates C kinase activity in a calcium-dependent manner. We were able to separate the two biochemical activities from a crude human placental fraction by gel filtration. These factors may play an important role in modulation of tumour promotion.

(*g*) *Inhibition of junctional intercellular communication as a possible short-term test to detect tumour-promoting agents* (Dr H. Yamasaki, Mr M. Zeilmaker and Mr F. Katoh; in collaboration with Dr T. Masui, Dr S. Fukushima and Dr N. Ito, Nagoya City University, Nagoya, Japan)

In an attempt to establish an in-vitro short-term test to detect tumour-promoting agents, we

[41] Hamel, E., Martel, N., Tayot, K.J.L. & Yamasaki, H. (1984) *Carcinogenesis, 5*, 15–21

studied the effects of these agents on junctional intercellular communication in cultured Chinese hamster V79 cells using a microinjection-dye transfer technique. When Lucifer Yellow CH solution is injected into a cell, the average number of cells that becomes fluorescent after 10 min is 11.6 ± 7.8 (SD). When TPA (100 ng/ml) was used as a positive control, the extent of dye transfer was reduced to 2.9 ± 2.1 cells within 2 h. Nine chemicals that have been reported to have or suspected of having tumour-promoting activity in experimental animals were tested at different doses and after different incubation times. 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane, lindane (1,2,3,4,5,6-hexachlorocyclohexane), phenobarbital and butylated hydroxyanisole showed inhibitory properties in V79 cells, but with kinetics different from that of TPA. With 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane and lindane, exposure for 24 h resulted in full blockage of dye transfer; with phenobarbital, a treatment time of 96 h was necessary to achieve this effect; and butylated hydroxyanisole was more active after 48 h than after 24 or 72 h incubation. Five of the reported or suspected tumour-promoting agents — benzoyl peroxide, anthralin, deoxycholic acid, lithocholic acid and butylated hydroxytoluene — had no effect on communication between V79 cells at noncytotoxic doses; deoxycholic acid, lithocholic acid and butylated hydroxytoluene, but not anthralin, inhibited communication only at cytotoxic doses. Our results indicate that we can detect several, but not all, types of tumour-promoting agents[42].

We also examined the effect of the rat bladder tumour promoters, sodium L-ascorbate, uracil and butylated hydroxyanisole, on junctional intercellular communication of BALB/c 3T3 cells. The last inhibited intercellular communication, while sodium L-ascorbate and uracil did not. Since administration of sodium L-ascorbate and several other bladder tumour promoters is known to result in increased urinary pH[43], we investigated the effect of the pH of the culture medium on intercellular communication and found that it is inhibited in proportion to the increase of pH. These results suggest that butylated hydroxyanisole acts directly on cells, while sodium L-ascorbate and uracil do not. In the case of sodium L-ascorbate, increased extracellular pH would play an important role.

5. PERINATAL CARCINOGENESIS

(*a*) *Multigeneration effects of carcinogens after exposure of males* (Dr V.S. Turusov, Mrs. D. Galendo, Miss M.P. Desvaux, Miss M. Laval, Mrs N. Lyandrat, Dr L. Tomatis and Dr J.R.P. Cabral; in collaboration with Professor N.P. Napalkov, N.N. Petrov Research Institute of Oncology, Leningrad, USSR, DEC/81/33; and Dr B.N. Hemsworth, Life Sciences Laboratory, Teeside Polytechnic, Cleveland, UK, DEC/82/01)

A study designed to assess the incidence of TPA-induced tumours in control BALB/c mice and F1 and F2 offspring of *N*-ethyl-*N*-nitrosourea(ENU)-treated adult fathers is currently under way. Groups of BALB/c mice received a single administration of ENU, and, two weeks later, ENU-treated males were mated with untreated females. Their offspring (F1 generation) were divided into two groups of about 50 males and 50 females; one group received TPA at eight weeks of age. When untreated males and females of the F1 generation reached the age of eight weeks, they were mated to obtain the F2 generation. These mice will again be divided into two groups, one receiving TPA and the other not. The ENU-treated males were mated with untreated females a second time, 25 weeks after treatment. The offspring of this mating will also be divided into two groups, one receiving the TPA and the other not. Adequate control groups are available.

[42] Zeilmaker, M. & Yamasaki, H. (1986) *Cancer Res., 46,* 6180–6186

[43] Fukushima, S., Shibata, M., Shirai, T., Tamano, S. & Ito, N. (1986) *Cancer Res., 46,* 1623–1626

A further study is in progress in which tumour incidence is being studied in the offspring of male mice treated with *N*-methyl-*N*-nitrosourea and mated subsequently. To date, some 1500 Swiss mice offspring, representing three generations, have been obtained. Histopathological examination was begun in January 1987.

(*b*) *Role of promoting factors in possible carcinogenic effect of 5-bromodeoxyuridine* (Professor N.P. Napalkov, N.N. Petrov Research Institute of Oncology, Leningrad, USSR, DEC/81/33)

The progeny of 400 BALB/c 3T3 mice were used. Male mice were treated with ENU (80 mg/kg intraperitoneally) and two weeks later were mated with untreated females to produce F1 progeny. Control F1 progeny were produced using untreated males. Groups of TPA-promoted and control groups were formed similarly, and other experimental F1 and F2 groups (postnatal treatment with phenobarbital, butyl hydroxytoluene and X-irradiation) and appropriate control groups as well as collection of autopsy material is in progress.

(*c*) *Multigeneration effects of carcinogens after exposure of females* (Dr H. Yamasaki, Dr J.R.P. Cabral, Mrs D. Galendo, Mrs M.P. Desvaux, Miss M. Laval, Mrs N. Lyandrat and Dr L. Tomatis)

Pregnant CD1 mice were injected with 100 ng/kg bw or 10 mg/kg bw DMBA on gestation day 15; offspring were painted with acetone or TPA (93 mg/application, twice a week). Skin papillomas and carcinomas developed only in TPA-treated groups, but Ha-*ras* mutation was found in all carcinomas and in 50% of papillomas tested. In order to investigate the multigeneration effects of DMBA and perhaps a constitutive cHa-*ras* mutation in F2 mice, indicative of a germ-cell mutation, we mated offspring of mice treated *in utero* with DMBA. Due to infertility of the F1 females, however, we obtained only male F2 offspring. If germ cells contain a mutated gene that is critical for the initiation of tumours, we would expect a vast number of skin tumours after TPA treatment, since all somatic cells should have inherited the same mutation. No such multigeneration effect was observed: no F2 mouse had more than two papillomas after TPA treatment. In addition, no Ha-*ras* mutation (A to T transversion at the 61st codon) was seen in skin tumours produced on F2 mice or in the tails of selected animals. Thus, this experiment provides evidence of a critical gene mutation in germ cells.

(*d*) *Antioxidants and transplacental neuro-oncogenesis* (Dr J.R.P. Cabral, Mrs D. Galendo, Miss M.P. Desvaux, Miss M. Laval and Mrs N. Lyandrat)

The scope of the present studies is to determine whether initiation of transplacental neuro-oncogenesis can be modulated by treatment with antioxidants. Preliminary studies were undertaken to measure the level of fetal brain DNA alkylation in the progeny of animals pretreated with antioxidants and then injected with either directly-acting alkylnitrosureas or indirectly-acting dialkylphenyltriazenes. In the second part of these experiments, it is planned to assess the effects of antioxidant pretreatment on the neuro-oncogenicity of different compounds.

III. DATA COLLECTION AND RESEARCH METHODS

1. IMPROVEMENT OF EPIDEMIOLOGICAL DATA COLLECTION

(*a*) *Cancer registries*

(i) *International Association of Cancer Registries* (Dr C.S. Muir and Miss S. Whelan; in collaboration with Professor K. Shanmugaratnam, Singapore Cancer Registry, DEB/73/016)

The Agency continues to provide a secretariat to the Association, now comprising 245 members. Regular newsletters inform members about:

- developments in cancer registration throughout the world,
- projects and meetings arising from the Association's status as a nongovernmental organization in official relations with WHO,
- the progress of collaborative studies between members of the Association and Agency, and
- relevant literature, including abstracts of annual reports and other registry publications.

Members of the Association have collaborated with the Agency in many of the studies described in sections I.1(*a–f*) and I.3(*h*), and in putting forward suggestions to the WHO Committee responsible for revision of the neoplasm sections of the International Classification of Diseases. The Association sends representatives to many relevant meetings at the WHO Headquarters and in the Regions, to which it is invited as a nongovernmental organization in official relations with WHO.

The 1985 annual scientific meeting of the Association was held in Hartford, Connecticut, USA, with the theme 'Cancer Registries and Multiple Primary Tumours'. The two-and-one-half-day meeting, hosted by the Connecticut Tumor Registry in conjunction with its own 50th anniversary, addressed both methodological factors in the registration of multiple tumours, their significance in the study of common etiological factors and the carcinogenic effects of tumour therapy. The 1986 meeting, organized in collaboration with the National Cancer Institute, Budapest, had as its theme 'The Cancer Registry in the Service of the Community'. The programme included presentations on the role of the cancer registry in cancer control in both developed and developing countries, as well as historical reviews of the growth of registration in different parts of the world.

The 1987 meeting of the Association is to be held in Copenhagen, at the invitation of the Danish Cancer Registry. The main topic of the meeting will be 'The Cancer Registry and Environmental Cancer'.

(ii) *Advice given to cancer registries* (Dr D.M. Parkin, Mrs J. Nectoux and Miss S. Whelan)

Treatment, analysis and publication of data collected within the small area of Ardèche du Nord, France, covering a population of about 50 000 persons, are carried out at the Agency. The third report will be issued in December 1987, covering a period of four years, with approximately 600 cases.

Advice is given both to organizations wishing to set up cancer registries, and to established registries, on methodology. Several commonly used computer programs are available to

registries free of charge, including common verification checks (sex-site, age-site, site-histology) an ICD-O-to-ICD-9 conversion program (based on a conversion devised by Percy and van Holten[1], with the addition of rules to deal with metastases and neoplasms of the haemoatopoietic and reticuloendothelial systems), and programs for converting ICD coded cases into the categories of the classification scheme for childhood cancer (I.3.*h*.i).

Registries are encouraged to send copies of any reports published to the Agency. Abstracts of all such reports are prepared for the International Association of Cancer Registries' Newletters, and the report references and abstracts have now been entered onto computer to facilitate retrieval of the information and permit search for specific items by combining parameters of interest and interrogating the system.

(iii) *Confidentiality in cancer registries* (Dr C.S. Muir and Mrs E. Démaret)

Following a survey of the legal basis of cancer registration carried out among the members of the International Association of Cancer Registries in 1982, it became clear that many registries would like an internationally accepted code of practice in relation to confidentiality. The objectives of such a code would be to provide a general set of rules, and to supply a framework to registries wishing to produce their own confidentiality document, suitable for their country and environment, and in accordance with existing national regulations. A working party was established to prepare such a code of confidentiality, and a first draft was circulated to members of the Association. After comments had been received from the registries, the text was revised and a second draft circulated in 1986.

While a number of registries are in agreement with the existing document, others fear that such a code might be counterproductive, in that the rules outlined may be more restrictive than those under which they now operate. For the moment, the project has been suspended and no firm decision has been taken to publish the code. The document is available from the Agency for those interested.

(iv) *Cancer Registration and its Techniques* (Dr D.M. Parkin and Dr C.S. Muir; in collaboration with Dr O. Møller Jensen, Danish Cancer Registry, Copenhagen; and Dr R. MacLennan, Queensland Institute of Medical Research, Brisbane, Australia)

The first volume of *Cancer Registration and Its Techniques*[2], published in 1978, has become the standard work of reference in this field. With increasing automation in cancer registries, it was felt that the time had come to produce a second edition, on the assumption that the principal registration processes would be carried out by computer (retaining, nevertheless, a description of manual card-filing methods). The new volume will contain chapters on the purposes of registration, data items collected, sources of data, classification and coding, quality control, reporting of results, statistical methods for registries, registries in developing countries, the hospital registry, and confidentiality and legal aspects. The methods used in several very different registries will be presented as a series of appendices.

It is anticipated that publication will take place in 1988.

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[1] Percy, C. & van Holten, V., eds (1979) *Conversion of Neoplasms by Topography and Morphology from the International Classification of Diseases for Oncology (ICD-O) to Chapter II, Neoplasms, 9th Revision of the International Classification of Diseases (ICD-9) 1975 (NIH Publication No. 80-2007)*, Washington DC, US Government Printing Office

[2] MacLennan, R., Muir, C.S., Steinitz, R. & Winkler, A., eds (1978) *Cancer Registration and Its Techniques (IARC Scientific Publications No. 21)*, Lyon, International Agency for Research on Cancer

(*b*) *Computers and cancer registration*

(i) *A microcomputer system for cancer registries* (Mr A. Bieber and Dr D.M. Parkin; in collaboration with Mr H. Menck, University of Southern California, Cancer Surveillance Program, Los Angeles, CA, USA)

The cancer registration programs (CANREG) reported upon earlier[3] had become obsolete by 1985, when Mr H. Menck was asked to adapt the system he had developed for hospital registries in the USA to be suitable for smaller population-based registries. A new version of CANREG was created, to be used with the computer operating system MS-DOS, written in the BASIC programming language[4].

In 1986, support was provided from the Governing Council Special Fund for further development of CANREG and its installation in cancer registries in developing countries, where funds and technical skills are often in short supply. By July 1987, CANREG had been installed in three centres in Africa (Gabon, Mali and Zimbabwe) and in three in Asia (Manila, Shanghai, China and Singapore). Current plans are for installations in Bermuda, Viet Nam (Hanoï) and two centres in Thailand (Khon Kaen and Bangkok).

Improvements and enhancements to CANREG continue, and future versions will provide additional options for data verification and data analysis.

(ii) *Directory of Computer Resources for Cancer Registries* (Dr D.M. Parkin, Mrs E. Démaret, Dr C.S. Muir and Mr M. Smans; in collaboration with Mr H.R. Menck, University of Southern California, Cancer Surveillance Program, Los Angeles, CA, USA; Dr P. Crosignani, National Institute for the Study and Treatment of Tumours, Milan, Italy; and Dr R. Skeet, Thames Cancer Registry, Sutton, Surrey, UK)

This project, which was started in 1984, involved production of a volume describing the computer equipment and software used by cancer registries throughout the world, with a view to providing a source of reference for researchers wishing to install a computerized system, or to locate software useful for specific registry functions. Information was collected by means of questionnaires sent to registries throughout the world, and their responses were abstracted in a standard format. The *Directory* contains abstracts from over 60 registries, together with sections describing in some detail systems of registration in which both batch and on-line access to main-frame machines and microcomputers are used. The *Directory* also contains an extensive bibliography, comprising all published work on the use of computers in relation to cancer registration.

The *Directory* was published in 1986 as a joint IARC/International Association of Cancer Registries volume[5]. Following a review of user response to the *Directory*, the value of issuing revisions or updates will be decided.

(*c*) *Classification and nomenclature: standardization*

(i) *Tenth revision of the International Classification of Diseases (ICD-10)* (Dr C.S. Muir and Miss S. Whelan; in collaboration with Mrs C. Percy, National Cancer Institute, Bethesda, MD, USA; and Dr L. Teppo, Finnish Cancer Registry, Helsinki)

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[3] IARC (1985) *Annual Report 1985*, Lyon, p. 104

[4] Bieber, A. & Parkin, D.M. (1986) *CANREG Cancer Registration Using a Microcomputer* (*IARC intern. Tech. Rep. 86/003*), Lyon, IARC

[5] Menck, H.R. & Parkin, D.M. (1986) *Directory of Computer Systems Used in Cancer Registries*, Lyon, International Agency for Research on Cancer

As for the 9th Revision[6], the Agency was asked by WHO to take responsibility for advising on the content of the neoplasms chapter of the 10th revision of the *International Classification of Diseases, Injuries and Causes of Death* (ICD-10). The first and second drafts, elaborated at meetings held in Lyon on 18–22 February 1985 and in Washington DC on 3–5 December 1985, have been sent to all members of the International Association of Cancer Registries for comment.

In these proposals, advantage has been taken of the decision by WHO to expand the number of rubrics available in the ICD by the adoption of an alpha-numeric system. There are now 150 three-digit rubrics available for malignant disease, rather than the 100 provided in the 9th revision, permitting recognition at the three-digit level of many sites previously allotted a four-digit number. Connective tissue tumours, including peripheral nerves, have been grouped in view of the increasing epidemiological interest in these neoplasms, as have the various sites of mesothelioma. The most noteworthy change has been expansion of the number of rubrics available for lymphomas, which, although possibly useful for histopathologists and cancer registries, may prove much too detailed for mortality coding. The Agency was represented at meetings of the heads of WHO collaborating centres for the classification of diseases, held in Titchfield, UK, on 6–10 April 1987 by Mrs C. Percy (National Cancer Institute) and in Leningrad, USSR, from 2–9 June 1987 by Dr L. Teppo (Finnish Cancer Registry).

(ii) *The International Classification of Diseases for Oncology (ICD-O)* (Dr C.S. Muir)

The ICD-O has been developed in parallel with the 10th revision of ICD. This has involved scrutiny of all existing terms, deletion of those no longer used and provision of new code numbers to meet new concepts in tumour histopathology, notably for malignant lymphomas and leukaemias. Mrs C. Percy of the SEER Programme (National Cancer Institute, Bethesda, MD, USA) has coordinated a very extensive field trial of the proposed revised ICD-O. It is anticipated that this evaluation will be completed by the end of 1987.

(iii) *Study of death certificate coding* (Dr C.S. Muir; in collaboration with Mrs C. Percy, National Cancer Institute, Bethesda, MD, USA)

In 1978, Percy and Dolman[7] reported on the coding in seven countries of the same set of death certificates bearing the mention of cancer. Attention was drawn to the sizeable international differences in the application of coding rules. Thus, cancer of the breast was considered to be the underlying cause of death on 95 death certificates in France, in contrast to the Federal Republic of Germany where only 65 were so assigned.

Following the redrafting of the coding rules for death certificates mentioning cancer in the 9th revision of the ICD, it was decided to repeat the earlier study by coding the same set of death certificates. Conscious of the criticism that the coding of death certificates written in the terminology of the USA could give rise to a sizeable bias, a selection of death certificates was assembled in the participating countries, and these too were circulated. The preliminary results of this second enquiry (Table 41) show that, although there has been an improvement in comparability, much still remains to be done. Clearly, the comparison of international mortality statistics needs to take into account national differences in death certificate coding.

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[6] World Health Organization (1977) *Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death, Vol. 1 (Tabular List)* and *Vol. 2 (Index), 1975 Revision*, Geneva

[7] Percy, C. & Dolman, A. (1978) *Publ. Health Rep., 93*, 335–350

Table 41. Comparison of coding of same set of US death certificates mentioning cancer by the 8th and 9th Revisions of ICD for selected countries[a]

Site	ICD Revision	USA	France	Canada	England & Wales	Federal Republic of Germany	USSR
Stomach	8th	31	32	29	29	32	31
	9th	29	28	29	32	32	31
Rectum	8th	30	34	32	30	35	36
	9th	32	37	32	34	34	36
Lung	8th	77	96	95	91	90	82
	9th	82	92	82	88	95	80
Breast	8th	84	95	89	87	65	86
	9th	86	98	87	91	88	89
Prostate	8th	47	56	48	39	45	56
	9th	49	64	49	54	51	53

[a] Percy, C., personal communication of preliminary data

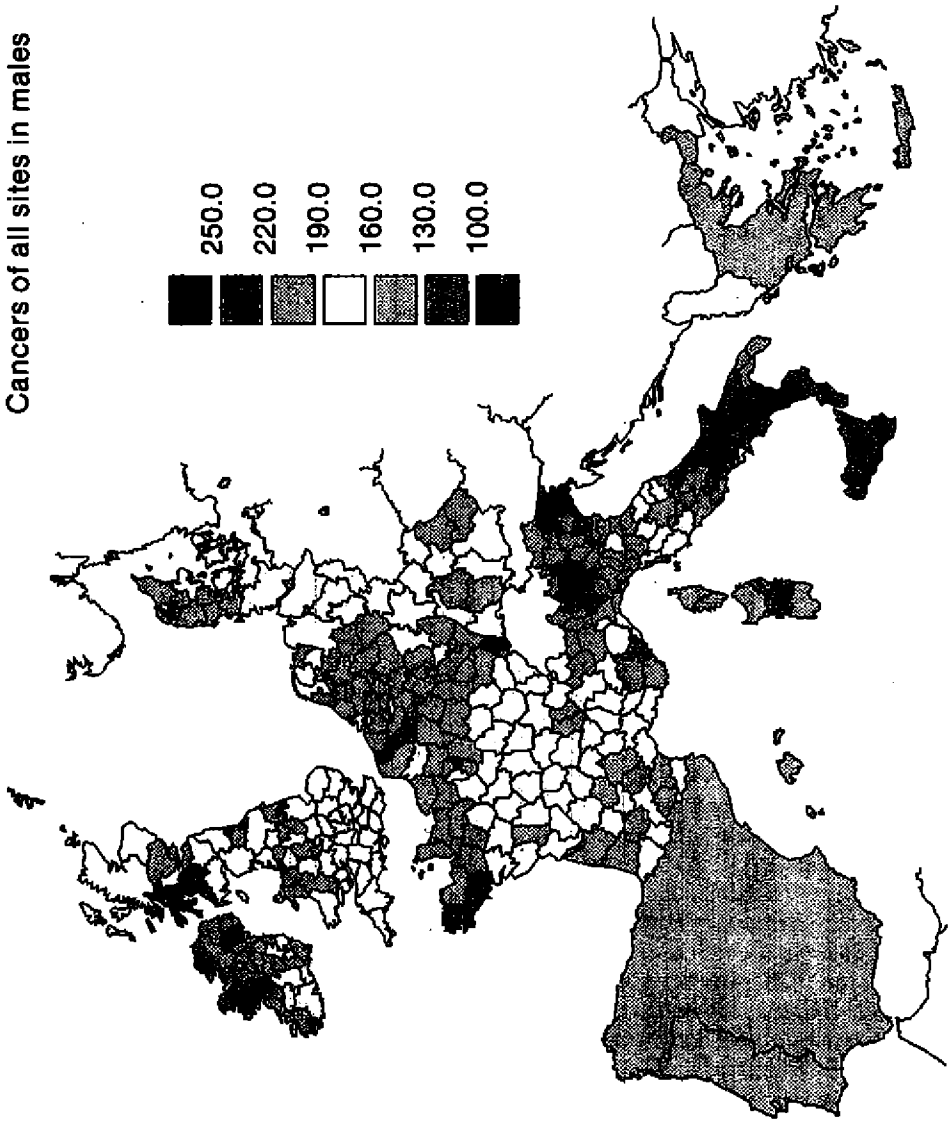
(*d*) *Cancer registration and cancer epidemiology in Latin countries* (Dr A. Tuyns and Dr J. Estève; in collaboration with Mr L. Raymond, Geneva Tumour Registry, Switzerland; and Dr R. Zanetti, Piedmont Tumour Registry, Turin, Italy)

The annual meeting of the group of Latin cancer registries was held in 1986 in Strasbourg, France, at the invitation of Dr P. Schaffer, and in 1987 in Ponza, Italy, at the invitation of Professor M. Crespi. Results from cancer registries and from analytical epidemiological studies presented at these meetings are available from the Agency.

(*e*) *The mapping of cancer* (Dr C.S. Muir, Mr M. Smans, Dr P. Boyle and Dr J. Estève; in collaboration with Mr A. Doneux, National Institute of Statistics, Brussels; Dr H. Bille, National Board of Health, Copenhagen; Dr M.H. Pejovic and Dr A. Rezvani, Gustave Roussy Institute, Villejuif, France; Dr K. Kern, Federal Statistics Bureau, Wiesbaden, Federal Republic of Germany; Mr J. Stephens, Central Statistics Office, Dublin; Dr P. Morganti, Central Statistical Institute, Rome; Mr P. Henckes, Health Statistics Service, Luxembourg; Dr J.K.S. van Ginnekan, Netherlands Central Bureau of Statistics, Voorburg, Netherlands; Mr L.H. Anderson, Department of Health and Social Services, Belfast, Northern Ireland, UK; Professor M.R. Alderson, Office of Population Censuses and Surveys, London; Mr D. Salmond, General Register Office for Scotland, Edinburgh, Scotland, UK; Dr W. Hunter, European Economic Community, Luxemburg; Dr Z. Péter, National Institute of Oncology, Budapest; Dr C. Cislaghi, Institute of Biometrics and Medical Statistics, Milan, Italy; and Dr W.H. Mehnert, National Cancer Registry, Berlin-Johannisthal)

Since publication of the *Atlas of Cancer in Scotland, 1975–1980* in 1985[8], work has continued on the production of several further cancer atlases.

[8] Kemp, I., Boyle, P., Smans, M. & Muir, C., eds (1985) *Atlas of Cancer in Scotland 1975–1980: Incidence and Epidemiological Perspective* (*IARC Scientific Publications No. 72*), Lyon, International Agency for Research on Cancer


Cancers of all sites in males
250.0
220.0
190.0
160.0
130.0
100.0

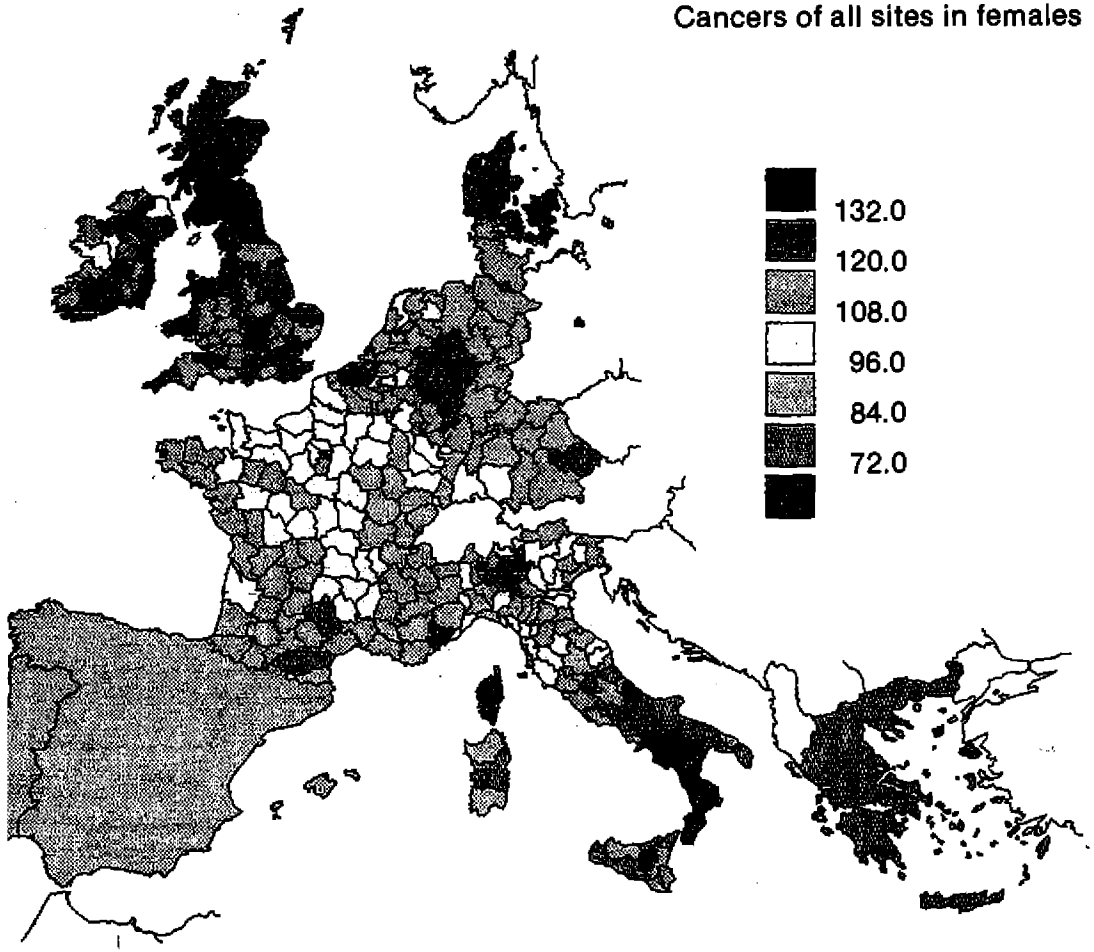


Fig. 16. Standardized mortality rates for cancer of all sites in males (a) and females (b) for the 12 countries of the European Communities

The colour scale shows standardized mortality rates per 100 000 standardized to the world population

(i) *Mortality atlas for the European Economic Community*

This atlas presents mortality data from 355 'level-II' regions of the European Economic Community, such as departments of France and counties of England and Wales, for the late 1970s. The first publication will be limited to the nine countries that were members of the community in 1984 when the study started. Discussions are under way for expanding this work to cover the 12 countries of the Community and for presenting a more recent period.

A fascinating and diverse spectrum of patterns emerges (Fig. 16). For oesophageal cancer, the highest levels of risk are to be found in the north and west of France, with somewhat lower but still elevated levels seen elsewhere, extending into the north of Italy, but ending abruptly at the Belgian and German borders. In contrast, in women, the excess risk is to be seen in the British Isles, notably Scotland, and in the Republic of Ireland. Gastric cancer is rare in France, particularly in the south, the highest levels being recorded in people of each sex in the north of Italy and in Bavaria.

Contributing nations have been asked to provide a commentary on the collection and reliability of death certificates and to indicate the geographic and economic features that may have bearing on cancer risk. The bulk of this material is now available.

The Community has contributed financially towards the preliminary work involved in preparation of the atlas.

(ii) *Other atlases*

An atlas presenting cancer mortality for Italy, which was prepared with the support of the Agency, was presented at a meeting held in Milan in 1986.

Cancer mortality data from Hungary, from 1980 to 1985 have now been received and preliminary maps sent to Dr Z. Péter, so that he may write an appropriate commentary.

An atlas presenting cancer incidence for the German Democratic Republic is in preparation. The maps will depict incidence data for 1975–1980 both at the level of *kreis* and at the level of *land*, in order to facilitate interpretation of incidence patterns, which show on average a smaller variability than in Scotland.

Work on the statistical evaluation of geographical clustering continues (see III.2.*b*.ii).

(*f*) *Clearing-house for on-going research in cancer epidemiology* (Dr C.S. Muir, Dr D.M. Parkin, Mrs E. Démaret, Mrs A. Nagy-Tiborcz and Miss S. Whelan; in collaboration with Professor G. Wagner, Professor J. Wahrendorf and Mr K. Schlaefer, German Cancer Research Centre, Heidelberg, Federal Republic of Germany, DEB/74/003; partially supported by Contract No NO1-CO-55195 with the National Cancer Institute, USA)

The clearing-house for on-going research in cancer epidemiology was created in 1974 by the Agency and the German Cancer Research Centre, Heidelberg, Federal Republic of Germany, and operates with partial support from the International Cancer Research Data Bank of the National Cancer Institute of the USA. The clearing-house publishes an annual *Directory* of current research projects, each entry containing, in addidtion to a general description of the project, the name and address of the principal investigator, name of collaborator(s), keywords and the expected time span of the study. Extensive indexes facilitate access to the information in the book. Although the content has more than doubled since the first issue in 1976, in the last years the number of new studies has more or less equalled the number of completed ones.

The 1986 *Directory* contains 1352 projects, the 1987 *Directory*, 1321. Projects were reported from some 70 countries; the USA and the UK are still the largest contributors, followed by

Japan, Italy and Canada, but an increased epidemiological effort is seen in several parts of the world, notably in China, Scandinavia and South America. No significant change in the cancer sites studies has been observed, and lung, breast, cervix and gastrointestinal tract are still the most frequently studied sites. The 1987 issue shows an increase in the number of studies relating cancer to occupational exposures. The number of intervention studies is also growing, and there is increasing interest in the possible role of viruses in the etiology of cancer. More studies than previously seem to make use of laboratory methods.

In 1985, an evaluation survey was carried out among *Directory* users; the results are published in the 1986 *Directory*. Since one of the aims of the clearing-house is to promote contacts between research workers, it was of interest to note that over 50% of the respondents said they had contacted other investigators listed in the *Directory*, and 48% that they had been contacted by others about their projects; 47% of respondents said that, after consulting the *Directory*, they had obtained ideas for their own studies, and 10% that they had abandoned a study they were planning. A total of 120 respondents made suggestion on how to improve the *Directory*, the most frequent requests being in relation to the inclusion of references to completed studies and to interim reports.

Since 1985, in collaboration with the International Commission for Protection against Environmental Mutagens and Carcinogens, the directories have included studies in the field of mutation epidemiology. The number of projects is still limited — about 50 annually. The inclusion of a list of banks of biological materials, giving addresses and information on the holdings, seems to be a valuable feature. As usual, the names and addresses of active population-based cancer registries are included.

2. STATISTICAL METHODOLOGY

(*a*) *Dissemination of statistical methods for cancer research*

Volumes II and III of *Statistical Methods in Cancer Research*, which cover, respectively, the analysis of cohort studies[9] and the design and analysis of long-term animal experiments[10], are now available. A monograph on statistical methods in descriptive epidemiology will be published soon.

The dissemination of statistical expertise is also undertaken through direct collaboration with research institutes in different countries. Important examples of such collaboration are described below.

(i) *Large-bowel cancer and polyps* (Dr J. Estève; in collaboration with Dr J. Faivre, Burgundian Register of Digestive Tumours, Dijon, France)

We have participated in the design of a case-control study of intestinal polyps and cancer of the large bowel, launched under the auspices of the European Cancer Prevention Group and coordinated by the Cancer Registry of Dijon. This project also has a clinical component, consisting of the study of bile-acid metabolites in faeces and of cell proliferation abnormalities. The inclusion of polyps of various sizes in the design may permit assessment of a possible promoting effect of diet after comparison with the cancer groups.

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[9] Breslow, N.E. & Day, N.E. (1986) *Statistical Methods in Cancer Research, Vol. II, The Design and Analysis of Cohort Studies* (*IARC Scientific Publications No. 82*), Lyon, International Agency for Research on Cancer

[10] Gart, J.J., Krewski, D., Lee, P.N., Tarone, R.E. & Wahrendorf, J. (1986) *Statistical Methods in Cancer Research, Vol. III, The Design and Analysis of Long-term Animal Experiments* (*IARC Scientific Publications No. 79*), Lyon, International Agency for Research on Cancer

(ii) *Breast cancer in Argentina* (Dr J. Kaldor, Mr S. Shiboski and Mrs A. Arslan; in collaboration with Dr J. Iscovich, La Plata, Argentina)

A statistical analysis was carried out for a study of breast cancer and diet in La Plata, Argentina. The Argentine investigators had recorded 150 cases of breast cancer, selected one neighbourhood and one hospital control for each case, and conducted a detailed interview of each study subject, with a principal focus on diet. Breast cancer incidence in La Plata is one of the highest recorded in the world, and this study represents one of the first attempts to study the role of diet and cancer outside Europe and North America.

Table 42. Relative risk for breast cancer of food consumption by quantile

Food group		Quartile of food consumption				Z trend test	Mean annual frequency[a]		
		1	2	3	4		Cases	Hospital controls	Neighbourhood controls
Processed meat (fat)	H[b]	1.0	0.91	1.1	1.3	0.76	108	93	79
	N	1.0	0.77	1.2	1.6	1.6			
Processed meat (lean)	H	1.0	1.3	1.6	2.4*	2.3	60	37	34
	N	2.3	1.4	1.8	2.6*	2.3*			
Seafood	H	1.0	1.2	0.38	1.6	0.61	26	21	20
	N	1.0	1.4	0.41	2.2	1.3			
Eggs	H	1.0	1.7	2.1*	6.0*	4.9	138	76	72
	N	1.0	0.87	2.6*	7.1*	5.0*			
Bread, cakes (with butter)	H	1.0	0.89	0.96	1.6	1.4	86	177	159
	N	1.0	1.3	1.5	2.3*	2.2*			
Grains	H	1.0	1.6	2.2*	3.3*	3.7*	163	132	129
	N	1.0	0.44	1.2	2.3*	2.5*			
Green leafy vegetables	H	1.0	0.53	0.27*	0.32*	−3.5	144	172	185
	N	1.0	0.54	0.17*	0.15*				
Citrus fruit	H	1.0	0.98	1.0	0.75	−0.63	186	204	234
	N	1.0	0.43*	0.27*	0.58	−2.5*			
All desserts	H	1.0	1.0	2.0	3.1*	3.5	476	327	273
	N	1.0	1.4	2.3	3.7*	3.4*			

[a] Obtained by multiplying reported seasonality by monthly frequency of consumption

[b] H: Hospital controls; N: Neighbourhood controls

* $p < 0.05$

Table 42 shows the relative risks estimated for selected food items, by quartile of annual consumption in controls. The relative risks are adjusted for age, age at first pregnancy and level of education, but not for other food items. The most impressive trends occur for consumption of butter and meat, and for fruit and green vegetables, both of which had protective effects.

A multivariate analyses, consumption of green vegetables and meat emerged as the strongest independent predictors of breast cancer risk.

(iii) *Cohort study of workers in the Nylon and Tergal industry* (Dr E. Cardis, Mr X. Nguyen-Dinh and Dr J. Estève; in collaboration with Dr M. Hours and Dr J. Fabry, Faculty of Medicine, Lyon, France)

A study of a cohort workers in the Tergal industry in Lyon reported a slight excess of cancers weakly related to exposure category but not related to length of exposure[11]. An analysis of the follow-up to 1986 is under way; it is thought that this additional follow up might confirm or refute the excess noted previously, thus clarifying the potential hazards associated with work in the Tergal polyester industry.

(iv) *Cancer mortality and incidence in a cohort of alcoholics* (Dr E. Cardis; in collaboration with Dr E. Bjelke, University of Bergen, Norway; and Dr P. Sundby, Cancer Registry, Oslo)

An analysis of the follow-up until 1986 of a cohort of alcoholics identified in 1925–1940 in Norway has been performed. It confirms previous reports[12] of an elevated incidence of oesophageal cancer. The elevated incidence of cancer of the stomach and intestine, reported previously, is no longer apparent.

(*b*) *Development of statistical methodology* (Dr N.E. Day, Dr J. Estève, Dr E. Cardis, Mr P. Damiecki, Dr J. Kaldor, Mr M. Smans and Dr J. Wahrendorf; in collaboration with Mr D. Clayton, University of Leicester, UK; Dr C. Portier, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; Dr E. Schifflers, Department of Mathematics, Faculty of Science, Namur, Belgium; and Dr H. Tomasson, Icelandic Cancer Registry, Reykjavik)

(i) *Empirical Bayes methods for estimating a large number of parameters*

Research has continued on empirical Bayes methods for descriptive epidemiology[13]. The goal of the methods is to produce estimates of an ensemble of parameters, such as rates of cancer by geographical region, that are not distorted by differences in precision among the individual estimates. A technical paper in which the methods are applied to cancer mapping has been accepted for publication[14], and the ideas are being extended to analyses of time trends and proportionate mortality. Empirical Bayes methods also have potential application in analytical epidemiology, particularly for investigations that involve a large number of closely related risk factors. Examples are studies of nutrition, which typically involve the collection of information on hundreds of food items, and the creation of dozens of derived variables; studies of oral contraceptives, which may occur in many formulations; and studies of occupational risk, in which individuals may be classified as working in one or more of a variety of job categories. Again, there is a wide range in the precision with which the effect of each variable is estimated, depending on the distribution of variables in the study population. An empirical Bayes solution has been proposed and is being developed further[15].

(ii) *Statistical methods in descriptive epidemiology*

Efforts are being made to improve the statistical tools available in descriptive epidemiology. A test aiming at detecting aggregative patterns of cancer rates[16] has been developed. Motivated

[11] Hours, M., Bertholon, J., Estève, J., Cardis, E., Freyssinet, C.L., Quelin, P. & Fabry, J. (1986) *Scand. J. Work Environ. Health, 12,* 455–460

[12] Sunby, P. (1967) *Alcoholism and Mortality,* Oslo, Universiteitsforlaget

[13] IARC (1985) *Annual Report 1985,* Lyon, p. 110

[14] Kaldor, J. & Clayton, D. (1987) *Biometrics* (in press)

[15] Kaldor, J. (1987) In: Dwyer, J., ed., *Longitudinal Methods in Health Research,* Oxford, Oxford University Press (in press)

[16] Kemp, I., Boyle, P., Smans, M. & Muir, C., eds (1985) *Atlas of Cancer in Scotland 1975–1980: Incidence and Epidemiological Perspective (IARC Scientific Publications No. 72),* Lyon, International Agency for Research on Cancer

by a forthcoming monograph on cancer trends (see I.1.*d*), research on the analysis and presentation of time trends is being carried out following ideas discussed at a meeting held in April 1987.

(iii) *Measurement error in cancer epidemiology*

Following our earlier work on misclassification of risk factors[17], we are developing methods for estimating and correcting errors in the measurement of risk factors. We are also investigating the design implications of repeated measurements in case-control studies.

(iv) *Risk scores from logistic regression*

Logistic regression has become a very widely used tool for analysing case-control studies, since it facilitates the simultaneous analysis of many covariates. In nutrition studies, in particular, many food items are included in the model equation, and scores are often derived in order to estimate risks attributable to combinations of items. It is known that risks derived in this way may be seriously biased, and research is being carried out to obtain unbiased procedures[18].

(v) *Model for carcinogenesis*

Using as a starting point a two-mutation model of carcinogenesis[19] we have derived mathematical models for initiation and promotion effects[20] and applied them to data from animal carcinogenesis experiments and epidemiological studies of occupational carcinogens[21]. This work is being developed in two directions: (1) statistical methods for distinguishing tumours that are genetically inherited from those arising spontaneously, and application of the methods to data on second primary cancers (in collaboration with Dr F. de Vathaire, Gustave Roussy Institute, Villejuif, France), and (2) improvement and evaluation of a statistical test for initiating *versus* promoting action of a carcinogenic agent (in collaboration with Dr C. Portier).

(*c*) *Evaluation of early detection programmes* (Dr N.E. Day, Dr D.M. Parkin and Dr A.J. Sasco)

(i) *Screening for cancer of the cervix*

The results of an Agency working group on cervical cancer screening and of a UICC project group on the evaluation of screening programmes for cancer[22] have now been published[23,24]. Future studies will include an analysis of the effectiveness of cervical cytology screening and the derivation of natural history parameters in Chinese populations.

Work previously reported on the use of computer simulation in the planning of screening programmes[25] was completed with a publication examining the costs and benefits of different strategies in England and Wales[26].

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[17] IARC (1985) *Annual Report 1985,* Lyon, p. 109
[18] Tomasson, H. & Day, N.E. (in preparation)
[19] Moolgavkar, S.H. & Knudson, A.G. (1981) *J. natl Cancer Inst., 66,* 1037–1052
[20] Cardis, E. & Crowley, J. (in preparation)
[21] Cardis, E. (1985) *Modelling the Effect of Exposure to Environmental Carcinogens on Incidence of Cancer in Populations,* PhD Thesis, Ann Arbor, MI, USA, University Microfilms
[22] IARC (1985) *Annual Report 1985,* Lyon, p. 111
[23] Hakama, M., Miller, A.B. & Day, N.E., eds (1986) *Screening for Cervical Cancer (IARC Scientific Publications No. 76),* Lyon, International Agency for Research on Cancer
[24] IARC Working Group (1986) *Br. med. J., 293,* 659–664
[25] IARC (1985) *Annual Report 1985,* Lyon, p. 114
[26] Parkin, D.M. & Moss, S.M. (1986) *J. Epidemiol. Commun. Health., 40,* 143–153

(ii) *Screening for cancer of the breast* (Dr N.E. Day; in collaboration with Dr L. Tabár, Mammography Department, Falun Hospital, Sweden)

Further analysis has been performed of data from the two-county (Kopparberg and Ostergotland), randomized, controlled trial of screening for breast cancer by single-view mammography, particularly in relation to the sensitivity and sojourn-time distribution at different ages[27].

A UICC meeting was held in Helsinki on 7–9 April 1986 to review the current state of the art on breast cancer screening. The proceedings are to appear as a UICC monograph, and a summary has been published[28]. The main conclusions were:

(1) For women over 50 years of age, screening by mammography alone every two years should reduce mortality by about 40%. More frequent screening should provide further benefit, but the extent is not known.

(2) The role of physical examination in this age group, either alone or in conjunction with mammography, is unclear.

(3) Among women under 50 years of age, the benefit of screening is still in doubt.

(4) Breast self-examination has never been adequately evaluated.

(iii) *Screening for cancer of the colon and rectum* (Dr D.M. Parkin and Dr J. Estève; in collaboration with Dr J. Wahrendorf, German Cancer Research Centre, Heidelberg, Federal Republic of Germany; and Professor G. Dohm, University of Saarland, Homburg, Federal Republic of Germany)

Although much work has been done on the characteristics of the screening tests available for the early detection of colon cancer, which detect the presence of occult blood in faeces, to date there is no information available on whether such screening can reduce mortality rates from colorectal cancer. Regular testing for faecal occult blood has for several years been part of the routine health examination that is reimbursed by the social security system of the Federal Republic of Germany. These conditions are ideal for conducting a case-control study. A pilot study is presently under way to investigate whether it is feasible to obtain past screening history on deceased cases.

Further investigations will be undertaken in order to find other places where screening for colorectal cancer has been in operation for several years and where follow-up facilities exist.

(iv) *Screening for nasopharyngeal cancer*

Programmes for early detection of nasopharyngeal cancer have already been started in several provinces of south-eastern China, involving serological determinations of EBV antibodies in blood samples. Evaluation of the efficacy of screening should be conducted in order to estimate the impact on mortality. The feasibility of conducting a population-based study is now being assessed. Cohorts could be identified in Zongshan county of Guangdong province and followed up for determination of incidence and mortality rates of this cancer among seropositive and seronegative individuals. Rates of seroconversion and retroversion could also be assessed.

(v) *Theoretical developments*

The use of case-control studies for assessing screening[29] and for estimating underlying parameters has been proposed[30]. Methodological issues pertaining to the criteria for the choice

[27] Tabár, L., Faberberg, G., Day, N.E. & Holmberg, L. (1987) *Br. J. Cancer, 55,* 547–551

[28] Day, N.E., Brines, C.J., Chamberlain, J., Hakama, M., Miller, A.B. & Prorok, P. (1986) *Int. J. Cancer, 38,* 303–308

[29] Sasco, A.J., Day, N.E. & Walter, S.D. (1986) *J. chron. Dis., 39,* 399–405

[30] Brookmeyer, R., Day, N.E. & Moss, S. (1986) *Stat. Med., 5,* 127–138

of case and control series, definition of exposure, avoidance of biases and estimation of effect measures and history of disease[29,31,32] have been dealt with in several papers. Such studies have been used for assessment of the efficacy of screening for cervical[33,34] and breast cancer[35,36].

3. METHODS FOR DETECTING CARCINOGENS

(*a*) *International network of carcinogenicity testing* (Mr J. Wilbourn, Dr H. Vainio, Dr J.R.P Cabral and Dr R. Montesano)

Over the past several years, the Agency, in collaboration with the WHO/ILO/UNEP International Programme on Chemical Safety (IPCS), has established a network of laboratories in which chemicals are tested for carcinogenicity. The aims of this project are to select chemicals of high priority for study and to coordinate their testing within various collaborating laboratoires and, to a limited extent, within the Agency's facilities. The majority of studies involve the long-term testing of chemicals for carcinogenicity in rodents, although some deal with the development and validation of new tests *in vivo*, and studies of transplacental carcinogenesis.

Priorities for testing are selected by taking into account evaluations made in *IARC Monographs*, studies already under way in various national toxicology programmes and in other laboratories reporting to the IARC survey of chemicals being tested for carcinogenicity, and priorities established by the IPCS.

Carcinogenicity testing, including the design of protocols, is carried out by the collaborating laborties in accordance with guidelines given in Supplement 2 to the *IARC Monographs*[37] to ensure standardization of the quality of the testing procedures. Supplement 2 was updated to incorporate new principles in methods for long-term and short-term assays for carcinogens, and the deliberations of the meeting were published recently[38]. Collaboration with participating laboratories is implemented through ad-hoc research agreements drawn up for limited periods of time covering the specific task to be performed. The selection of laboratories is under continuous review. The principal investigators and the studies published, under way or planned in the collaborating laboratories in the network are listed in Table 43.

(*b*) *Determination of alkylated nucleic acid bases by immunoassay* (Dr D.E.G Shuker; in collaboration with Dr P.B. Farmer, MRC Toxicology Unit, Carshalton, Surrey, UK)

The major products arising from alkylation of DNA by low-molecular-weight carcinogens are guanine and adenine adducts. These adducts (e.g., 7-methyldeoxyguanosine and 3-methyldeoxyadenosine) are relatively unstable, and the corresponding alkyl bases are released

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[32] Sasco, A.J., *J. chron. Dis.* (submitted for publication)

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Table 43. Principal investigators and studies completed, under way or planned in the international network of carcinogenicity testing

Börzönyi, M. (National Institute of Public Health, Budapest, DEC/81/35):
Long-term study on atrazine by oral administration to rats — paper in preparation
Long-term study on simazine by oral administration to rats — prechronic study in progress
Cabral, R. (IARC):
Long-term study on deltamethrin by oral administration to mice and rats — preliminary findings reported[a,b]
Long-term study on fenvalerate by oral administration to mice — histological evaluation in progress
Griciute, L. (Oncological Institute of the Ministry of Health of the Lithuanian SSR, Vilnius, Lithuania, USSR, DEC/81/09):
Long-term study on ftorafur (a chemotherapeutic agent) in mice — in progress
Holmberg, B. (National Board of Occupational Safety and Health, Solna, Sweden, DEC/84/01):
Long-term study on ethanol by oral administration in isocalorific liquid diets to rats — in progress
Ito, N. (Nagoya City University Medical School, Japan, DEC/85/02):
Promoting activity of chlordane in a two-stage rat liver model — completed
Küng, A. (Institute of Experimental and Clinical Medicine, Ministry of Health of the Estonian SSR, Tallinn, Estonia, USSR, DEC/81/08):
Modifying effects of Estonian oil-shale ash on benzo[*a*]pyrene-induced carcinogenesis in rats — in progress
Roberfroid, M. (Unit of Toxicological Biochemistry, Catholic University, Brussels, DEC/82/06):
Study of diazepam and oxazepam in a two-stage rat liver model for promoting activity — results on oxazepam published[c]; tests on diazepam in progress
Study of the peroxisome proliferators, clofibrate, nafenopin, diethyl hexylphthalate, 2,4-D and 2,4,5-T in a two-stage rat liver model for promoting activity — in progress
Turusov, V. (Oncological Research Centre, Moscow, DEC/86/07):
Multigeneration follow-up study in offspring of mice treated with diethylstilobestrol during gestation — in progress
Van der Heijden, C.A. (National Institute for Public Health, Bilthoven, The Netherlands, DEC/86/05):
Long-term study on bis(tri-*n*-butyltin)oxide by oral administration to rats — paper in preparation
Long-term study on 2-nitropropane by oral administration to rats — prechronic study in progress

[a] Cabral, J.R.P. & Galendo, D. (1985) *Toxicologist, 5*, 16 (Abstr. 63)

[b] Cabral, J.R.P., Galendo, D., Laval, M. & Lyandrat, N. (1986) *VIth International Congress of Pesticide Chemistry, IUPAC, 10–15 August, Ottawa* (Abstr. 8A/7E-02)

[c] Préat, V., de Gerlache, J., Lans, M. & Roberfroid, M. (1987) *Carcinogenesis, 8*, 97–100

by mild heating of DNA *in vitro* or by the action of specific glycosylases *in vivo*, resulting in urinary excretion, without further metabolism, of the alkyl base. The ready determination of low-molecular-weight DNA adducts in DNA or urine would be extremely useful in the development of 'molecular epidemiology'. We have therefore chosen to develop immunoassay methods that have the advantage of being simple, rapid and relatively cheap to carry out.

7-Methylguanine (7-meGua)-modified methylated bovine serum albumin (MBSA), which was prepared using a novel synthetic analogue of 7-meGua which facilitates covalent binding to the protein, was used to immunize rabbits using a standard protocol. Antisera were obtained, after several months, which recognized 7-meGua-ovalbumin (4 ng/well) at high dilution ($1:10^5$) in an

ELISA procedure. 7-meGua itself could be reliably detected at a level of 1 pmol/well (20% inhibition, 50% inhibition at 10 pmol/well). Studies with a range of purines showed that an intact methyl imidazole ring was required for recognition; for example, 7-methylxathine was detected quite well (50% inhibition at 250 pmol/well) compared to 7-methyluric acid (20% inhibition at >10 000 nmol/well).

7-MeGua in DNA was detected using the competitive ELISA method following heating at neutral pH and precipitation of partially depurinated DNA. The limit of detection is currently 10–100 mmol 7-meGua/mol Gua. Work is under way to improve the sensitivity of the assay by use of monoclonal antibodies.

3-Methyladenine (3-meAde) antisera were prepared by essentially the same procedure as that described for 7-MeGua, in that a novel analogue of 3-meAde was covalently bound to MBSA for use as the antigen. The antisera recognizes 3-meAde, and in a competitive ELISA it is reliably detected at about 1 pmol/well (50% inhibition at 100 pmol/well). Adenine cross-reacts quite strongly (50% inhibition at 5 nmol/well). This antisera is being used to develop an assay for 3-meAde in human urine as a marker of exposure to methylating carcinogens[39].

Antisera have been raised against the methylated purine bases 7-meGua and 3-meAde using a novel method of antigen preparation[40]. This procedure is suitable for other alkylated adenines and guanines which are products of alkylation damage in DNA. The availability of rapid and sensitive ELISA methods for low-molecular-weight carcinogen adducts will greatly facilitate the development of 'molecular epidemiology'.

The urinary levels of methylated purine bases are now being determined in subjects living in high-risk areas for cancers of the stomach and oesophagus.

(*c*) *Methods for biological monitoring of exposure to vinyl chloride* (Mr A. Barbin and Mrs F. Ciroussel; in collaboration with Professor C. Trepo, INSERM U 271, Lyon, France; Professor M.F. Rajewsky and Dr G. Eberle, Institute of Cell Biology, University of Essen, Federal Republic of Germany; Professor M. Gérin, Department of Occupational and Environmental Medicine, University of Montréal, Québec, Canada; and Dr R.J. Laib, Institute for Occupational Physiology, University of Dortmund, Federal Republic of Germany)

A project has been initiated to develop sensitive methods for biological monitoring of workers exposed to vinyl chloride (VC). The following three endpoints will be investigated, firstly in rodents and then, if feasible, in humans exposed during their work to VC: (1) urinary excretion of alkylated nucleobases; (2) production of cirulating antibodies to serum albumin-VC conjugates; and (3) urinary excretion of specific thioethers.

During the past two years, our work has been centered on the development of methods for detecting adducts of VC and nucleic acids. Chemical synthesis of the reference compounds, 7-(2-oxoethyl)guanine, N^2,3-ethenoguanine and 3,N^4-ethenodeoxycytidine, has been performed, and high-performance liquid chromatography (HPLC) analysis of VC-modified nucleosides and nucleobases has been optimized. Using a reverse-phase column and a gradient elution, we are now able to separate in a single run and short time (60 min) all the known VC-DNA adducts that can be recovered following enzymatic or acid hydrolysis of modified DNA, i.e., 1,N^6-ethenoadenine, 1,N^6-ethenodeoxyadenosine, 3,N^4-ethenodeoxycytidine, N^2,3-ethenoguanine and 7-(2-oxoethyl)guanine. This technique has been applied for the analysis of DNA treated *in vitro* either with chloroethylene oxide, the ultimate carcinogenic metabolite of VC, or with

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chloroacetaldehyde, the rearrangement product of chloroethylene oxide. Using ultraviolet detection, 7-(2-oxoethyl)guanine was identified as the major modification, and 1,N^6-ethenodeoxyadenosine, N^2,3-ethenoguanine, 3,N^4-ethenodeoxycytidine as minor products in enzymatic hydrolysates of choroethylene oxide-treated double-stranded DNA. However, analysis of adducts formed *in vivo* requires much more sensitive detection methods. To this aim, we have raised monoclonal antibodies (MAb) to 1,N^6-ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine. Hybridoma cell lines secreting MAb were established by fusion of murine myeloma cells with spleen cells of mice immunized against conjugates of 1,N^6-ethenoadenosine or 3,N^4-ethenocytidine with keyhole limpet haemocyanin. We obtained 34 clones secreting MAb directed against 3,N^4-ethenodeoxycytidine and six clones producing anti-1,N^6-ethenodeoxyadenosine antibodies. These antibodies show affinity constants ranging from 1.6×10^7 to 1.7×10^9 l/mol and cross-react with the corresponding ethenobases and ethenoribonucleosides. Currently, the formation of ethenobases in nucleic acids of various organs from VC-exposed rats (provided by R.J. Laib) is under investigation, using a combination of HPLC and immunoanalytical procedures. The methods will then be adapted for the detection of ethenobases (putatively excreted) in the urine of VC-treated rats. Experimental animals will be exposed to VC by inhalation, and for this purpose we have constructed an inhalation exposure system working under dynamic conditions (adapted from Barrow and Steinhagen[41]).

Recent data obtained in several laboratories have shown the feasibility of biological monitoring in humans, based on the detection of circulating antibodies directed against DNA or protein adducts[42,43]. This approach will be tested in rats exposed to VC, using conjugates of serum albumin and chloroethylene oxide as antigens.

Two major VC metabolites, thiodiglycolic acid and *N*-acetyl-*S*-(2-hydroxyethyl)cysteine, have been identified in the urines of exposed individuals. In several studies, the measurement of urinary thiodiglycolic acid has been used as a biological indicator of exposure to VC[44,45]. However, due to natural endogenous formation, there is no significant increase of thiodiglycolic acid excretion at exposure levels below 1.5 ppm[44]. Therefore we have initiated, in collaboration with M. Gérin, a pilot study to check if the other urinary metabolite, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine, could be used as a biological indicator of VC exposure below 1 ppm levels[46,47].

(*d*) *Development and use of microencapsulated trapping agents for carcinogens in the gastrointestinal tract* (Dr I.K. O'Niell and Dr A.C. Povey; partly supported by the National Cancer Institute Grant No. RO1 CA 39417-01)

Semipermeable microcapsules containing both polyethyleneimine (PEI) as a nucleophilic trap and a ferrifluid derivative to enable magnetic recovery from faeces[48–51] have been further

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developed and applied. The principal aim is to trap carcinogens *in vivo* within the lower gastrointestinal tract. There is no method for biological monitoring at this site. The microcapsules are 15–45 μm in diameter and are given in doses of several millions, in order to provide a large surface area and for wide dispersal through the gut contents. They have been subject to development which have been filed in patent applications[52]. Most of the work of the past year has concerned their use *in vivo,* principally with human diets and on their safety, and for trapping hitherto unknown genotoxic agents.

(i) *Differential trapping of carcinogens in regions of the gastrointestinal tract* (with Mrs I. Brouet)

In a series of experiments, microcapsules were administered intragastrically to rats, bound to 0.006% ^{14}C-1,2-dimethylhydrazine (DMH) administered intraperitoneally, and up to 0.02% or 0.04% ^{14}C-*N*-methyl-*N*-nitrosourea (MNU), administered intragastrically and intrarectally, respectively[53]. The results demonstrate that the small amount of microcapsules used can trap a useful proportion of carcinogenic products transported through the lumen or formed therein, the amount trapped known to be proportional to the number of microcapsules.

(ii) *Trapping of endogenous nitrosating agents* (with Mrs I. Brouet and Dr M. Castegnaro)

The PEI inside the microcapsules is a polymeric analogue of piperazine, known to be a readily *N*-nitrosatable substrate. *In vitro,* it was found[54] that the PEI microcapsules display several properties highly desirable for biological monitoring of *N*-nitrosating agents; (1) nitrosation of microcapsules is linearly proportional to [NO_2^-]; (2) 40–70% of nitrosating agent is collected at 37°C in 2 h in the pH range 1.3 to 3.1; (3) the *N*-nitroso product is protected form metabolic damage. Total *N*-nitroso analysis[55] by thermal energy analyser was adapted for direct assay of the microcapsules.

Microcapsules were administered[54,56] to male BDIV rats that received 1000 ppm nitrite in drinking-water commencing either 15 h before, during, or 6 h after microcapsule dosing. Relative to this dose, the yield of excreted *N*-nitrosated PEI was much higher (0.76–4.9%) than the yields reported previously for urinary excretion of *N*-nitrosoproline and other nitrosated products in urine[57]. The yield of *N*-nitrosated PEI was dependent both on the number of microcapsules administered and the relative timing of nitrite administration.

(iii) *Deitary modulation of mucosal exposure to carcinogen metabolites* (with Mr J.-C. Bérézíat, Mrs I. Brouet and Dr E. Cardis; in collaboration with Dr S. Bingham, Dunn Clinical Nutrition Centre, Cambridge, UK)

As an approach to using microcapsules for eventual identification of hitherto unknown carcinogens and their dietary sources, arising from metabolism within the gut lumen,

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microcapsules were used to study the dietary modulation of mucosal exposure to metabolites from a model carcinogen, benzo[*a*]pyrene (BaP), principally excreted in the faeces and known to produce DNA adducts and chromosomal aberrations in the colon mucosa. Preliminary work[58] had shown the pattern of BaP metabolites trapped by microcapsules to be different from those excreted in the faeces. On the basis of the hypothesis that conventional animal diets are of little value in simulating human dietary exposure, eight complete human diets were prepared to permit isocaloric feeding and to vary beef, fat and non-starch polysaccharide (NSP, fibre) independently while maintaining all essential nutritional components within the normal range.

The protocol for the five groups was: day 14, gavage with ^{14}C-BaP and microcapsules; days 15 and 16, microcapsules; days 21 and 22, copper phthalocyanine trisulphonate (CPTS) microcapsules. A parallel set of five animal groups was administered: on days 14 and 15, CPTS microcapsules; day 21, ^{14}C-BaP and microcapsules; days 22 and 23, microcapsules. Faeces and urine were collected individually for three days following ^{14}C-BaP administration (7 μCi; 0.12 μmol) and for two days following CPTS microcapsule administrations; BaP metabolites were analysed both for distribution between faecal solids, faecal liquids, microcapsules and urine and by HPLC for alterations in pattern.

NSP in bran fibre appeared to increase metabolite binding. Beef diminished binding and increased the presence of BaP-1,6-dione, which is known to cause DNA-strand breakage through the formation of hydroxyl radicals[59]. Within the human diet range, a three-fold enhancement of the availability of BaP metabolites to mucosa was found.

(iv) *Effects of diet on microcapsule trapping and carcinogen metabolism* (with Mr J.-C. Bérézíat, Mrs A. Ellul, Miss F. El Ghissassi and Miss J. Michelon; in collaboration with Dr S. Bingham, Dunn Clinical Nutrition Centre, Cambridge, UK; and Dr A. Hubert, National Centre for Scientific Research, Paris)

Fat, caloric intake and origin of diet are believed to be important in the etiology of human cancer. The microcapsule-^{14}C-BaP protocol reported in the previous section was thus utilized (1) with the corresponding set of high-fat diets and one low-fat diet as control, and (2) with one diet fed at two-thirds daily intake. The data are presently being analysed; however, substantial effects on faecal weight, fraction excretion in urine and clearance rate were found.

(v) *Comparison of effects of diet and of gut bacteria on microcapsule trapping and carcinogen metabolism* (with Mrs A. Ellul and Miss F. El Ghissassi)

Diet affects bacterial populations[60], and it is believed that the bacterial flora of the gut can have major effects on the metabolism of xenobiotics. Using the same protocol described above for microcapsule trapping of [^{14}C]-BaP metabolites, two human diets (high-NSP/low-meat/low-fat and high-NSP/high-meat/high-fat) were fed to groups of inbred Fischer rats as described above, but also to two identical groups treated with antibiotics in the drinking-water and thus with no gut bacteria, as confirmed by faecal enzyme assays. Faecal weight was increased in germ-free animals, although body-weight gain was the same after adaptation, and the rate of BaP excretion greatly slowed. The experiment shows, apart from substantially reduced recovery of microcapsules and diminished excretion of BaP by germ-free animals, differences arising from bacterial action that are more pronounced for groups consuming the high-meat/high-fat diets.

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[59] Lorentzen, R.J. & Ts'o, P.O.P. (1977) *Biochemistry, 16,* 1467–1473
[60] Drasar, B.S. & Hill, M.J. (1974) *Human Intestinal Flora,* London, Academic Press, pp. 18–20

(vi) *Effect of human diets on appearance of cross-linking agents in the gastrointestinal* tract (with Miss Mme A. Ellul and Miss F. El Ghissassi)

Many cross-linking agents, i.e., bifunctional substances capable of cross-linking DNA strands and proteins, are known carcinogens. It had been noticed[43] that whenever microcapsules had passed through the rodent gut, the core:membrane ratio for microcapsule PEI (after ultrasonication) was substantially reduced, consistent with cross-linking. Microcapsules were labelled with ^{14}C-CH_3 using ^{14}C-methyl iodide, i.e., with the radiolabel attached to both membrane and core, and administered to inbred male Fischer rats that had been adapted for four weeks to human diets. The results show that cross-linking agents may arise in the intestine both from gut bacteria and from diet-dependent metabolites.

(vii) *Trapping of mutagenic fæcapentaene*-12 (in collaboration with Dr S. Plummer and Dr C.C. Harris, National Cancer Institute, Bethesda, MD, USA; supported by award of ICRETT fellowship to Dr A. Povey)

Fæcapentaenes were isolated by Hirai *et al.*[61] as apparently mutagenic components of faeces which were easily oxidized to nonmutagenic products. Fæcal mutagenicity has been shown to be eliminated by dietary supplementation with bran fibre[62]. Initial studies show that microcapsules can bind approximately 10% of oxidized fæcapentaene-12, the amount being dose-dependent; the microcapsules showed cross-linking, as had been anticipated from previous findings that suggest that fæcapentaene-12 cross-links DNA[63]. The adduct in the microcapsule core showed ultra-violet absorption at 323 and 285 nm. Further work is under way to find whether fæcepentaene-12 itself binds to microcapsules.

(viii) *Studies to confirm safety for potential human use* (with Dr J.R.P. Cabral, Miss M.P. Desvaux and Miss J. Michelon; in collaboration with Dr D. Godanesche, National Institute for Health and Medical Research, Clermont Ferrand, France)

The human gut is exposed *via* normal human foods and mucociliary clearance of the respiratory system to particulate matter of the same size as the microcapsules. Extremely low proportions of 2-μm latex particles have been reported by LeFevre *et al.*[64] to be retained by Peyer's patches in the rodent gut, whereas 15-μm latex particles were not. To establish positively that microcapsules are neither retained in the gut mucosa or lymph nodes nor translocated to other organs, two related experiments were undertaken. In the first, groups of rats were administered each (a) either five, ten or 25 daily doses of microcapsules of mean diameter 30 μm (total, 10, 20 or 50 million) and then sacrificed, or (b) 25 daily doses of microcapsules of mean diameter 19 μm (total, 50 million) or suspensions of solid latex microparticles of mean diameter 2.5 μm or (c) saline solution. After sacrifice, the gut is being examined macroscopically and microscopically for inflamed lymph nodes and the liver, spleen and lungs for the presence of iron oxide particles.

In the second experiment, microcapsules were labelled by reaction with ^{14}C-methyl iodide, and doses of 1.8×10^6 microcapsules (equivalent to 2 μCi per animal) were administered to rats. The rats were maintained in air-tight metabolic cages to permit collection of ^{14}C-CO_2 in exhaled air and separate collection of urine and faeces. After periods of 1 to 72 h, animals were

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[61] Hirai, W., Kingston, D.G.I., Van Tassell, R.L. & Wilkins, T.D. (1982) *J. Am. chem. Soc., 104,* 6149–6150

[62] Reddy, B.S., Sharma, C., Simi, B., Engle, A., Laakso, K., Puska, P. & Karpela, R. (1987) *Cancer Res., 47,* 644–648

[63] Plummer, S.M., Grafstrom, R.C., Yang, L.L., Curren, R.D., Linnainmaa, K. & Harris, C.C. (1986) *Carcinogenesis, 7,* 1607–1609

[64] Lefevre, M.E. & Joel, D.D. (1977) *Life Sci., 21,* 1403–1408

sacrificed. Total analyses of radioactivity in urine, faeces, stomach and intestinal contents and in 11 organs were performed, together with autoradiography of several tissue sections. Radioactivity found was 98.6% in faeces, 0.3% in exhaled air and 0.4% in urine; this was consistent with apparently complete faecal excretion of the microcapsules and some acid hydrolysis of the bound radiolabel within the stomach. After 72 h, the radioactivity present in the liver, spleen, kidney, blood or Peyer's patches was no more than 0.01% of the administered dose. No evidence of concentrated radioactivity, i.e., microcapsules, was found outside the gut in any of the autoradiographed slices.

(*e*) *Testing of compounds in* Salmonella *mutagenicity assays and in the SOS chromotest* (Dr C. Malaveille, Mrs G. Brun, Mrs A. Hautefeuille and Dr H. Bartsch)

Most of the compounds listed in Table 44 were assayed in relation to specific projects described in other sections of this *Annual Report*. All the other compounds were tested because of possible human exposure and to investigate mechanistic aspects of their (metabolic) activation into genotoxic derivatives.

Table 44. Results of *Salmonella* assays and SOS chromotests on compounds tested in 1986–1987

Compound	Occurrence/Use	Assay system	Mutagenic/genotoxic effect (±S9)[a]
Aqueous extract of *Swartzia madagascariensis*	Molluscicide	SOS chromotest	– (±S9)
Aqueous extract of *Phytolacca dodecandra*	Molluscicide	SOS chromotest	– (±S9)
Four saponins isolated from *Swartzia madagascariensis*	Molluscicide	SOS chromotest	2 – (±S9) 2 + (–S9)
Seven saponins isolated from *Phytolacca dodecandra*	Molluscicide	SOS chromotest	2 – (±S9) 5 + (–S9)
12 Glycosylamines and their *N*-nitroso derivatives	Synthetic compounds	*Salmonella* assay	See section III.3.*f* of this *Annual Report*
Three hydroxyphenanthrene derivatives	Opium/morphine pyrolysate	*Salmonella* assay	+ (+S9); see also section I.2.*e* of this *Annual Report*
Ochratoxin A	Mycotoxin	SOS chromotest	+ (–S9)
Ochratoxin B	Mycotoxin	SOS chromotest	– (±S9)
Ochratoxin a	Metabolite of ochratoxin	SOS chromotest	– (±S9)
Chloroxin; 4-chloro-*m*-cresol; chloroxylenol; 5-chloro-8-quinolinol	Synthetic compounds structurally related to ochratoxin	SOS chromotest	+ (–S9)
5-Chlorosalicylic acid	Synthetic compound structurally related to ochratoxin	SOS chromotest	– (±S9)
Aqueous/organic extracts of smoked fish and derived chromotographic/HPLC fractions	Nitrosated *in vitro*	SOS chromotest	See section I.2.*d*.ix of this *Annual Report*

[a] S9, 9000 × *g* supernatant fraction of rat liver

(*f*) *Induction of mutation, sister chromatid exchange and in-vitro cell transformation by particulates from Athens air* (Dr H. Yamasaki and Mrs C. Piccoli; in collaboration with Dr K. Athanasiou, National Hellenic Research Foundation, Athens)

Airborne particulates were collected within in the Athens basin over a period of 12 months, using Hi-Vol samplers. After *n*-hexane extraction, samples were tested in a battery of in-vitro tests: for their ability to induce mutation in bacteria and to induce mutation, sister chromatid exchange and morphological transformation in cultured mammalian cells. Positive results were found for mutagenicity in *Salmonella typhimurium* strain TA98, for induction of sister chromatid exchange in CHO cells and for transformation of BALB/c 3T3 cells in culture. A weak, non-dose-related induction of ouabain resistance in BALB/c 3T3 cells was also observed[65].

(*g*) *Synthesis and genotoxic activity of* N-*nitrosated glycosylamines* (Dr B. Pignatelli, Dr C. Malaveille, Dr M. Friesen, Mrs A. Hautefeuille and Dr H. Bartsch; in collaboration with Professor G. Descotes, Claude Bernard University/College of Industrial Chemistry, Lyon, France; Professor J. Sokolowski and Dr D. Piskorska, University of Gdansk, Poland; and Professor T. Matsushima, Institute of Medical Sciences, University of Tokyo)

Glycosylamines and Amadori compounds are present in many food items and are formed during the early stages of nonenzymatic browning (Maillard) reactions. The secondary amino group present in these compounds can be nitrosated. Although a few synthetic *N*-nitroso-fructuse-amino acids have been reported to be direct-acting mutagens in *Salmonella typhimurium his*$^-$ strains, the reaction mechanism has not been described.

The aims of the study were to elucidate the structural parameters that determine the mutagenic activity of *N*-nitrosoglycosylamines and *N*-nitroso-Amadori compounds and to investigate the nature of the ultimate mutagen(s) derived from the parent compound. Model compounds for early Maillard reaction products that may occur in food, i.e., reaction products between *p*-toluidine, *p*-aminobenzoic acid, *p*-nitroaniline and tryptamine, and sugars such as pentoses and fructose, and their *N*-nitrosated derivatives were synthesized, characterized and tested for their mutagenicity in *S. typhimurium his*$^-$ strains[66]. The structures of these substances were ascertained by spectroscopic analyses.

Three of nine glycosylamines were found to be weakly mutagenic in *S. typhimurium* TA100 strain; all the other glycosylamines and the Amadori compound were devoid of mutagenic activity. The *N*-nitrosation of seven out of ten of these compounds converted them into direct-acting mutagens. The activity of some of the *N*-nitroso compounds was similar to that of *N*-ethyl-*N*-nitrosourea. Comparative studies on *N*-nitrosated glycosyl-*p*-nitroanilines show that their mutagenic activity is dependent on the structure of the sugar moiety and requires the presence of free hydroxyl groups in that moiety. A comparison of the mutagenic activity exerted by *N*-nitrosoxylosyl derivatives of different amines showed that it also depends on the structure of the amine moiety. The mutagenicity of *N*-nitrosoglycosylamines was attributed to their hydrolysis to diazonium cations. Their formation was detected by azo-coupling with *N*-ethyl-1-naphthylamine using spectrophotometric and mass spectrometric analyses. Our data indicate that the mutagenicity of *N*-nitrosoglycosylamines and the *N*-nitroso-Amadori compound is attributable mainly to their hydrolytic decomposition into phenyl (alkyl) diazonium and/or phenyl (alkyl) cations.

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[65] Athanasiou, K., Arzimanoglou, I., Piccoli, C. & Yamasaki, H. (1987) *Cell Biol. Toxicol.* (in press)

[66] Pignatelli, B., Malaveille, C., Friesen, M., Hautefeuille, A., Bartsch, H., Piskorska, D. & Descotes, G. (1987) *Food Chem. Toxicol.* (in press)

(*h*) *Analysis of environmental carcinogens and analytical quality assurance*

(i) *International mycotoxin check sample programme* (Dr M. Friesen, Mrs L. Garren and Mrs M.-B. D'Arcy; supported in part by the Joint FAO/WHO Food Contamination Monitoring Programme and the Mycotoxin Working Group of the IUPAC Commission on Food Chemistry)

This continuing programme provides an opportunity for laboratories engaged in the analysis of mycotoxins in foodstuffs to compare their own analytical results with those of other laboratories around the world. Participants analyse identical portions of a homogeneous food sample for a given mycotoxin, using the analytical method of their choice. Results are collected and evaluated statistically at the Agency before redistribution to the individual laboratories. At present, the trial, which is free of charge to participants, is carried out once a year. In the most recent study, samples of aflatoxin-contaminated maize (corn), peanuts and milk were analysed by 204 laboratories in 49 countries. As shown in Figure 17, an increasing number of laboratories participate in the programme each year.

A subgroup of laboratories also participated as part of the Joint FAO/WHO Food Contamination Monitoring Programme, to help assure the quality of results generated by laboratories in the 22 countries collaborating in this effort. A programme of follow-up training and provision of necessary supplies and small equipment has been initiated for laboratories who require support in maintaining the quality of their analytical results.

(ii) *International N-nitrosamine check sample programme* (Dr M. Castegnaro and Mrs Z. Schneider; in collaboration with the IUPAC Commission on Food Chemistry)

Thirty-five laboratories participated in this study, the aim of which was to offer laboratories engaged in the field of analysis of nitrosamines in foodstuffs a way of comparing their results with those of laboratories around the world. Each laboratory received two samples (one of beer and one of malt) and three solutions of reference compounds (*N*-nitrosodimethylamine[NDMA], *N*-nitrosopyrrolidine[NPYR] and *N*-nitrosoproline[NPRO]. Each sample was to be analysed for the three compounds. All laboratories analysed NDMA and nearly all NPYR, but only 13 of them analysed NPRO. All the laboratories used the thermal energy analyser as a detector, except for two that used mass spectrometry.

Statistical evaluation of the results is presented in Table 45.

Results in beer: In the analysis of NDMA, seven laboratories out of 35 (20%) were rejected as outliers. These included the two mass spectrometry results, which seemed to be grossly

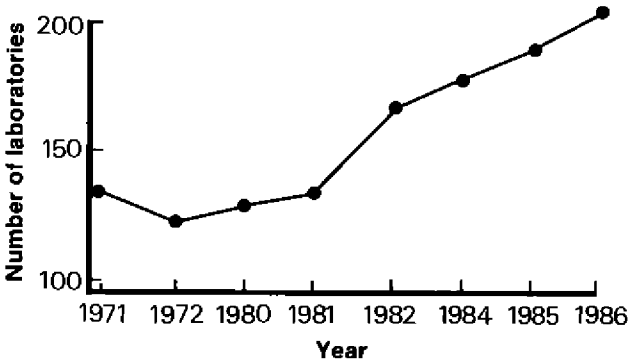

Fig. 17. Participation in analyses of maize and peanuts for aflatoxin levels

Table 45. Statistical parameters of the first check sample survey for analysis of *N*-nitrosamines

Sample	*N*-Nitrosamine	Median (μg/kg)	Mean (μg/kg)	Standard deviation (μg/kg)	Coefficient of variation (%)	Total number	Number of outliers
Beer	NDMA	0.16	0.15	0.06	38.58	35	7
	NPYR	0.05	0.16	0.16	103.35	32	2
	NPRO	1.00	1.32	1.27	96.23	13	0
Malt	NDMA	2.25	2.60	1.63	62.70	35	1
	NPYR	1.00	1.14	1.01	88.14	32	3
	NPRO	17.18	21.71	16.13	74.31	13	0

overestimated. Having excluded the outlying results, the statistical parameters are excellent for an analysis at this level of contamination, with a coefficient of variation of less than 40%. In the analysis of NPYR, only two outlying results were rejected, but the data show a much greater dispersion. For NPRO, only 13 laboratories analysed the sample, and six reported no detectable level.

Results in malt: In the analysis of NDMA, there was only one outlying result and a distinct grouping of the results between 1 and 4 μm/kg. The coefficient of variation, approximately 63%, compares quite well with those obtained in similar studies, i.e., for detection of aflatoxins at comparable levels.

A greater variability is encountered in the analysis of NPYR with a large proportion of 'non-detected' (about 30%). When these zero data are discarded, the results are quite good. Mean (1.49 μg/kg) and median (1.50 μg/kg) figures agree well, and the standard deviation is excellent (0.66 μg/kg), with a coefficient of variation of less than 45%.

Two studies of this type are foreseen by the end of 1987 and 1988 for the analysis of *N*-nitrosodiethanolamine in cosmetic products and of the same nitrosamines in the same matrixes. This latter study is being organized to determine whether comparability of the results has improved.

(iii) *Analysis of total N-nitroso compounds in biological samples* (Dr B. Pignatelli, Miss I. Richard and Dr H. Bartsch; in collaboration with Dr Y. Minaire, Dr R. Lambert, Dr B. Moulinier and Dr J. Forichon, Edouard Herriot Hospital, Lyon, France; and Professor M. Crespi, Regina Elena Institute, Rome)

Recent applications of the two avialable methods for group determination of total *N*-nitroso compounds (NOC), implicated in gastric cancer etiology, gave rise to contradictory findings, and the reported concentrations of NOC in human gastric juice showed large interlaboratory variations. Both methods have been subject to criticism.

By modifying previous methods, we have developed an improved procedure for the analysis of total NOC in human gastric juice[67]. The gastric juice sample, treated with sulphamic acid to remove nitrite, is injected directly into refluxing ethyl acetate containing either acetic acid for determining interfering thermal energy analyser-(TEA)-responsive compounds that are thermo/acetic acid-labile (TAC), or into hydrogen bromide for the analysis of both TAC and NOC. The nitric oxide levels are measured by chemiluminescence by TEA, and the difference

[67] Pignatelli, B., Richard, I., Bourgade, M.-C. & Bartsch, H. (1987) *Analyst* (in press)

between the two determinations represents the concentrations of NOC in gastric juice. This method is not affected by nitrate concentrations up to 1000 mmol/l.

To verify the suitability of this analytical method: (1) the efficiency of sulphamic acid and hydrazine sulphate as nitrite scavengers was examined in nitrite-spiked gastric juice samples; (2) the stability of NOC following these two treatments and the influence of time and storage conditions on NOC concentrations were examined and (3) absence of artefactual *N*-nitrosation was verified after spiking gastric juice samples with 2,6-dimethylmorpholine. The method was found to be superior to other available methods. It is (1) highly selective for NOC, (2) reproducible (coefficients of variations from triplicate measurements, 5–10%), (3) sensitive (detection limit, 0.02 mmol/l and (4) rapid. It requires only small volumes of gastric juice (2 ml for duplicates of both TAC and NOC determinations). It is performed without prior extraction (to avoid errors and poor recoveries of certain NOC). The difficulties and limitations, leading to false measurements, of previous methods having been eliminated, this method can accurately distinguish NOC from other TEA-responsive species.

Seventy-three gastric juice samples, obtained from patients before and after operation for duodenal ulcer ($n = 64$) or with chronic atrophic gastritis ($n = 9$) were analysed for NOC. Because of small sample volume, some gastric juices with similar pH were pooled. The means and ranges of TEA responses for the determined TAC, and calculated NOC concentrations in the gastric juice samples analysed, were compared for several pH ranges. Although the sample size was small, the following conclusions can be drawn: (1) concentrations (means) of NOC were not significantly different in gastric juice samples with extremely acidic or basic pH (0.6 *vs* 0.5 μmol/l, respectively); (2) in contrast, samples at pH 3.6–7.0 had higher (mean) NOC concentrations (1.3 μmol/l); (3) the concentrations profoundly influenced total TEA-responsive compounds, since the ratio [NOC]:[TAC] varied among individual samples from 0.2 to 5.

This method is now being applied to various groups of subjects with precancerous conditions of the stomach with the aim of resolving current controversies concerning the role of endogenously formed NOC in gastric cancer. This procedure will also be compared with the determination of NOC by HPLC/photohydrolyser developed by Shuker and Tannenbaum[68].

(iv) *Determination of total* N-*nitroso compounds in environmental samples* (Dr M. Castegnaro; in collaboration with Dr C.L. Walters, Department of Biochemistry, University of Surrey, Guildford, UK; and Dr R. Massey, Ministry of Agriculture, Fisheries and Food, Norwich, UK)

The results of the collaborative study for determination of total NOC have been evaluated statistically (see Table 46) and show a distinct improvement over those of a previous study[69]. The method for analysis of total NOC in organic extracts is now reliable, and future efforts will be directed to their determination in aqueous media.

(v) *Determination of volatile nitrosamines in rubber nipples and pacifiers* (Dr M. Castegnaro and Dr M. Friesen; in collaboration with Dr J.B. Westin, Tel-Aviv University, Sackler School of Medicine, Tel-Aviv, Israel; and Dr J.R.A. Pollock, Pollock and Pool Ltd, Reading, UK)

Sixteen types of children's pacifiers and baby-bottle nipples, bought in shops in Israel but produced both there and elsewhere, were analysed for their content of *N*-nitrosamines[70] and of

[68] Shuker, D. & Tannenbaum, S. (1983) *Anal. Chem. 55*, 2152
[69] Castegnaro, M., Massey, R.C. & Walters, C.L. (1987) *Food Addit. Contam., 4*, 37–43
[70] Westin, J.B., Castegnaro, M. & Friesen, M. (1987) *Environ. Res.* (in press)

Table 46. Results reported for analysis of total *N*-nitroso compounds by collaboratoring laboratories in second trial

Laboratory no.[a]	Amount of nitric oxide in whole samples (ng)			
	32.9 ng added	32.9 ng added	146.4 ng added	146.4 ng added
1	40.7	35.5	155.9	146.4
2	47.4	39.6	178.8	167.7
3	34.7	50.6	157.3	261.7
4	23	21	164	166
5	45.7	60.1	182	186
6a	50.5	38.2	101.5	172
6b	34.1	41.1	141.4	140.6
Overall mean[b]	40.58		170.32	
Standard deviation[b]	11.33		35.87	
Coefficient of variation[b]	27.9%		21.1%	

[a] 6a, using 8 ml of the provided sample and adjusting for 10 ml; 6b, modified procedure using 1.0–1.5 ml of the provided sample and adjusting for 10 ml

[b] Excluding results from 6b

nitrosatable amines, using two extraction methods. A number of volatile nitrosamines were detected in addition to the nitrosatable amines. Upon nitrosation in artificial saliva, these amines produced not only the related *N*-nitrosamines but also relatively high levels of the corresponding *N*-nitramines[71]. Thus, half of the samples tested failed to meet the regulations of either the USA or the Federal Republic of Germany, and more than 80% failed to comply with the even stricter Dutch standard.

(vi) *Collaborative study of methods of analysis of volatile nitrosamines in rubber nipples and pacifiers* (Dr M. Castegnaro; in collaboration with Dr J.R.A. Pollock, Pollock and Pool Ltd, Reading, UK; Dr L. Rossi, Commission of European Communitities, Brussels; and the IUPAC Commission of Food Chemistry)

At the request of the Commission of the European Community, a collaborative study to determine levels of NOC in rubber nipples and pacifiers has been initiated. Twelve samples and a standard solution for quantification have been circulated to 21 laboratories. The samples had to be tested by two methods: that described by Havery and Fazio[72] (1982) and that of Speigelhalder[73] (1983).

Results have been received from 14 laboratories and are currently being evaluated for the two methods. The statistical parameters for quantification of the standard solution are presented in Table 47.

[71] Castegnaro, M., Pollock, J.R.A. & Friesen, M. (1987) In: Bartsch, H., O'Neill, I.K. & Schulte-Herman, R. eds, *Relevance of N-Nitroso Compounds to Human Cancer: Exposure and Mechanisms* (*IARC Scientific Publications No. 84*), Lyon, International Agency for Research on Cancer, pp. 377–379

[72] Havery, D.C. & Fazio, T. (1982) *Food Chem. Toxicol., 20,* 939–944

[73] Spiegelhalder, B. (1983) In: Preussman, R., O'Neill, I.K., Eisenbrand, G., Spiegelhalder, B. & Bartsch, H., eds, *Environmental Carcinogens: Selected Methods of Analysis*, Vol. 6 (*IARC Scientific Publications No. 45*), Lyon, International Agency for Research on Cancer, pp. 265–273

Table 47. *N*-Nitrosamines in rubber nipples and pacifiers; results from 14 laboratories

	Nitrosamine analysed[a]				
	NDMA	NDEA	NDBA	NPIP	NMOR
Actual content (mg/ml)	1.2	1.5	0.8	0.8	2.3
Mean (mg/ml)	1.04	1.314	0.917	0.594	2.788
Standard deviation (mg/ml)	0.051	0.072	0.086	0.034	0.205
Coefficient of variation	4.87	5.48	9.37	5.81	7.35

[a] NDMA, *N*-nitrosodimethylamine; NDEA, *N*-nitrosodiethylamine; NDBA, *N*-nitrosodi-*n*-butylamine; NPIP, *N*-nitrosopiperidine; NMOR, *N*-nitrosomorpholine

Good interlaboratory agreement with coefficient of variation below 10% has been achieved for all samples, despite two individual results provided by separate laboratories which were double the spiking level of NDBA and NMOR. This taken into account, the variation of the results from the two methods will have only a minor component of the variability from the end determination technique.

(vii) *Environmental Carcinogens: Methods of Analysis and Exposure Measurement* (Dr I.K. O'Niell and Mrs B. Dodet; in collaboration with Dr L. Fishbein, ENVIRON Corporation, Washington DC; and Dr A. Mackenzie-Peers, St Alvère, France)

This series of volumes aims to improve assessment and measurement of exposure to carcinogens (known or suspected) in the environment, by providing selected methods of sampling and analysis and background data on occurrence, toxicological aspects and epidemiology. The Editorial Board met for the eleventh time in September 1986 to discuss priorities and Agency requirements for these volumes; present were the Chairman of the IARC Scientific Council, Dr B. Armstrong, and the Chairman of the Editorial Board, Dr L. Fishbein. It was decided that present work and planning should continue on:

(1) publication of volume 9 on passive smoking;

(2) finalization of volume 10 on benzene and alkylated benzenes;

(3) continuation of volume 11 on dioxins, polychlorinated dibenzofurans and biphenyls;

(4) a review board on indoor air (volume 12); and

(5) the next priority subject — biological monitoring techniques.

In 1986, volume 8, *Some Metals: As, Be, Cd, Cr, Ni, Pb, Se, Zn* was published, the Review Board for volume 11 (see below) met and designed the outline for dioxins, polychlorinated dibenzofurans and biphenyls. The Review Board for volume 12 was convened (see below) and decided on a volume outline, with authors and reviewers. Comments were invited from several organizations, including the IUPAC Commission on Atmospheric Chemistry, WHO EURO and the Netherlands Ministry for the Environment, before proceeding with this volume.

The Review Board for volume 11 (dioxins, polychlorinated dibenzofurans and biphenyls) met on 20–21 September 1985 in Bayreuth, Federal Republic of Germany, and comprised the following members: Dr K. Ballschmiter, University of Ulm, Federal Republic of Germany; Dr H. Buser, Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture, Wädenswil, Switzerland (co-chairman); Dr W. Crummett, Dow Chemical Co., Midland, MI, USA; Dr L. Fishbein, National Center for Toxicological Research, Jefferson, AR, USA;

Dr H. Hagenmaier, University of Tübingen, Federal Republic of Germany; Dr O. Hutzinger, University of Bayreuth, Federal Republic of Germany; Dr R.K. Mitchum, US Environmental Protection Agency, Las Vegas, NV, USA; Dr J. Norstrom, National Wildlife Research Centre, Ottawa; Dr S. Safe, Texas A. & M. University, College Station, TX, USA; Dr C. Rappe, University of Umea, Sweden (co-chairman); Dr J.J. Ryan, Health and Welfare Canada, Ottawa; and Dr J.D. Wilson, Monsanto Company, St Louis, MO, USA.

The review board for volume 12 on indoor air, held in Essen, Federal Republic of Germany on 21–22 October 1986, included the following members: Dr K.D. Brunnemann, Naylor Dana Institute for Disease Prevention, Valhalla, NY, USA; Dr R. Koenig, Batelle Institute, Frankfurt, Federal Republic of Germany; Dr H.W. de Koning, WHO, Geneva, Switzerland; Dr B. Seifert, Institute for Water, Soil and Air Hygiene, Berlin (West), Federal Republic of Germany (Chairman); Dr A. Swedjemark, National Institute of Radiation Protection, Stockholm; and Dr L. Wallace, Harvard University School of Public Health, Boston, MA, USA

(*i*) *Long-term and Short-term Assays for Carcinogens: A Critical Appraisal*

A meeting was held in Lyon (2–6 December 1985), in collaboration with the International Programme on Chemical Safety and the Commission of the European Communities, to review the conduct and use of long-term and short-term assays for the detection of chemical carcinogens, in the light of progress made in the understanding of the carcinogenesis process and of the scientific basis of the various endpoints used in these assays. The meeting was attended by 44 experts in the fields of carcinogenesis and mutagenesis, whose task was to discuss and prepare a series of reports to be published by the Agency[74]. In the report on long-term carcinogenicity assays, particular attention was given to (1) the role of pharmacokinetic data in their design and evaluation; (2) early preneoplastic lesions in carcinogenesis; and (3) the possibility of discriminating between the relative contributions that carcinogens may make to the various stages of the carcinogenesis process. Discussions on short-term assays used in mutagenicity testing was summarized in a series of reports that include the principle and scientific basis of each assay and their relevance to the detection of carcinogens and to the multistage process of carcinogensis.

(*j*) *International conference on detection methods for DNA-damaging agents in man: applications in cancer epidemiology and prevention* (Dr H. Bartsch and Dr I. O'Niell; in collaboration with the National Cancer Institute and the Food and Drug Administration, Bethesda, MD, USA; the Commission of European Community, Brussels; and the Finnish and Swedish Environment Funds)

This conference sponsored jointly by the Agency, the Institute of Occupational Health, Helsinki, the National Cancer Institute, Bethesda, MD, USA, the Food and Drug Administration of the USA, the CEC in Brussels and the Finnish and Swedish Environment Funds, will promote critical appraisal of methods for detecting DNA-damaging agents and their immediate effects in humans, their application in research into the causes of human cancer, and their use in monitoring exposure of humans to known carcinogens. A particular objective of the conference is to foster, through multidisciplinary discussions, the use of these methods in future research in cancer epidemiology. Measurement methods discussed will include analysis of human tissues

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[74] Montesano, R., Bartsch, H., Vainio, J., Wilbourn, J. & Yamasaki, H., eds (1986) *Long-term and Short-term Assays for Carcinogens: A Critical Appraisal* (*IARC Scientific Publications No. 83*), Lyon, International Agency for Research on Cancer

and body fluids for adducts of carcinogens with DNA, RNA and protein, and analyses for carcinogen-modified nucleobases, thioethers, indicator nitrosamines and mutagenic metabolites.

The subject matter of the conference will be covered through addresses by 40 invited speakers and two panel discussions on future requirements. One session on measurement of the effects of alkylating agents will be dedicated to the honour of Professor L. Ehrenberg's scientific contributions to this and related subjects. The proceedings will be published in the *IARC Scientific Publications* series.

(*k*) *IARC survey of chemicals being tested for carcinogenicity* (Mrs M.J. Ghess, Mr J. Wilbourn, Dr A. Tossavainen and Dr H. Vainio)

The IARC survey of chemicals being tested for carcinogenicity was initiated in 1973 in collaboration with the National Cancer Institute of the USA. Twelve *Information Bulletins* have been published to date. The main aim of this project is to continue to survey, at intervals of approximately two years, the status of long-term carcinogenicity testing throughout the world and to follow-up the stages of the experiments from their inception to the publication of final results. The survey also identifies chemicals for which future *IARC Monographs* should be prepared.

Each *Bulletin* contains lists of chemicals under investigation, use categories, animal species, strain and number of animals; purity of the substance tested; route of exposure and dose levels; date and stage of experiments; principal investigator(s) and references to published reports on completed studies. Survey results are arranged alphabetically by country, within each country by city and within each city by institute.

In September 1985, the twelfth questionnaire was sent to the 90 institutes who reported in *Bulletin No. 11,* asking for updated information on experiments already in progress or on experiments recently started or planned. Special attention was given to obtaining information on completed studies and on whether the results had been published (and, if so, complete reference citations). If they had not been published, enquiries were made as to whether this was because results were negative or because the experiment had been inadequate. Questionnaires were also sent to nine newly identified laboratories that expressed willingness to report details of their long-term testing programmes.

Data on synonyms and trade names, Chemical Abstract Services Registry Numbers and use categories were added by IARC staff when they were not provided by the investigators. The *Bulletin* was produced using computer programmes to generate indexes of principal investigators, laboratories, synonyms and trade names, Chemical Abstract Services Registry Numbers and published studies.

Information *Bulletin No. 12,* published in autumn 1986, gives data received from 95 institutes in 20 countries on a total of 998 chemicals or complex mixtures. Published studies were reported for 216 chemicals. A cross index of names (chemical names and synonyms) is included for all studies reported in *Bulletin No. 12* as well as those reported in *Bulletins 9, 10* and *11* that have been completed and published, completed and unpublished, completed but unpublished because the results were negative or discontinued.

Each *Bulletin* contains a section on epidemiological studies in progress on cancer risks in human populations possibly exposed to chemicals. Of the 1229 projects underway in 66 countries listed in the 1985 *IARC Directory of On-Going Research in Cancer Epidemiology,* about 263 are wholly or partly concerned with 66 individual chemicals or chemical substances listed in *Bulletin No. 12.*

A questionnaire on the usefulness of the *Bulletin* was sent out to all 237 participating laboratories as well as other interested scientists. A total of 97 replies indicates that the *Bulletin* is a valuable source of information.

The thirteenth Survey questionnaire will be sent out in autumn 1987 to all previous participants and to any newly identified investigators/institutes undertaking long-term carcinogenicity testing in experimental animals.

4. DESTRUCTION OF CARINOGENIC WASTES FROM LABORATORIES AND SAFE HANDLING OF CHEMICAL CARCINOGENS

(*a*) *Chemical degradation of methotrexate* (Dr M. Castegnaro; in collaboration with Dr J. Benvenuto, Texas Medical Center, Houston, TX, USA)

After the 1985 meeting to finalize the document on degradation of antineoplastic agents, it was decided to organize a complementary collaborative study to validate the two amended methods from Dr Benvenuto's laboratory for decontamination of wastes containing methotrexate. In view of the successful results, they have been accepted and incorporated in volume 73 of the *IARC Scientific Publications*[75].

(*b*) *Chemical degradation of melphalan and mutagenicity testing of residues* (Dr M. Castegnaro, Mrs I. Brouet and Dr C. Malaveille; in collaboration with Dr J. Barek and Dr J. Zima, Charles University, Prague)

The previous method using oxidation by potassium permanganate in the presence of sulphuric acid generated mutagenic residues[75]. Two alternative methods have therefore been tested using: (1) oxidation by potassium permanganate under alkaline conditions; and (2) oxidation by potassium permanganate first under alkaline then under acid conditions. While residues generated with method (2) were slightly mutagenic, method (1) provided completely nonmutagenic residues[76].

(*c*) *Chemical degradation of nitrosourea drugs and mutagenicity testing of residues* (Dr M. Castegnaro, Mrs I. Brouet and Dr C. Malaveille; in collaboration with Dr J. Barek, Charles University, Prague)

The previous method using oxidation by potassium permanganate under acidic conditions gave mutagenic residues for most of the nitrosourea drugs tested; the oxidation was therefore tested using alkaline conditions. Mutagenic residues were detected with *S. typhimurium* strains TA1530 and TA1535 with and without metabolic activation for carmustine, semustine, lomustine and PCNU.

(*d*) *Methods for degradation of new compounds, bibliographic searching* (Dr M. Castegnaro)

Bibliographic searches were carried out on another 22 antineoplastic agents. Research into their degradation will be initiated, subject to the availability of extramural funds. Potential sources of such funds are being approached actively.

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[75] Castegnaro, M., Adams, J., Armour, M.A., Barek, J., Benvenuto, J., Confalonieri, C., Goff, U., Ludeman, S., Reed, B., Sansone, E.B. & Telling, G., eds, (1985) *Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Antineoplastic Agents* (*IARC Scientific Publications No. 73*), Lyon, International Agency for Research on Cancer

[76] Barek, J., Castegnaro, M., Malaveille, C., Brouet, I. & Zima., J. (1987) *Microchem. J.* (in press)

(*e*) *Safe handling of chemical carcinogens* (Dr M. Castegnaro; in collaboration with Dr E.B. Sansone, Frederick Cancer Research Facility, Frederick, MD, USA)

A handbook giving guidelines for the handling of chemical carcinogens, the minimum requirements for setting up a laboratory for handling them and describing their disposal techniques was published in 1986[77].

[77] Castegnaro, M. & Sansone, E.B. (1986) *Chemical Carcinogenes: Some Guidelines for Handling and Disposal of Small Quantities of Laboratory Wastes*, Berlin (West), Springer

IV. TECHNICAL SUPPORT

1. COMPUTING AND BIOSTATISTICAL SUPPORT (Mr M. Smans, Mrs B. Charnay, Mr P. Damiecki, Mr X. Nguyen-Dinh, Mrs A. Arslan, Mrs D. Magnin, Mr M. Jaboulin, Mrs B. Kajo, Dr E. Cardis, Dr J. Kaldor, Dr J. Wahrendorf and Dr J. Estève)

After a thorough study of hardware requirements and the announcement of a series of new machines by Digital Equipment, it was decided to replace the obsolete VAX 11/780 by a VAX 8300, which has two processors of the same speed as the previous computer. In the meantime a 'Microvax' was connected to our VAX 11/780 to decrease its load while waiting for installation of the new machine, which was done in October 1986. Since this new computer runs under the same operating system and accepts our previous peripherals, all changes were made with minimal interruption of the work. Our computer configuration is gradually evolving towards a local network which would have the VAX 8300 as its main frame and several Microvaxes and other smaller microcomputers for some specialized purposes. The first Microvax will be upgraded to serve several word-processing terminals with the newer word-processing software of Digital (WPS+). This system will gradually replace the former units based on independent microcomputers, the Decmates, which are now both obsolete and worn out.

Computerized printing, handling of bibliographic collections, building of data bases for storing and retrieving information (e.g., biological data bank, fellowships programme) are an important complement to scientific activities, which should increase in the future because of the wider applicability of computer-based methodology. This progression is, however, dependent on availability of computer specialists and on regular training of our present staff.

Investigations and tests on new statistical packages are in progress, leading occasionally to the installation of new computing tools.

Computing support to several epidemiological studies, including technical supervision of computer assistants, is given regularly.

Statistical advice for the design, analysis and interpretation of epidemiological and experimental studies is provided regularly to all units of the Agency. Statistical expertise is made available at each meeting on the evaluation of carcinogenic risks to humans (*IARC Monographs*).

2. BIBLIOGRAPHIC SUPPORT

(*a*) *Library services* (Mrs A. Nagy-Tiborcz and Mrs L. Ossetian)

The Library provides active support to all scientific programmes of the Agency, and to the local medical and scientific community.

Currently, the library receives 285 journals and serial subscriptions; the present stock of bound journals is approximately 8500. A total of 8200 books are held, many of which were purchased with funds provided by voluntary donors.

The *Library Bulletin* continues to list papers published by staff members, annual reports received from other organizations and all recent book acquisitions.

The Librarian participates in preparation of the *Directory of On-Going Research in Cancer Epidemiology*.

(*b*) *Computerized bibliographic services* (Mrs M. Coudert)

Since December 1986, the Agency's terminal has been linked to a new host, SUNIST (Isle d'Abeau, France), which gives access to the French C.C.N. (National Collection Catalogue), making it possible to locate journals held by French libraries.

The workload of the service has increased considerably over the past two years, as shown in Table 48.

Table 48. Workload of computerized bibliographic services

	1984–1985	1985–1986	1986–1987
Searches	399	545	680
Monthly updatings	69	96	78

3. COMMON LABORATORY SERVICES (Dr J.R.P. Cabral, Dr H. Yamasaki, Miss M. Laval and Mrs N. Lyandrat)

These services include animal breeding and maintenance of the animal house, the histology laboratory and the glass-washing service. The Agency's scientists use animals bred in-house for the majority of their work, since they now have considerable detailed knowledge of the spontaneous tumour rates in the strains used — BDIV and BDVI rats and C57B1/6 mice. Facilities for the maintenance of nude mice and rabbits are also available.

The histology laboratory processes all the histological material from experimental animals in the Agency as well as biopsy material sent by Agency researchers doing field work abroad.

The glass-washing facility is a unified service for the experimental work carried out in chemistry, biochemistry and cell culture.

V. EDUCATION AND TRAINING

1. RESEARCH TRAINING FELLOWSHIPS (Dr R. Montesano, Mrs M. Davis and Mrs E. El Akroud)

(*a*) *The Fellowships Selection Committee*

The Fellowships Selection Committee met twice in Lyon over the period to review applications; the members of the Committee were:

Dr D. Bootsma (1986–1987)	Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands
Dr T. Kakunaga (1987)	Oncogene Research Center, Osaka University, Osaka, Japan
Dr A. Likhachev (1986–1987)	N.N. Petrov Research Institute of Oncology, Leningrad, USSR
Dr T.M. Mack (1986)	Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA
Dr B. Mansourian (1986)	Office of Research Promotion and Development, WHO, Geneva, Switzerland
Dr A.B. Miller (1987)	National Cancer Institute of Canada, Epidemiology Unit, Toronto, Ontario, Canada
Dr E. Pastorelo (1986)	National Campaign for the Fight Against Cancer, Rio de Janeiro, Brazil
Dr T.J. Slaga (1986–1987)	University of Texas System Cancer Center, Smithsville, TX, USA (representative of UICC)
Dr M. Terada (1986)	Serology Division, National Cancer Center Research Institute, Tokyo, Japan

The Agency representatives were Dr R. Montesano (Chairman), Dr H. Bartsch (1987), Dr G. Lenoir (1986), Dr C.S. Muir (1986) and Dr D.M. Parkin (1987).

In 1986, a total of 12 fellowships were awarded out of approximately 55 applications; and, in 1987, 11 out of 44 eligible candidates received fellowships. In 1986, one fellowship was tenable at the IARC; in 1987, two were available.

The distribution of fellowships awarded by discipline is given in Table 49; the list of Fellows is given in Table 50.

(*b*) *Analysis of fellowships programme (1966–1987)*

The main conclusions of the evaluation[1] of the fellowships programme can be summarized as follows:

(i) 79.5% of IARC fellows are still actively engaged in cancer research.

(ii) 49.5% of IARC fellows consider that the IARC fellowship played a decisive role in their career in cancer research.

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[1] Sohier, R., Cole, P. & Montesano, R. (1986) *IARC Internal Technical Report No. 86/002*

Table 49. Distribution of Research Training Fellowships awarded by discipline

Scientific discipline	No. of fellowships		
	1986	1987	1966–1987
Epidemiology and Biostatistics	3	4	64
Chemical Carcinogenesis	1	2	55
Viral Carcinogenesis	3	—	39
Cell Biology, Cell Differentiation and Cell Genetics	3	4	42
Biochemistry and Molecular Biology	2	1	51
Others			70
Total	12	11	321

(iii) 82.5% of IARC fellows returned to their home country.

(iv) The quality of the fellows, as judged by their publications' records, supervisors' reports and present positions, is very good.

(v) The majority of applications come from developed countries (see Table 51).

(vi) The IARC is one of the few institutions that provides basic training in cancer epidemiology and has contributed substantially to the development of this discipline in various countries (e.g., Colombia, France, India, Italy, Japan, the USSR).

(vii) Training in the epidemiology of chronic diseases is available in few countries. This probably reflects the lack of training in this discipline at the predoctoral level in other countries.

(*c*) *Visiting Scientist Awards*

This Award was given in 1986 to Dr D. Shuker (Medical Research Council, Toxicology Unit, Carshalton, Surrey, UK), who spent a period of one year in the Unit of Environmental Carcinogens and Host Factors, and in 1987 to Dr C.-C. Hsieh (Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA), who will spend one year in the Unit of Analytical Epidemiology.

2. TRAINING COURSES (Dr W. Davis and Mrs C. Déchaux)

During the period 1986–1987, the programme included both basic and advanced courses in cancer epidemiology, a course on methods of surveillance of mutagen and carcinogen exposure, and for the first time, a course on molecular biology for cancer epidemiologists.

(*a*) *Advanced course on quantitative methods in cancer epidemiology, IARC, Lyon, 26 August–6 September 1985*

The third course in this series was organized under the direction of Dr N.E. Day (IARC). Faculty members included Professor N.E. Breslow (University of Washington, Seattle, WA, USA), Dr D. Clayton (University of Leicester, UK), Professor D. Trichopoulos (University of Athens), Dr L. Bernstein (University of Southern California, Los Angeles, CA, USA), Professor M.C. Pike and Dr M. Coleman (University of Oxford, UK), Dr J. Estève, Dr J. Wahrendorf, Dr J. Kaldor and Dr G. Lenoir from IARC.

Table 50. Fellowships awarded in 1986 and 1987

Name	Institute of origin	Host institute
1986		
DEGAN, P.	National Institute for Research on Cancer, Genoa, Italy	Unit of Mechanisms of Carcinogenesis, IARC, Lyon, France
DOKHELAR, M.-C.P.	UA CNRS 1156, Gustave Roussy Institute Villejuif, France	Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA
EWERTZ, M.	The Danish Cancer Registry, Institute of Cancer Epidemiology, Copenhagen	MRC Biostatistics Unit, Cambridge, UK
FRIDMAN, R.A.	Department of Radiation & Clinical Oncology, Hadassah University Hospital, Jerusalem	Laboratory of Developmental Biology & Anomalies, National Institute of Dental Health, NIH, Bethesda, MD, USA
GERYK, J.	Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague	Biology Section, Curie Institute, Orsay, France
KOUZARIDES, T.	MRC Laboratory of Molecular Biology, Cambridge, UK	Department of Biochemistry, New York University Medical Center, New York, NY, USA
LU, X.	Department of Cell Biology, Cancer Institute, Chinese Academy of Medical Sciences, Beijing	Laboratory of Growth Control & Development, Imperial Cancer Research Fund Laboratories, London
MATOS, E.L.	Chemical & Environmental Carcinogenesis Service, "Angel H. Roffo" Institute of Oncology, University of Buenos Aires, Buenos Aires	Department of Epidemiology SC-36, University of Washington, School of Public Health & Community Medicine, Seattle, WA, USA
MULHERKAR, R.	Cancer Research Institute, Tata Memorial Centre, Bombay, India	NCI, Frederick Cancer Research Facility, Frederick, MD, USA
PAVLISH, O.A.	Laboratory of Viral Carcinogenesis, All-Union Cancer Research Center, USSR Academy of Medical Sciences, Moscow	Institute for Virology, Health Centre, Freiburg, Federal Republic of Germany
ROOMAN, R.P.A.	Department of Paediatrics, University of Antwerp, Antwerp, Belgium	Laboratory of Endocrinology, Department of Paediatrics, University of North Carolina, Chapel Hill, NC, USA
SOBUE, T.	Division of Epidemiology, Department of Field Research, The Center for Adult Diseases, Osaka, Japan	The Johns Hopkins University, School of Hygiene & Public Health, Baltimore, MD, USA

1987

BOUTRON, M.	Burgundian Digestive Tumour Registry, Dijon, France	London School of Hygiene and Tropical Medicine, London
GOOT, I.T.	Institute for Oncology Problems, Academy of Sciences of the Ukrainian SSR, Kiev, USSR	Protein Chemistry Laboratory, Imperial Cancer Research Fund Laboratories, London
ISHWAD, C.S.	Comparative Oncology Unit, Cancer Research Institute, Tata Memorial Centre, Bombay, India	Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA, USA
KAPRIO, J.A.	Department of Public Health, University of Helsinki, Helsinki	(1) Department of Family & Preventive Medicine, USC School of Medicine, Los Angeles, CA, USA; and (2) Department of Psychology, Twin Studies, Laboratory for Behavioral Medicine, University of Indiana, Bloomington, IN, USA
KLAUDE, M.E.	Wenner-Gren Institute, University of Stockholm, Stockholm	Unit of Mechanisms of Carcinogenesis, IARC, Lyon, France
LEY, S.C.	MRC Mechanisms in Tumour Immunology Unit, MRC Centre, Cambridge, UK	Department of Molecular Immunology, Dana-Farber Cancer Institute, Boston, MA, USA
NIKITIN, A.Y.	N.N. Petrov Research Institute of Oncology, Leningrad, USSR	Institute for Cell Biology (Cancer Studies), University of Essen, Essen, Federal Republic of Germany
PIERANI, A.	National Research Council, Centre for the Study of Pharmacology of Cellular Infrastructure, Milan, Italy	Laboratory of Biochemistry & Molecular Biology, The Rockefeller Institute, New York, NY, USA
STRAND, D.J.	Department of Genetics, University of Georgia, Athens, GA, USA	Department of Genetics, Johannes Gutenberg University, Mainz, Federal Republic of Germany
VERREAULT, R.	Department of Social and Preventive Medicine, Faculty of Medicine, Laval University, Ste-Foy, Quebec, Canada	Department of Epidemiology, University of Washington, School of Public Health & Community Medicine, Seattle, WA, USA
ZHANG, Z.-F.	Department of Epidemiology, School of Public Health, Shanghai Medical University, Shanghai, China	Unit of Descriptive Epidemiology, IARC, Lyon, France

Table 51. Geographic distribution of IARC fellowships awarded 1966–1986 inclusive

TO \ FROM	ARGENTINA	AUSTRALIA	AUSTRIA	BANGLADESH	BELGIUM	BRAZIL	BULGARIA	BURMA	CANADA	CHILE	CHINA	COLOMBIA	CZECHOSLOVAKIA	DENMARK	EGYPT	FINLAND	FRANCE	GERMANY, FED. REP.	GREECE	HUNGARY	INDIA	IRAN
ARGENTINA																						
AUSTRALIA											1											
BELGIUM																	2				1	
CANADA					1		1															
FRANCE	1	1					1						5				1	1	1		1	
GERMANY, FED. REP.		2					1															
JAPAN											1											
NETHERLANDS																		1				
NEW ZEALAND																						
NORWAY																		1				
SPAIN																						
SWEDEN		1	1				1		1		1		1				3	1			2	
SWITZERLAND																						
UNITED KINGDOM	1	5			1	1	2				1	1	1	2	1		2				3	1
USA	2	4	1	1	5		1				4	4	3		2	3	20	4		7	6	
IARC							1	1	1	1	1							2			1	
TOTAL	4	13	2	1	7	1	8	1	2	1	9	5	10	2	3	3	28	10	1	7	14	1

With an exceptionally large number of applications, 65 participants from 22 countries were admitted to the course.

(*b*) *Course on cancer epidemiology, Holzhau, Saxony, German Democratic Republic, 28 April–9 May 1986*

This course, intended for participants from northern and eastern Europe, brought together 40 students from nine countries. It was held at the invitation of the Central Institute for Cancer Research of the Academy of Sciences of the German Democratic Republic (Director: Professor S. Tanneberger) in the Academy guest house. The course director was Professor O. Møller

ISRAEL	ITALY	IVORY COAST	JAPAN	LEBANON	NETHERLANDS	NEW ZEALAND	NIGERIA	NORWAY	PERU	POLAND	PORTUGAL	ROMANIA	SAUDI ARABIA	SINGAPORE	SPAIN	SWEDEN	SWITZERLAND	THAILAND	TUNISIA	TURKEY	UGANDA	USSR	UNITED KINGDOM	USA	YUGOSLAVIA	TOTAL
																								1		1
																										1
																										3
			1																							3
		1	2						1	1	1						1					4				23
			2																			2		3		10
																										1
																								1		2
																								2		2
																										1
															1											1
1			5							3						1								4	1	27
																						1		2		3
	4		5							1			1			1	1			1	1	5	1	8	1	52
15	18		15	1	5	1	3	2		6		2		1	2	2	2	1	1			2	11	11		168
	1		1													1						8	3	2		24
16	23	1	31	1	5	1	3	2	1	11	1	2	1	1	3	5	4	1	1	1	1	22	15	34	2	322

Jensen (Danish Cancer Registry, Copenhagen); the other external faculty members were Professor S. Grufferman (Duke University, Durham, NC, USA), Professor J.M. Elwood (University of Nottingham, UK), Dr F. Merletti (University of Turin, Italy) and Dr D. Clayton (University of Leicester, UK). The local organization was in the hands of Dr W.H. Mehnert (National Cancer Registry, Berlin-Johannisthal). Other local faculty members were Professor K. Ebeling (Central Institute for Cancer Research, Berlin), Dr G. Enderlein (Central Institute for Occupational Medicine, Berlin), Professor W. Jaenisch (Humboldt University, Berlin), Dr M. Moehner (National Cancer Registry, Berlin-Johannisthal) and Dr D. Panzer (Institute for Medical Statistics and Data Processing, Berlin).

(c) Cancer epidemiology course, Kuala Lumpur, 23 June–4 July 1986

In collaboration with the Regional Office for the Western Pacific, this course was held in the Institute for Medical Research, Kuala Lumpur (Director: Dr Lim Teong Wah). Thirty participants from eight countries in the region attended the course, organized under the direction of Dr C.S. Muir (IARC) with Dr J. Osborn (London School of Hygiene and Tropical Medicine, UK), Dr Gao Yu Tan (Shanghai Cancer Institute, China), Dr C.D.J. Holman (Health Department of Western Australia, Perth, Australia), Dr A.J. McMichael (Commonwealth Scientific and Industrial Research Organization, Adelaide, Australia), Professor K. Shanmugaratnam (National University of Singapore) and Professor T. Yoshimura (University of Occupational and Environmental Health, Kitakyushu, Japan). The Malaysian faculty included Dr Ng Kok Han (Institute of Medical Research, Kuala Lumpur) and Dr Perdaman Singh (Institute of Radiotherapy, Oncology and Nuclear Medicine, Kuala Lumpur).

(d) Molecular biology for cancer epidemiologists, IARC, Lyon, 15–24 July 1986

Professor J. Cairns (Harvard School of Public Health, Cambridge, MA, USA) and Dr R. Saracci (IARC) were the joint coordinators of an innovative course in which the other faculty members were Dr J. Mullins (Harvard School of Public Health, Cambridge, MA, USA), Professor I.B. Weinstein (College of Physicians and Surgeons, Columbia University, New York, NY, USA), Professor B. Griffin (Postgraduate Medical School, London), Dr L. Gissman (German Cancer Research Centre, Heidelberg, FRG), Dr G.B. de-Thé (National Centre for Scientific Research, Lyon, France), Dr B. Ponder (Institute of Cancer Research, Haddow Laboratories, Sutton, Surrey, UK), Dr S. Venitt (Institute of Cancer Research, London) and Dr H. Bartsch, Dr G. Lenoir and Dr R. Montesano (IARC). A total of 51 participants from 14 countries attended the course.

(e) Course on cancer epidemiology (in French), Rabat, 6–17 October 1986

Professor B. Terracini (University of Turin, Italy) was director of the course, intended for participants from the francophone countries of Africa and Europe. The course was held at the Sidi Mohamed Ben Abdellah Institute of Oncology, at the invitation of its Director, Professor A. El Hafed. The faculty included Dr J. Estève and Dr A. Tuyns (IARC), Dr E. Benhamou and Dr H. Sancho-Garnier (Gustave-Roussy Institute, Villejuif, France) and Mr L. Raymond (Geneva Cancer Registry, Switzerland). The local faculty included Professor B. El Gueddari and Dr M. Odda from the guest institute. The 36 participants came from five countries, with especially strong representation from Italy and Spain.

(f) Health monitoring of human populations exposed to mutagens and carcinogens, Bombay, India, 3–14 November 1986

The course, directed by Dr H. Vainio (IARC), was held in the Cancer Research Institute, Tata Memorial Centre, Bombay, India (Research Director: Dr M.G. Deo). The International Programme on Chemical Safety, WHO (Manager: Dr M. Mercier) and the International Association of Environmental Mutagen Societies (President: Professor F.H. Sobels) jointly sponsored the course. The faculty from outside India included Dr M. Sorsa and Dr K. Hemminki (Institute of Occupational Health, Helsinki), Professor C. Ramel (Wallenberg Laboratory, Stockholm) and Professor A.N. Natarajan (Silvius Laboratories, Leiden, The Netherlands). The Indian faculty consisted of Dr C.R. Krishna Murti (Scientific Commission of Bhopal Gas Leakage, New Delhi), Dr S.S. Agarwal (King George's Medical College, Lucknow), Dr A.N. Bhisey (Cancer Research Institute, Bombay), Dr (Mrs) S.V. Bhide (Cancer Research Institute, Bombay), Dr P.S. Chauhan, Dr D.S. Joshi and Dr K. Sundaram (Bhabba

Atomic Research Centre, Bombay), Professor S.P. Modak (University of Pune) and Dr Sharat Chandra (Indian Institute of Science, Bangalore).

There were 32 participants — one from Australia and the remainder from India.

(*g*) *Advanced methods in cancer epidemiology, Heidelberg, Federal Republic of Germany, 15–26 June 1987*

The course was held at the Institute of Epidemiology and Biometry of the German Cancer Research Centre, Heidelberg (Director: Professor H. zur Hausen). A total of 51 participants from 19 countries attended the course; the programme coordinator was Dr N.E. Day (MRC Biostatistics Unit, Cambridge, UK). The faculty comprised Professor N.E. Breslow (University of Washington, Seattle, WA, USA), Dr D. Clayton (University of Leicester, UK), Professor D. Trichopoulos (University of Athens), Professor P.G. Smith and Dr V. Beral (London School of Hygiene and Tropical Medicine, UK), Professor J. Wahrendorf (Institute of Epidemiology and Biometry, German Cancer Research Centre, Heidelberg) and Dr J. Kaldor (IARC).

3. PUBLICATIONS [Mrs E. Heseltine (until 30 June 1986), Dr W. Davis (from 15 April 1987), Mrs M. Coudert, Mrs M.-M. Courcier, Mrs E. El Akroud, Mrs A. Romanoff and Mrs J. Thévenoux]

In November 1985, an Advisory Committee on Publications was set up, under the chairmanship of the Deputy Director, which reviews all proposals for IARC publications with regard to their relevance to Agency programmes. The manuscripts included in meetings proceedings published by the Agency now undergo peer review before publication, in order to maintain the high standards of this series.

(*a*) *New titles*

Since publication of the last *Annual Report*[2], the following publications have appeared:

Age-related Factors in Carcinogenesis (*IARC Scientific Publications No. 58*)

Interpretation of Negative Epidemiological Evidence for Carcinogenicity (*IARC Scientific Publications No. 65*)

The Role of the Registry in Cancer Control (*IARC Scientific Publications No. 66*)

Transformation Assay of Established Cell Lines: Mechanisms and Application (*IARC Scientific Publications No. 67*)

Environmental Carcinogens. Selected Methods of Analysis, Vol. 7, *Some Volatile Halogenated Hydrocarbons* (*IARC Scientific Publications No. 68*)

Directory of On-going Research in Cancer Epidemiology 1985 (*IARC Scientific Publications No. 69*)

The Role of Cyclic Nucleic Acid Adducts in Carcinogenesis and Mutagenesis (*IARC Scientific Publications No. 70*)

Environmental Carcinogens. Selected Methods of Analysis, Vol. 8, *Some Elements: As, Be, Cd, Cr, Ni, Pb, Se, Zn* (*IARC Scientific Publications No. 71*)

Atlas of Cancer in Scotland, 1975–1980. Incidence and Epidemiological Perspective (*IARC Scientific Publications No. 72*)

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[2] *IARC* (1985) *Annual Report 1985*, Lyon, pp. 148–152

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Haloethers (*IARC Scientific Publications No. 73*)

Tobacco: A Major International Health Hazard (*IARC Scientific Publications No. 74*)

Cancer Occurrence in Developing Countries (*IARC Scientific Publications No. 75*)

Screening for Cancer of the Uterine Cervix (*IARC Scientific Publications No. 76*)

Hexachlorobenzene. Proceedings of an International Symposium (*IARC Scientific Publications No. 77*)

Carcinogenicity of Alkylating Cytostatic Drugs (*IARC Scientific Publications No. 78*)

Statistical Methods in Cancer Research, Volume III: The Design and Analysis of Long-term Animal Experiments (*IARC Scientific Publications No. 79*)

Directory of On-going Research in Cancer Epidemiology 1986 (*IARC Scientific Publications No. 80*)

Long-term and Short-term Assays for Carcinogens: A Critical Appraisal (*IARC Scientific Publications No. 83*)

The Relevance of N-*Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (*IARC Scientific Publications No. 84*)

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 36, *Allyl compounds, aldehydes, epoxides and peroxides*

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 37, *Tobacco habits other than smoking; betel-quid and areca-nut chewing; and some related nitrosamines*

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 38, *Tobacco smoking*

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 39, *Some chemicals used in plastics and elastomers*

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 40, *Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation*

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 41, *Some halogenated hydrocarbons and pesticide exposures*

Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity No. 12

Directory of Computer Systems used in Cancer Registries

A full list of IARC publications is given at the back of this *Report.*

(*b*) *Publications in preparation*

The following titles are being prepared for publication or are in press:

Environmental Carcinogens: Methods of Analysis and Exposure Measurement. Vol. 9, *Passive Smoking* (*IARC Scientific Publications No. 81*)

Statistical Methods in Cancer Research. Vol. II, The Design and Analysis of Cohort Studies (*IARC Scientific Publications No. 82*)

Environmental Carcinogens. Methods of Analysis and Exposure Measurement, Vol. 10, *Benzene and Alkylated Benzenes* (*IARC Scientific Publications No. 85*)

Directory of On-going Research in Cancer Epidemiology 1987 (*IARC Scientific Publications No. 86*)

Table 52. Distribution and sales of IARC publications up to 30 June 1987

	Official distribution	Sales		Official distribution	Sales
Scientific Publications					
No. 1	799	870	No. 42	1348	648
2	882	1276	43	1566	488
3	1043	963	44	1118	398
4	1011	872	45	992	383
5	1145	1742	46	983	274
6	998	1380	47	925	170
7	1142	740	48	956	312
8	1133	1124	49	1512	403
9	1076	847	50	888	229
10	1111	1044	51	1088	504
11	1183	690	52	444	326
12	1350	1061	53	704	487
13	1062	893	54	1624	457
14	1054	727	55	1619	460
15	1101	1101	56	636	449
16	1176	816	57	689	441
17	1058	445	58	599	235
18	1093	635	59	1093	440
19	1218	605	60	704	487
20	1004	471	61	634	396
21	1412	1027	62	449	448
22	1050	498	63	671	334
23	1135	1062	64	815	441
24 part 1	946	427	65	642	490
24 part 2	947	470	66	638	450
25	1197	623	67	540	406
26	1214	411	68	587	424
27	1161	692	69	837	400
28	1017	331	70	666	154
29	1028	603	71	794	127
30 2 volumes	1246	643	72	805	193
31	1129	479	73	1463	173
32	2198	3675	74	510	181
33	1430	1555	75	603	203
34	978	607	76	584	194
35	670	445	77	617	115
36	981	366	78	541	106
37	1813	461	79	586	128
38	948	377	80	551	139
39	1267	356	83	576	86
40	1443	107	84		
41	1248	394			
Non-serial Publications					
Alcool et cancer	684	199	Information Bulletin No. 8	367	344
Cancer Morbidity and Causes of Death among Danish Brewery Workers	749	472	Information Bulletin No. 9	236	360
			Information Bulletin No. 10	451	260
			Information Bulletin No. 11	359	301
Directory of Computer Systems used in Cancer Registries	250	44	Information Bulletin No. 12	354	35

Table 52. (*Continued*)

	Official distribution	Sales		Official distribution	Sales
Monographs Series					
1	2638	2099	24	2510	1239
2	2081	2439	25	2339	1211
3	2162	2388	26	2397	1090
4	2025	2364	27	2396	1192
5	1871	2010	28	2528	1141
6	2047	2024	29	2475	1305
7	2272	1850	30	2449	983
8	2228	1823	31	2341	1060
9	2232	1659	32	2418	1470
10	2245	1832	33	2437	1123
11	2386	1530	34	2395	1064
12	2291	1701	35	2079	1101
13	2256	1505	36	1621	903
14	2461	2193	37	1697	811
15	2357	1717	38	1959	1092
16	2321	1616	39	2099	820
17	2462	1493	40	2656	102
18	2418	1574	41	2664	87
19	2389	1579	Suppl. 1	2470	1440
20	2309	1583	Suppl. 2	2645	1806
21	2355	1195	Suppl. 3	2195	922
22	2341	1259	Suppl. 4	2879	2022
23	2501	1432	Suppl. 5	1253	542

International Incidence of Childhood Cancer (*IARC Scientific Publications No. 87*)

Cancer Incidence in Five Continents, Vol. V (*IARC Scientific Publications No. 88*)

Detection Methods for DNA Damaging Agents in Man (*IARC Scientific Publications No. 89*)

Non-occupational Exposure to Mineral Fibres (*IARC Scientific Publications No. 90*)

Trends in Cancer Incidence in Singapore 1968–1982 (*IARC Scientific Publications No. 91*)

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 42, *Silica and some silicates*

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 43, *Man-made mineral fibres and radon*

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 44, *Alcohol and alcoholic beverages*

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 6, *Genetic and related effects: An updating of selected IARC Monographs from Volumes 1–42*

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7, *Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1–42*

(c) *Distribution and sales*

Up to 30 June 1987, the numbers of copies of IARC publications that had been distributed free of charge and those that had been sold were as outlined in Table 52.

(*d*) *Scientific illustrations* (Mr Déchaux and Mr G. Mollon)

Illustrations for IARC publications and for journal articles, lectures and poster presentations of the scientific staff, as well as for other purposes are prepared by a draughtsman and a photographer. Photographic work is also carried out in connection with various laboratory activities.

Annex 1

PARTICIPATING STATES AND REPRESENTATIVES AT THE TWENTY-SEVENTH SESSION OF THE IARC GOVERNING COUNCIL 29–30 April 1986

Australia

Dr D. De Souza
Deputy Secretary and Chief
Commonwealth Medical Officer
Department of Health, Woden, A.C.T.

Dr W. Langsford
Medical Director, Australian Embassy
Paris

Belgium

Dr J. Francois
Director-General
Ministry of Public Health and the Family
Brussels

Canada

Dr E. Somers (*Chairman*)
Director-General
Environmental Health Directorate
Health and Welfare Canada
Ottawa

Professor R. Simard
Scientific Director
Montreal Cancer Institute
Montreal, Quebec

Federal Republic of Germany

Mr H. Voigtländer
Director
International Health Relations Section
Federal Ministry for Youth, Family
Affairs and Health
Bonn

Finland

Professor J. Rantanen
Director-General
Institute of Occupational Health
Helsinki

Dr M. Ruokola
Director-General
National Board of Health
Helsinki

France

Dr G. Martin-Bouyer (*Vice-chairman*)
Technical Adviser to the Ministry of
Social Affairs and National Solidarity
Directorate-General for Health
Paris

Dr M. Bruaire
Technical Adviser
Ministry of Health
Paris

Mrs F. Burnol
Head, Department of Life Sciences
Responsible for Research and Higher
Education
Ministry of National Education
Paris

Professor P. Louisot
Faculty of Medicine Lyon-Sud
Oullins

Mrs M. STAKIC
Responsible for Life Sciences
Ministry of External Relations
Paris

Italy

Professor M. COLOMBINI
Director, International Relations Office
Ministry of Health
Rome

Professor F. POCCHIARI
Director-General
National Institute of Health
Rome

Professor L. SANTI
Director, Institute of Oncology
Genoa

Japan

Dr K. HASEGAWA
Director-General
Statistics and Information Department
Ministry of Health and Welfare
Tokyo

Dr H. NAKATANI
Deputy Director
International Affairs Division
Ministry of Health and Welfare
Tokyo

The Netherlands

Dr R. KROES
Director
National Institute of Public Health and Environmental Hygiene
Bilthoven

Mr A.P.M. BERSEE
Deputy Head
International Health Affairs Division
Ministry of Welfare
Public Health and Cultural Affairs
Leidschendam

Sweden

Professor H. DANIELSSON
Secretary-General
Swedish Medical Research Council
Stockholm

Union of Soviet Socialist Republics

Academician N.N. BLOKHIN
President
Academy of Medical Sciences of the USSR
Director, Cancer Research Center
Moscow

Dr T.A. SHAMARO
Senior Inspector
External Relations Board
Ministry of Health of the USSR
Moscow

United Kingdom

Sir James LEARMONTH GOWANS
Medical Research Council
London

Dr J. METTERS (*Rapporteur*)
Deputy Chief Scientist
Department of Health and Social Security
London

United States of America

Dr P.J. FISCHINGER
Deputy Director
National Cancer Institute
Bethesda, MD

Mr N.A. BOYER
Director for Health and Transportation Programs
Bureau of International Organization Affairs
Washington DC

World Health Organization

Dr H. MAHLER
Director-General

Dr LU RUSHAN
Assistant Director-General

Mr D. DEVLIN
Office of the Legal Counsel

Mr A. IMBRUGLIA
Director
Division of Budget and Finance

Dr J. STJERNSWÄRD
Chief, Cancer Unit

Observers

Professor H.J. EVANS
Outgoing Chairman, Scientific Council

Norway

Mr O.J. SANDVAND
Head of Department
Council for Medical Research
Norwegian Research Council for Science and Humanities
Oslo

PARTICIPATING STATES AND REPRESENTATIVES AT THE TWENTY-EIGHTH SESSION OF THE IARC GOVERNING COUNCIL 29–30 April 1987

Australia

Dr D. DE SOUZA
Deputy Secretary and Chief
Commonwealth Medical Officer
Commonwealth Department of Health
Woden, A.C.T.

Belgium

Dr J. FRANCOIS
Director-General
Ministry of Public Health and the Family
Brussels

Canada

Dr E. SOMERS (*Chairman*)
Director-General
Environmental Health Directorate
Health and Welfare Canada
Ottawa

Dr P. BOIS
Medical Research Council of Canada
Ottawa

Federal Republic of Germany

Mr H. VOIGTLÄNDER
Director
International Health Relations Section
Federal Ministry for Youth, Family Affairs, Women and Health
Bonn

Finland

Dr M. RUOKOLA
Director-General
National Board of Health
Helsinki

Ms A. VUORINEN
First Secretary
Permanent Mission of Finland to the United Nations Office and other International Organizations at Geneva
Geneva

France

Professor J.-F GIRARD
Director-General
Directorate-General for Health
Paris

Ms A. DERLICH
Under-Secretary for the Budget
and Financial Affairs
Paris

Mr J.-C. LECLERC
Ministry of Foreign Affairs
Paris

Mr R. LECLERC
Ministry of Economy, Finance
and Privatization
Paris

Professor P. LOUISOT
Faculty of Medicine Lyon-Sud
Oullins

Italy

Professor M. COLOMBINI
Director, International Relations Office
Ministry of Health
Rome

Professor F. POCCHIARI
Director-General
National Institute of Health
Rome

Japan

Dr K. FURUICHI
Director-General
Statistics and Information Department
International Affairs Division
Ministry of Health and Welfare
Tokyo

Dr H. NAKATANI
Deputy Director
International Affairs Division
Ministry of Health and Welfare
Tokyo

Dr T. HASEGAWA (*Rapporteur*)
Chief, Planning Office
National Cancer Center
International Affairs Division
Ministry of Health and Welfare
Tokyo

The Netherlands

Dr R. KROES
Director
National Institute of Public Health and
Environmental Hygiene
Bilthoven

Mr F.H. DE MAN
Deputy Head
International Health Affairs Division
Ministry of Welfare
Health and Cultural Affairs
Rijswijk

Norway

Dr B.M. AASEN
State Secretary
Department of Health and Social Affairs
Oslo

Dr C. LYCHE
Head of Division
Ministry of Scientific and Cultural Affairs
Oslo

Professor H. PIENE
Vice-President
Medical Research Council of Norway
Oslo

Mr O.J. SANDVAND
Director
Medical Research Council of Norway
Oslo

Sweden

Professor H. DANIELSSON (*Vice-chairman*)
Secretary-General
Swedish Medical Research Council
Stockholm

Union of Soviet Socialist Republics

Academician N.N. BLOKHIN
President
Academy of Medical Sciences of the
USSR
Director, Cancer Research Center
Moscow

Dr T.A. SHAMARO
Senior Inspector
External Relations Board
Ministry of Health of the USSR
Moscow

United Kingdom

Sir James LEARMONTH GOWANS
Medical Research Council
London

Dr J. METTERS
Deputy Chief Scientist
Department of Health and Social Security
London

Mr A.J. VITTERY
Medical Research Council
London

United States of America

Dr I.J. MASNYK
Acting Associate Director for International Affairs
National Cancer Institute
Bethesda, MD

Mr N.A. BOYER
Director for Health and Transportation Programs
Bureau of International Organization Affairs
Washington DC

World Health Organization

Dr LU RUSHAN
Assistant Director-General

Mr D. DEVLIN
Office of the Legal Counsel

Mr A. IMBRUGLIA
Director
Division of Budget and Finance

Dr E. SHIGAN
Director
Division of Noncommunicable Diseases

Dr J. STJERNSWÄRD
Chief, Cancer Unit

Observers

Professor B.K. ARMSTRONG
Outgoing Chairman, Scientific Council

Dr P. SELBY
Executive Director, UICC

Professor R. SIMARD
Incoming Chairman, Scientific Council

Annex 2

MEMBERS OF THE IARC SCIENTIFIC COUNCIL AT ITS TWENTY-SECOND SESSION, 27–30 JANUARY 1986

Professor H.J. EVANS (*Chairman*)
Director
Medical Research Council Clinical and Population Cytogenetics Unit
Western General Hospital
Edinburgh, Scotland
UK

Dr R. KROES (*Vice-chairman*)
Director
National Institute of Public Health and Environmental Hygiene
Bilthoven
The Netherlands

Dr B.K. ARMSTRONG[1] (*Rapporteur*)
University of Washington
School of Public Health and Community Medicine
Seattle, WA
USA

Professor L.L. GRICIUTE
Director
Cancer Research Institute of Lithuania
Vilnius
USSR

Dr B.E. HENDERSON
Director, Norris Cancer Center
University of South California
Los Angeles, CA
USA

Professor D. HENSCHLER
Director
Pharmacology and Toxicology Institute
Department of Medicine
Bayerische Julius Maximilians University
Würzburg
Federal Republic of Germany

Professor T. MATSUSHIMA
Professor and Chairman
Department of Molecular Oncology
Institute of Medical Science
University of Tokyo
Japan

Professor R. MONIER
Director
Laboratory of Molecular Oncology
Gustave Roussy Institute
Villejuif
France

Professor J. PONTÉN
Professor and Chairman
Department of Pathology
University of Uppsala
Sweden

Professor R. SIMARD
Scientific Director
Cancer Institute of Montreal
Montreal, Quebec
Canada

Professor B. TERRACINI
Professor of Cancer Epidemiology
Institute of Morbid Anatomy
University of Turin
Italy

[1] As from July 1986: Director, National Health and Medical Research Council, Research Unit in Epidemiology and Preventive Medicine, University of Western Australia, Nedlands, WA, Australia

Professor A. WAMBERSIE
Unit of Radiobiology and
Radioprotection
Catholic University of Louvain
Faculty of Medicine
Brussels

Observer

UICC represenative:

Professor R. FLAMANT
Chief, Medical Statistics Department
Gustave Roussy Institute
Villejuif
France

World Health Organization

Dr V. GRABAUSKAS
Director
Division of Noncommunicable Diseases

Dr LU RUSHAN
Assistant Director-General

Dr J. STJERNSWÄRD
Chief, Cancer Unit

MEMBERS OF THE IARC SCIENTIFIC COUNCIL AT ITS TWENTY-THIRD SESSION, 12–15 JANUARY 1987

Dr B.K. ARMSTRONG (*Chairman*)
Director and Professor
National Health and Medical Research
Council Research Unit in Epidemiology
and Preventive Medicine
Department of Medicine
University of Western Australia
Nedlands, WA
Australia

Professor D. HENSCHLER (*Vice-chairman*)
Director
Pharmacology and Toxicology Institute
Bayerische Julius Maximilians University
Würzburg
Federal Republic of Germany

Professor F. DE WAARD
Head, Department of Epidemiology
National Institute of Public Health
and Environmental Hygiene
Bilthoven
The Netherlands

Professor L. GRICIUTE
Director
Cancer Research Institute of Lithuania
Vilnius
USSR

Professor T. MATSUSHIMA
Professor and Chairman
Department of Molecular Oncology
Institute of Medical Science
University of Tokyo
Japan

Professor R. MONIER
Director
Laboratory of Molecular Oncology
Gustave Roussy Institute
Villejuif
France

Professor J. PONTÉN
Professor and Chairman
Department of Pathology
University of Uppsala
Sweden

Professor R. SIMARD (*Rapporteur*)
Vice-Rector for Studies
University of Montreal
Montreal, Quebec
Canada

Professor B. TERRACINI
Professor of Cancer Epidemiology
Department of Biomedical Science and Human Oncology
University of Turin
Italy

Professor A. WAMBERSIE
Unit of Radiobiology and Radioprotection
Catholic University of Louvain
Faculty of Medicine
Brussels

World Health Organization

Dr K. STANLEY
Cancer Unit

Advisers

Professor E. SAKSELA
Professor and Chairman
Department of Pathology
University of Helsinki
Finland

Dr P.G. SMITH
Reader, Tropical Epidemiology Unit
London School of Hygiene and Tropical Medicine
London
UK

Annex 3

STAFF AT IARC
1 July 1985 – 30 June 1987

Office of the Director

Director	Dr L. TOMATIS
Deputy Director	Dr C.S. MUIR (from 1 November 1985)
Scientists	Dr G.T. O'CONOR (until 30 April 1986) Dr V. TURUSOV (from 1 September 1987)
Adviser (Scientist)	Dr G. MARTIN-BOUYER (from 1 January 1987)
Administrative Assistants	Mr C. AUGROS Mrs M. DAVIS Mrs A. GESER Mrs E. RIVIERE
Secretaries	Mrs C. DECHAUX Mrs W. FEVRE-HLAHOLUK Mrs L. NEYRET (until 10 May 1987)

Gambia Hepatitis Intervention Study

Project Leader	Dr A. HALL (from 17 January 1986)
Medical Officer	Dr F. LOIK (from 29 May 1986)
Statistician Programmer	Dr H. INSKIP (from 1 February 1986)

Editorial, Translation and Publication Services

Head, Editorial and Publications Services	Mrs E. HESELTINE (until 30 June 1986)
Translator	Mr Y. POLLET (until 30 April 1986) Miss M.-C. GRAN (from 14 September 1986)
Technical Assistant (Search Analyst)	Mrs M. COUDERT
Laboratory Technician (Photography)	Mr G. MOLLON
Secretaries	Mrs J. BAILLY Mrs E. EL AKROUD
Clerks	Mrs M.-M. COURCIER Mr J. DECHAUX Mrs A. ROMANOFF (from 1 June 1987) Mrs J. THEVENOUX

Education and Training	
Chairman, Fellowships Selection Committee	Dr R. Montesano
Administrative Assistant	Mrs M. Davis
Secretary	Mrs C. Dechaux
Library	
Librarian	Mrs A. Nagy-Tiborcz
Technical Officer (Information Systems)	Miss H. Miido
Clerk	Mrs L. Ossetian

Division of Scientific Activities

Unit of Analytical Epidemiology	
Chief	Dr R. Saracci
Scientists	Dr P. Boyle Dr G. Engholm (until 31 October 1985) Mr A. Fletcher (until 31 December 1986) Dr E. Johnson Dr E. Riboli Dr L. Simonato Dr J.P. Velema (until 30 March 1987) Dr D. Zaridze (until 31 October 1985)
Programmer/Statistician	Miss M. Blettner (until 31 December 1986)
Assistants (Statistics)	Ms G. Brunod (from 1 September 1985 — half-time) Miss R. Winkelmann
Clerk (Statistics)	Miss S. Seuchter (until 12 December 1986)
Technical Clerk	Mrs J. Lavellee-Hawken (until 31 January 1987)
Secretaries	Mrs S. Dartoy Miss A. Shannon Mrs S. Stallard Mrs A. Zitouni
Unit of Biostatistics Research and Informatics	
Chief	Dr N.E. Day (until 17 September 1986) Dr J. Esteve (from 1 January 1987)
Scientists	Dr J. Esteve (until 31 December 1986) Dr J. Kaldor Dr J. Wahrendorf (until 31 March 1986)
Computer Systems Manager	Mr M. Smans (from 1 October 1986)
Computer analysts	Ms B. Charnay Mr P. Damiecki Mr X. Nguyen-Dinh

Assistants (Statistics)	Mrs A. Arslan Miss D. Magnin
Secretaries	Mrs H. Biehe (from 13 April 1987) Miss J. Hawkins (until 20 February 1987) Mrs A. Rivoire
Clerk (Computer Operator)	Mr M. Jaboulin
Clerk	Mrs B. Kajo

Unit of Field and Intervention Studies

Chief	Dr N. Muñoz
Scientist	Dr F.X. Bosch (from 6 July 1986)
Secretaries	Mrs K. Essoulami (until 31 August 1985) Mrs K. Zouhair (from 1 September 1985)

Unit of Descriptive Epidemiology

Chief	Dr C.S. Muir (until 31 December 1985) Dr D.M. Parkin (from 1 March 1986)
Scientist	Dr D.M. Parkin (until 28 February 1986)
Assistants (Statistics)	Mr A. Bieber Miss F. Casset (from 1 September 1985) Mr M. Smans (until 30 September 1986)
Technical Assistants	Mrs E. Demaret Mrs J. Nectoux Miss S. Whelan
Secretaries	Miss O. Bouvy Miss A.-M. Corre Mrs C.F. Petit (half-time) Mrs A. Romanoff (until 31 May 1987)

Unit of Environmental Carcinogens and Host Factors

Chief	Dr H. Bartsch
Scientists	Dr M. Ahotupa Dr M. Castegnaro Dr M. Friesen Dr C. Malaveille Dr. I. O'Neill Mr H. Ohshima Dr P. Pignatelli
Laboratory Research Assistants	Mr A. Barbin Mrs G. Brun Miss A.-M. Camus Mrs L. Garren
Laboratory Technicians	Mr J.-C. Bereziat Miss M.-C. Bourgade (until 10 October 1986)

	Mrs I. Brouet
	Mrs A. Hautefeuille
	Miss J. Michelon
	Miss I. Richard (from 1 June 1987)
Secretaries	Mrs M.-B. D'Arcy
	Miss Y. Granjard
	Mrs Z. Schneider
	Mrs M. Wrisez
Unit of Mechanisms of Carcinogenesis	
Chief	Dr R. Montesano
Scientists	Dr J.P. Cabral
	Dr C. Drevon
	Dr K. Enomoto (from 26 April 1986)
	Dr D. Fitzgerald (from 17 May 1986)
	Dr V. Gurtsevitch
	Dr M. Hollstein
	Dr G. Lenoir
	Dr N. Mironov (from 8 February 1987)
	Dr B. Sylla (from 13 July 1986)
	Dr H. Yamasaki
Technical Assistant	Miss C. Bonnardel
Laboratory Research Assistants	Mrs A.-M. Aguelon-Pegouries
	Miss H. Bresil
	Mr F. Katoh (from 30 March 1986)
	Miss M. Laval
	Mrs M.-F. Lavoue
	Mrs G. Martel-Planche
	Mrs M. Vuillaume
Laboratory Technicians	Miss B. Chapot
	Miss M.-P. Desvaux
	Mrs G. Galendo
	Mr J. Garcia
	Mrs N. Lyandrat
	Miss N. Martel
	Mrs S. Pauly
	Mrs C. Piccoli
Secretaries	Mrs P. Collard-Bianchi
	Mrs C. Fuchez
	Mrs E. Perez (from 9 September 1985 — half-time)
Laboratory Aides	Mr J. Cardia-Lima
	Mr R. Dray
	Mrs M. Essertel
	Mr F. Faria
	Mrs N. Farina
	Miss M. Maranhao
	Mrs S. Veyre

Unit of Carcinogens Identification and Evaluation	
Chief	Dr H. VAINIO (Leave without pay from 1 April 1987)
Scientists	Mrs L. HAROUN Dr L. SHUKER Dr A. TOSSAVAINEN Mr J. WILBOURN
Technical Officers (Bibliographic Research)	Mr C. PARTENSKY Mrs M.-J. PETERSCHMITT
Technical Assistants	Mrs J. CAZEAUX Mrs M.-J. GHESS Mrs D. MIETTON
Secretaries	Mrs M. LEZERE Mrs M. MAINAUD (from 9 September 1985 — half time) Miss S. REYNAUD Mrs K. ZOUHAIR (until 31 August 1985)
Division of Administration and Finance	
Director	Mr K. SAITA (until 30 April 1987) Mr E. WESTENBERGER (from 1 May 1987)
Secretary	Mrs J. MARTINEZ
Personnel	
Assistant	Mrs A. ESCOFFIER
Clerk-stenographer	Mrs A.-M. MAILLOL (from 11 May 1987)
Budget and Finance	
Budget and Finance Officer	Mr M. JOHNSON
Finance Officer	Mr G. DALSTON (until 31 August 1986) Mr S. SAPRA (from 25 February 1987)
Assistant (Accounting)	Mrs M. HERIN
Assistant (Payments)	Mrs F. ROMAGNAN
Secretary	Mrs D. MARCOU-HANSSON
Clerk (Cashier)	Dr D. HORNEZ
Clerks (Finance)	Mrs D. LOMBARDO Mrs F. FLORENTIN (half-time)
Administrative Services	
Administrative Services Officer	Mr B. BORGSTROM
Administrative Assistant	Mrs R. SEXTIER
Switchboard Operator	Mrs R. KIBRISLIYAN
Driver	Mr J.-F. DURAND-GRATIAN
Usher (Messenger)	Mr M. JAVIN (until 28 February 1986) Mr W. CHEMOUL (from 1 February 1986 to 31 January 1987) Mr D. LAGARDE (from 13 April 1987)

Assistant (Building Maintenance)	Mr E. CATHY
Maintenance Technicians	Mr P. BARBIEUX
	Mr M. BAZIN
	Mr J.-P. BONNEFOND
	Mr G. THOLLY
Assistant (Registry)	Mrs M.-H. CHARRIER
Clerks	Mrs M. GREENLAND (half-time)
	Mrs M. MAINAUD (until 8 September 1985)
	Mrs E. PEREZ (until 8 September 1985)
	Mrs L. VIGIER (from 6 January 1986)
Assistant (Supplies)	Mrs J. POPOFF
Clerks	Mrs A. TROCHARD
	Mrs L. GRAVIER (half-time)
Storekeeper	Mr M. PRAT
Equipment Operators (Reproduction)	Mr K. AMIR (until 30 November 1986)
	Mr D. GRAIZELY
	Mr M. JAVIN (from 1 March 1986)

Documents and Stenographic Pool

Assistant	Mrs J. BORGSTROM
Clerk	Miss M. GEESINK
Clerk-stenographers	Miss S. ANTHONY
	Miss S. COTTERELL
	Mrs A.-M. MAILLOL (until 10 May 1987)
	Mrs L. NEYRET (from 11 May 1987)
	Miss J. NYAIRO (from 1 June 1986)

SHORT-TERM EMPLOYMENTS (CONSULTANTS AND TEMPORARY STAFF) 1 July 1985 – 30 June 1987

Office of the Director

Consultants	Mr P. DUNDERDALE*
	Dr A.B. MILLER
	Mrs M. DE MONLEON
	Dr G.T. O'CONOR
	Professor R. SOHIER*
	Dr V. TURUSOV*
Social Adviser	Mrs P. MALINDINE* (part-time)
Clerk-stenographer	Mrs A.-C. MORET* (part-time)

* Still on short-term employment on 30 June 1987

Education and Training and Editorial, Translation and Publications Services

Consultants	Dr W. Davis* Mr J. Stares

Division of Scientific Activities

Unit of Analytical Epidemiology

Consultants	Dr G. Engholm Mr G. Macfarlane* Dr A. Walker
Assistants (Statistics)	Mrs M. Charrel* (half-time) Mr P. Maisonneuve* (part-time)

Unit of Biostatistics Research and Informatics

Consultants	Dr B. Bjelke Dr E. Cardis* Mr S.W. Duffy Dr M. Hayashi Dr B. McKnight Dr P. Rosa

Unit of Field and Intervention Studies

Consultants	Dr F.X. Bosch Dr H. Casas-Cordero* Dr P. Correa
Laboratory Technician	Mrs F. Ciroussel

Unit of Descriptive Epidemiology

Consultants	Mr P. Delfosse Dr R. Gurevicius Ms A. Malhotra
Assistant (Statistics)	Mr P. Maisonneuve* (part-time)

Unit of Environmental Carcinogens and Host Factors

Laboratory Technician	Mrs F. Ciroussel
Technical Officer (Bibliographic Research)	Mrs E. Dodet* (part-time)
Laboratory Technicians	Miss F. El Ghissassi* Mrs A. Ellul*
Scientist	Dr A. Povey*

Unit of Mechanisms of Carcinogenesis

Consultants	Dr A. Lyubimov Dr J.M. Vasiliev

* Still on short-term employment on 30 June 1987

Laboratory Technicians	Miss J. Bonnet Miss V. Valverde
Research Laboratory Assistant	Mr F. Katoh
Laboratory Aides	Mrs Y. Delzoppo* Miss V. Desvaux A. Munia Miss S. Ottone
Unit of Carcinogens Identification and Evaluation	
Consultants	Dr A. Aitio Dr K. Hemminki
Technical Officer (Bibliographic Research)	Mrs E. Dodet* (part-time)
Clerks	Mrs J. Atherton* (half-time) Mrs M. Lepetit* (half-time) Mr T. LeMinh* (part-time)

Division of Administration and Finance

Budget and Finance	
Consultant	Mr P. Dunderdale
Clerks	Miss H. George (half-time) Miss A. Milone* (half-time)
Administrative Services	
Consultants	Dr W. Davis (part-time) Dr A. Geser Mr A. Sayour
Switchboard Operator	Mrs S. Magenties
Documents and Stenographic Pool	
Clerk-stenographers	Miss H. Biehe Miss A. Cousseau* Miss A. Dufournet* Mrs L. Neyret Miss J. Nyairo

Annex 4

VISITING SCIENTISTS, FELLOWS AND TRAINEES AT IARC 1 July 1985 – 30 June 1987

Visiting Scientists

Dr E. Cardis, Unit of Biostatistics Research and Informatics (from 20 November 1986)

Dr K. Chan, Unit of Environmental Carcinogens and Host Factors (20 May–3 June 1986)

Dr K. Hooper, Unit of Carcinogen Identification and Evaluation (16–27 September 1985)

Dr A.G. Knudson, Visiting Professor Award from the General Motors Cancer Research Foundation (25–29 May 1987)

Miss N. La Verda, Unit of Descriptive Epidemiology (July–August 1985)

Dr D. Ruiz Lopez, Unit of Environmental Carcinogens and Host Factors (February–April 1987)

Dr M. Serres, Unit of Mechanisms of Carcinogenesis (from November 1985)

Miss K. Vo Thi, Unit of Environmental Carcinogens and Host Factors (23–31 October 1985 and 8–12 April 1986)

Dr Shao Yi Ming, Unit of Environmental Carcinogens and Host Factors (August 1986–June 1987)

Fellows

Mr P. Arvela, Unit of Environmental Carcinogens and Host Factors, Fellowship from the Centre National de Recherche Scientifique (CNRS) (20 May–20 October 1985) and Fellowship from the Academy of Finland (March–April 1987)

Dr R. Becker, Unit of Mechanisms of Carcinogenesis, Fellowship from US National Cancer Institute (until December 1985)

Mr M. Billaud, Unit of Mechanisms of Carcinogenesis, Fellowship from the Association pour la Recherche sur le Cancer (from August 1986)

Mrs S. Calmels-Rouffet, Unit of Environmental Carcinogens and Host Factors, Fellowship from the Ligue Nationale contre le Cancer

Miss M. Cordier, Unit of Mechanisms of Carcinogenesis (from 9 September 1985), Fellowship from the Ligue Nationale Française pour la Recherche sur le Cancer (from January 1987)

Dr P. Degan, Unit of Mechanisms of Carcinogenesis, IARC Research Training Fellow (from 16 July 1986)

Dr U. Frixen, Unit of Mechanisms of Carcinogenesis, IARC Research Training Fellow (until February 1986)

Miss L. Giroldi, Unit of Mechanisms of Carcinogenesis, Fellowship from the Ligue Nationale Française contre le Cancer

Dr E. Hamel, Unit of Mechanisms of Carcinogenesis, Fellowship from the Fondation Mérieux

Dr D. Huang, Unit of Mechanisms of Carcinogenesis, Fellowship from the International Cancer Research Technology Transfer Programme (ICRETT) (October–December 1985)

Dr R. Klann, Unit of Mechanisms of Carcinogenesis, Fellowship from the International Cancer Research Technology Transfer Programme (ICRETT) (15 September 1986–15 October 1986)

Dr S.A. Kyrtopoulos, Unit of Environmental Carcinogens and Host Factors, Fellowship from European Science Foundation (April 1987)

Mr M. Mesnil, Unit of Mechanisms of Carcinogenesis, Fellowship from the Association pour la Recherche sur le Cancer

Dr C. Mutiro, Unit of Mechanisms of Carcinogenesis, Fellowship from the International Cancer Research Technology Transfer Programme (ICRETT) (May 1986)

Dr J. Nair, Unit of Environmental Carcinogens and Host Factors, IARC Research Training Award (until December 1985)

Dr D. Neal, Unit of Mechanisms of Carcinogenesis, Fellowship from the International Cancer Research Technology Transfer Programme (ICRETT) (21 July–8 August 1986)

Dr J.O. Nwanko, Unit of Environmental Carcinogens and Host Factors, Fellowship from the International Cancer Research Technology Transfer Programme (ICRETT) (September–October 1986)

Dr A. Pinter, Unit of Mechanisms of Carcinogenesis, Fellowship from the International Cancer Research Technology Transfer Programme (ICRETT) (March 1986)

Dr R. Sierra, Unit of Descriptive Epidemiology, Fellowship from the International Cancer Research Technology Transfer Programme (ICRETT) (January 1987)

Dr D. Shuker, Unit of Environmental Carcinogens and Host Factors, Royal Society Award (from May 1986)

Dr J.L. Wang, Unit of Mechanisms of Carcinogenesis, Fellowship from the International Cancer Research Technology Transfer Programme (ICRETT) (April–May 1986)

Miss Q. Wang, Unit of Mechanisms of Carcinogenesis, Fellowship from the Foundation Mérieux (from November 1985)

Dr C.P. Wild, Unit of Mechanisms of Carcinogenesis, IARC Research Training Fellow (until August 1986)

Trainees

Mr L. Broussolle, Unit of Environmental Carcinogens and Host Factors (March–July 1986)

Mrs V. Bussacchini-Griot, Unit of Environmental Carcinogens and Host Factors, Special Training Award (until March 1987)

Mr A. Calender, Unit of Mechanisms of Carcinogenesis, Special Training Award (from 1 June 1985)

Miss S. Calmels, Unit of Environmental Carcinogens and Host Factors (until November 1985)

Miss F. Casset, Unit of Descriptive Epidemiology (July–August 1985)

Mrs F. Ciroussel, Unit of Environmental Carcinogens and Host Factors, Special Training Award (from November 1986)

Mr J. Delzoppo, Unit of Biostatistics Research and Informatics (14 October–31 December 1985)

Miss B. Fischer, Unit of Descriptive Epidemiology (November 1986–June 1987)

Mr A. Guljas, Unit of Descriptive Epidemiology (February–May 1986)

Dr G. Maru, Unit of Environmental Carcinogens an Host Factors, Special Training Award (from March 1987)

Mrs V. Maru, Unit of Environmental Carcinogens and Host Factors, Special Training Award (from June 1987)

Mr P. Montagnier, Unit of Biostatistics Research and Informatics (20 May–20 August 1986)

Dr U. Nair, Unit of Environmental Carcinogens and Host Factors, Special Training Award (July–November 1985)

Miss R. Osowale, Unit of Environmental Carcinogens and Host Factors, Special Training Award (March 1986)

Miss S. Ottone, Unit of Mechanisms of Carcinogenesis (June 1986)

Dr F. Pionneau, Unit of Mechanisms of Carcinogenesis, Special Training Award (September 1985–August 1986)

Dr D. Piskorska, Unit of Environmental Carcinogens and Host Factors, Special Training Award (until July 1985)

Miss S. Poirier, Unit of Environmental Carcinogens and Host Factors, Fellowship from the Ligue Nationale Française contre le Cancer

Dr A. Povey, Unit of Environmental Carcinogens and Host Factors, Special Training Award (August 1985–January 1987)

Dr A. Rahimtula, Unit of Environmental Carcinogens and Host Factors, Special Training Award (from September 1986)

Miss I. Richard, Unit of Environmental Carcinogens and Host Factors (March–July 1986 and November 1986–May 1987)

Dr D. Ririe, Unit of Mechanisms of Carcinogenesis (July–October 1985)

Mr H. Sobol, Unit of Mechanisms of Carcinogenesis (from 1 November 1986)

Miss B. Verpillieux, Unit of Environmental Carcinogens and Host Factors, Special Training Award (from June 1987)

Mr P. Ziegler, Unit of Environmental Carcinogens and Host Factors, Special Training Award (March–April 1986), Unit of Descriptive Epidemiology (May–August 1986)

Annex 5

RESEARCH AGREEMENTS IN OPERATION BETWEEN IARC AND VARIOUS INSTITUTIONS
1 July 1985 – 30 June 1987

Collaborating centres

DEB/74/03	Institute for Documentation, Information and Statistics, German Cancer Research Centre, Heidelberg, Federal Republic of Germany (Clearing-house for on-going research in cancer epidemiology)
DEB/81/19	Regina Elena Institute for the Study and Therapy of Tumours, Rome (Reference centre for the epidemiology of precancerous lesions and environmental carcinogens)
DEB/83/28	University Hospital of Vaud, Lausanne, Switzerland (Multinational study on the epidemiology of lymphoid neoplasia)
DEB/85/27	All-India Institute of Medical Sciences, New Delhi (Multinational study on the epidemiology of lymphoid neoplasia)
DEB/85/28	National Institute of Cancer, Lima (Multinational study on the epidemiology of lymphoid neoplasia)
DEB/85/29	Armed Forces Institute of Pathology, Rawalpindi, Pakistan (Multinational study on the epidemiology of lymphoid neoplasia)
DEB/85/30	Kyoto University Hospital, Kyoto, Japan (Multinational study on the epidemiology of lymphoid neoplasia)
DEB/85/31	Aichi Cancer Center Hospital, Nagoya, Japan (Multinational study on the epidemiology of lymphoid neoplasia)

Cancer registries/Incidence studies

DEB/73/16	International Association of Cancer Registries (Provision of a secretariat and other supporting services)
DEB/81/28	Danish Cancer Registry, Copenhagen (Case-control study of cervical cancer patients in Denmark, to assess the risk of developing second primary tumours, other than leukaemia, in patients exposed to radiation)
DEB/83/09	Cancer Registry, Central Institute for Cancer Research, Berlin (Long-term carcinogenic hazards of chemotherapy treatment for cancer)
DEB/83/17	Cancer Registry, Central Institute for Cancer Research, Berlin (Preparation of cancer incidence atlas of the German Democratic Republic)

DEB/85/07	Department of Mathematics, University of Namur, Namur, Belgium (Time trends in cancer incidence and mortality)
DEB/85/09	Osaka Cancer Registry, Department of Field Research, Center for Adult Diseases, Osaka, Japan (Improvement of Death Certificate Only (DCO) and Histologically Verified (HV) indices in Japan)
DEB/85/32	Ministry of Health, Harare (Cancer registry of Harare)
DEB/85/35	Department of Epidemiology, London School of Hygiene and Tropical Medicine, London (Case-control studies of second malignancies in relation to cytotoxic therapy)
DEB/85/37	All-Union Cancer Research Centre of the USSR, Academy of Medical Sciences, Moscow (Descriptive epidemiology of cancer in the USSR)
DEB/85/41	Department of Anatomo-pathology, Faculty of Medicine, University of Rwanda, Butare (Establishment of a cancer registry)
DEB/85/42	Minstry of Health, Suva (Provision of a cancer registry service for the Fiji Islands)
DEB/85/46	Danish Cancer Registry, Copenhagen (Case-control studies of second malignancies in relation to cytotoxic therapy)
DEB/85/47	Cancer Control Agency of British Columbia, Vancouver, Canada (Case-control studies of second malignancies in relation to cytotoxic therapy)
DEB/85/48	Birmingham Cancer Registry, Birmingham, UK (Case-control studies of second malignancies in relation to cytotoxic therapy)
DEB/85/49	Cross Cancer Institute, Edmonton, Alberta, Canada (Case-control studies of second malignancies in relation to cytotoxic therapy)
DEB/85/51	Ontario Cancer Treatment and Research Foundation, Toronto, Canada (Case-control studies of second malignancies in relation to cytotoxic therapy)
DEB/85/52	Finnish Cancer Registry, Helsinki (Case-control studies of second malignancies in relation to cytotoxic therapy)
DEB/85/54	Department of Gynaecological Oncology, Karolinska Hospital, Stockholm (Case-control studies of second malignancies in relation to cytotoxic therapy)

BRI/87/01	Norwegian Cancer Registry, Oslo (Case-control studies of second malignancies in relation to cytotoxic therapy)
BRI/87/02	Department of Radiation Physics, University of Texas Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, TX, USA (Radiation dosimetry for cases and controls enrolled in the IARC international study of second malignancies in relation to cytotoxic therapy)
BRI/87/03	Cancer Institute of the Netherlands, Amsterdam (Case-control study of second leukaemia and myelodysplasia after Hodgkin's disease)
DEP/87/02	National Institute of Public Health, Bamako (Cancer registry of Mali)

Studies on cancers linked with herpesviruses

DEC/83/09	Laboratoire de Cytogenetique, Centre de Transfusion Sanguine, St Etienne, France (Characterization of cytogenetic anomalies observed in Burkitt-type lymphoma cells)
MCA/87/01	Cancer Research Center, Academy of Medical Sciences of the USSR, Moscow (Prevalence of anti-HLV-I antibodies in the population of the USSR from different geographic areas)

Studies on liver cancer

DEP/79/21	Department of Social Medicine and Public Health, University of Singapore (Cohort study on hepatitis B carriers and liver cancer)
DEB/84/10	Hadassah School of Public Health, Hebrew University, Jerusalem (Collaborative investigation of serum indicators of subsequent cancer risk)
DEB/85/23	Public Health Department of Barcelona, Barcelona, Spain (Epidemiological study on liver cancer in Catalonia)
DEB/86/12	National cancer Institute, Bangkok (Study of etiological factors for liver cancer in Thailand)
FIS/87/01	National Cancer Institute, Bangkok (Cohort study of HBsAg carriers in Bangkok)
DIR/86/01	Medical Reserach Council, London (Gambia Hepatitis Intervention Study)

Studies on nutrition and on cancer of the gastrointestinal tract

DEC/81/04	Leatherhead Food Research Assocation, Leatherhead, UK (Determination of total *N*-nitroso compounds in the gastric juice of patients with precancerous lesions)

DEB/81/40	Regina Elena Institute for the Study and Therapy of Tumours, Rome (Case-control study of adenomatous polyps of the large bowel)
DEB/81/41	Public Health laboratory Service, Centre for Applied Microbiology and Research, Salisbury, UK (Analysis of faeces and urine samples from the case-control study of adenomatous polyps of the large bowel in Rome)
DEB/83/14	Clinical Investigation Unit, Dudley Road Hospital, Birmingham, UK (Biochemical analyses of blood samples collected during a screening survey of precancerous lesions of oral cavity and oesophagus in Samarkand region, USSR)
DEB/83/16	All-Union Cancer Research Center of the USSR, Academy of Medical Sciences, Moscow (Chemopreventive trial of precancerous lesions of the mouth and oesophagus in Uzbek SSR (USSR))
DEB/83/21	Icelandic Cancer Registry, Reykjavik (Nutrition and breast cancer in Iceland)
DEB/84/01	Singapore Cancer Registry, Department of Pathology, University of Singapore, Singapore (Development of methodology for the conduct of diet-directed case-control studies in Singapore)
DEB/84/13	Central Institute of Cardiovascular Research, Academy of Sciences, Berlin (Feasibility investigation of dietary information)
DEC/85/01	Unit of Epidemiology, Curie-Sklodowska Institute of Oncology, Warsaw (Study on the endogenous formation of *N*-nitroso compounds and nutritional status in high- and low risk subjects for stomach cancer in Poland)
DEB/85/05	Department of Clinical Chemistry, University Hospital, University of Lund, Lund, Sweden (Chemical stability of nutrients in body fluids)
DEB/85/08	Department of Social Medicine, Faculty of Medicine, Federal University of Pelotas, Pelotas, Brazil (Oesophageal cancer in Rio grande do Sul, Brazil)
DEB/85/24	Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA (Identification of mutagenic agents in hot tea extracts)
DEB/85/25	Department of Epidemiology and Prevention of Cardio-vascular Diseases, Institute of Cardiology, Warsaw (Feasibility investigation of dietary information)
DEB/85/26	Department of Clinical Chemistry, Clinic of Metabolic Diseases, University of Krakow, Krakow, Poland (Feasibility investigation of dietary information)

DEB/85/36	Medical Clinic for Gastroenterology, University Clinical Centre, Ljubljana, Yugoslavia (Precancerous lesions of the stomach in Slovenia)
DEB/85/43	Department of Medical Nutrition of the Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden (Statistical analysis of the methological study on dietary assessment and related biochemical parameters, conducted in Malmö, Sweden)
DEB/85/44	Department of Pathology, Faculty of Medicine, National University, Montevideo (Case-control study of oesophageal cancer in Uruguay)
DEB/85/45	Faculty of Medicine, National University, La Plata, Argentina (Case-control study on oesophageal cancer in La Plata, Argentina)
DEB/86/02	Department of Medicine C, Glostrup Hospital, Glostrup, Denmark (Feasibility investigation of dietary information)
DEB/86/05	Institute for Documentation, Information and Statistics, German Cancer Research Centre, Heidelberg, Federal Republic of Germany (Pilot study to evaluate the feasibility of a case-control study to evaluate the effectivness of screening for colo-rectal cancer by means of testing for faecal occult blood)
DEB/86/13	Study Group on Colorectal Cancer, Academy of Medical Sciences of Catalonia and Baleares, Majorca, Spain (Case-control study of colorectal cancer in Majorca)
ECH/87/02	Institut Pasteur, Lyon, France (Analysis of gastric bacterial flora in patients with precancerous lesions of the stomach)

Studies on occupational cancer

DEB/84/14	Department of Community Health, Clinical School of Medicine, Wellington Hospital, University of Otago, Wellington (Establishment and maintenance of an international register of persons exposed to phenoxy acid herbicides and contaminants)
DEB/85/06	Department of Epidemiology, National Institute of Public Health, Bilthoven, The Netherlands (Establishment and maintenance of an international register of pesons exposed to phenoxy acid herbicides and contaminants)
DEB/85/10	Unit of Epidemiology, Faculty of Medicine, University of Melbourne, Melbourne, Australia (Establishment and maintenance of an international register of persons exposed to phenoxy acid herbicides and contaminants)
DEB/85/20	National Council of Scientific and Technical Investigations, Buenos Aires (Effect of nitrosatable pesticides on peripheral human lymphocytes)

DEB/85/21	Department of Community Medicine, University of Melbourne, Melbourne, Australia (Establishment and maintenance of an international register of persons exposed to phenoxy acid herbicides and contaminants)
DEB/85/40	MRC Environmental Epidemiology Unit, University of Southampton, Southampton, UK (Establishment and maintenance of an international register of persons exposed to phenoxy acid herbicides and contaminants)
DEB/85/50	National Cancer Registry, Central Institute for Cancer Research, Berlin (Epidemiological study of silica-exposed slate quarry workers in the German Democratic Republic)
DEB/86/03	Department of Preventive Medicine, Institute of Occupational Medicine, Milan, Italy (Establishment and maintanance of an international register of persons exposed to phenoxy acid herbicides and contaminants)

Studies on the effects of passive smoking

DEB/85/01	Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA (International study of urinary cotinine levels in nonsmokers)
DEB/85/02	Department of Hygiene and Epidemiology, School of Medicine, University of Athens, Athens (International study of urinary cotinine levels in nonsmokers)
DEB/85/03	Department of Epidemiology, Louisiana State University, New Orleans, LA, USA (International study of urinary cotinine levels in nonsmokers)
DEB/85/04	Department of Epidemiology, Local Health Unit, Turin, Italy (International study of urinary cotinine levels in nonsmokers)
DEB/85/11	Department of Family and Preventive Medicine, School of Medicine, University of Southern California, Los Angeles, CA, USA (International study of urinary cotinine levels in nonsmokers)
DEB/85/13	Curie-Sklodowska Institute of Oncology, Warsaw (International study of urinary cotinine levels in nonsmokers)
DEB/85/18	Epidemiology Unit, National Cancer Institute of Canada, Toronto, Ontario, Canada (International study of urinary cotinine levels in nonsmokers)
DEB/85/19	Department of Epidemiology and Statistics, Hosptial San Jaume y Santa Magdalena, Mataro, Spain (Exploratory study on the association between passive smoking and bladder cancer)
DEB/85/22	Italian Association for Research on Cancer, Udine, Italy (International study of urinary cotinine levels in nonsmokers)
DEB/85/33	Shanghai Cancer Institute, Shanghai, China (International study of urinary cotinine levels in nonsmokers)

DEB/85/34	Department of Public Health, Tohoku University, School of Medicine, Sendai, Japan (International study of urinary cotinine levels in nonsmokers)
DEB/85/38	Institute for Research on Preventive and Social Medicine, Bremen, Federal Republic of Germany (International study of urinary cotinine levels in nonsmokers)
DEB/85/39	Postgraduate Institute of Medical Education and Reserarch, Chandigarh, India (International study of urinary cotinine levels in nonsmokers)

Studies of various other cancer forms

DEC/78/13	Department of Clinical Genetics, University Hospital of Lund, Lund, Sweden (Study on the possibility of correlating the karyotypes of cancer cells to specific etiological factors)
DEB/82/19	Icelandic Cancer Registry, Reykjavik (Evaluation of familial factors by determining risk for cancers of the breast and other sites)
DEB/84/12	Curie–Sklodowska Institute of Oncology, Warsaw (Case-control studies on cancer of the pancreas and on childhood brain tumours in Poland)
DEB/85/14	Hospital Santa Caterina, Gerona, Spain (Pilot study on risk factors for cervical cancer)
DEB/85/15	Cancer Registry of Zaragoza, Zaragoza, Spain (Pilot study on risk factors for cervical cancer)
DEB/85/16	Department of Preventive and Social Medicine, University of Seville, Seville, Spain (Pilot study on risk factors for cervical cancer)
DEB/85/17	Foundation for Higher Education, Cali, Columbia (Pilot study on risk factors for cervical cancer)
DEB/86/06	Department of Health and Social Security, Vitoria, Spain (Case-control study on risk factors for cervical cancer)
DEB/86/07	Cancer Registry of Murcia, Regional Health Authority, Murcia, Spain (Case-control study on risk factors for cervical cancer)
DEB/86/08	Cancer Registry of Pamplona, Institute of Public Health, Pamplona, Spain (Case-control study on risk factors for cervical cancer)
DEB/86/09	Department of Epidemiology, Institute of Social Welfare, Salamanca, Spain (Case-control study on risk factors for cervical cancer)
DEB/86/10	Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA (Breast cancer and hormonal profile in Chinese and Chinese–American women)

DEB/86/11	Department of Medical Statistics and Epidemiology, Sun Yat Sen University of Medical Sciences, Guangzhou, China (Breast cancer and hormonal profile in Chinese and Chinese–American women)
DEB/86/14	Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA (Biochemical analyses for studies of (a) urinary levels of oestrogens and progesterone in relation to passive smoking in nonsmoking women, and (b) breast cancer and hormonal profile in males)
FIS/87/02	Department of Pathology, University of Aberdeen, Aberdeen, Scotland, UK (Human papilloma virus (HPV) and cervical cancer studies: in-situ hybridization test)

Studies on chemical carcinogenesis

DEC/79/06	Institute of Medical Sciences, University of Tokyo (Mutagenesis and neoplastic transformation *in vitro* of cultured cells by environmental chemicals)
DEC/79/10	Cancer Research Center, Academy of Medical Sciences of the USSR, Moscow (Investigation on the development of cellular and biochemical markers of in-vitro transformation of epithelial cells in culture)
DEC/80/13	Institute of Oncology, University of Genoa, Genoa, Italy (Long-term carcinogenicity testing of environmental chemicals)
DEC/81/02	Cancer Institute, Chinese Academy of Medical Sciences, Beijing (Detection in human tissues by specific antibodies of cellular macromolecule modifications induced by nitrosamines)
DEC/81/03	Institute for Cell Biology, University of Essen, Essen, Federal Republic of Germany (Detection in human tissues by specific antibodies of cellular macromolecular modifications induced by nitrosamines)
DEC/81/08	Institute of Experimental and Clinical Medicine, Tallin, Estonian SSR, USSR (Studies in the mutagenic and carcinogenic activities of fly ashes originating from the combustion of shale-oil)
DEC/81/09	Oncological Institute of the Ministry of Health, Ministry of Health of Lithuanian SSR, Vilnius, Lithuanian SSR, USSR (Long-term carcinogenicity testing of environmental chemicals)
DEC/81/33	N.N. Petrov Research Institute of Oncology, Leningrad, USSR (Study of the role of promoting factors in the possible carcinogenic effect of 5-bromodeoxyuridine)
DEC/81/34	Oncological Research Center, Academy of Medical Sciences, Moscow (Long-term carcinogenicity testing of environmental chemicals)
DEC/81/35	National Institute of Hygiene, Budapest (Long-term carcinogenicity testing of environmental chemicals)

DEC/82/01	Life Science Laboratory, Teeside Polytechnic, Cleveland, UK (Study on carcinogenic effects in the offspring of male Swiss mice treated with MNU or ENU before mating)
DEC/82/06	School of Pharmacy, Catholic University of Louvain, Brussels (Study on the promoting activity of diazepam and related compounds)
DEC/82/22	Joint Mass Spectrometry Center, Claude Bernard University, Lyon, France (Study on the development of methods of analysis of carcinogens by combined high-performance liquid chromatography-mass spectrometry)
DEC/83/01	Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, UK (Preparation and characterization of antibodies against DNA modifications induced by nitrosamines to be used for the determination of human exposure to that group of carcinogens)
DEC/83/02	Institute of Oncology, Ljubljana, Yugoslavia (Study on the role of carcinogenic agents in determining the metastatic potential of induced tumours)
DEC/83/03	Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford, UK (Studies on analgesic-associated renal pelvic and ureteral/urothelian hyperplasia and carcinoma)
DEC/83/05	N.N. Petrov Institute of Oncology, Leningrad, USSR (Study on the activation of chemical carcinogens by embryonal tissues at various stages of development)
DEC/83/07	Laboratory for Applied Research on Chemical Carcinogens, Institute for Scientific Research on Cancer, Villejuif, France (Investigation of quantitative initiation-promotion carcinogenesis on mouse skin)
DEC/83/10	Cancer Research Unit, University of York, York, UK (Detection of aflatoxin B_1 and metabolites by immunoassay in human biological materials)
DEC/83/11	Institute of Oncology, Medical Academy, Sofia (Mycotoxins and individual oxidative susceptibility in relation to endemic nephropathy and tumours of the urinary system)
DEC/83/12	Hepatitis Laboratory, INSERM U 45, 69372 Lyon Cedex 08, France (Interactions between chronic infection by duck hepatitis B virus and consumption of aflatoxin in the etiology of hepatocarcinoma)
DEC/84/01	Research Department, National Board of Occupational Safety and Health, Solna, Sweden (Long-term carcinogenicity testing of environmental chemicals)
DEC/84/03	Cancer Research Institute, Tata Memorial Centre, Bombay, India (Monitoring of carcinogen exposure in tobacco/betel-quid chewers and smokers)

DEC/84/04	I Institute of Pathology, Semmelweis Medical University, Budapest (Study of the capacity of UV light to induce DNA repair processes in human cells)
DEC/84/05	Laboratory of Pathology, N.N. Petrov Research Institute of Oncology, Leningrad, USSR (Early alterations in the kinetics of cell populations in the mucosa of rat intestine following administration of 1,2-dimethylhydrazine)
DEC/84/06	Faculty of Agriculture, Cario University, El-Fayoum, Egypt (Study on etiological factors involved in bladder cancer in bilharzia-infected patients in Egypt)
DEC/85/02	Department of Pathology, Nagoya City University Medical School, Nagoya, Japan (Testing of environmental chemicals in the rat-liver two-stage model)
DEC/85/03	Centre for Studies on Molecular Biology, University of the Punjab, Lahore, Pakistan (Study on the detection of alkylated damage and repair in human tissues)
DEC/85/04	François Baclesse Centre, Caen, France (Detection in human tissues by specific antibodies of markers of cellular macromolecules induced by nitrosamines)
DEC/85/05	Clinical Research Unit, University of Oulu, Oulu, Finland (Drug metabolism in man)
DEC/85/06	N.N. Petrov Research Institute of Oncology, Leningrad, USSR (Study on O^6-alkylguanine-DNA methyltransferase activities in human tissues)
DEC/85/07	N.N. Petrov Research Institute of Oncology, Leningrad, USSR (Study on tumour promotion in offspring of carcinogen-treated male mice)
DEC/85/08	Dunn Clinical Nutrition Centre, Cambridge, UK (Microcapsule trapping of carcinogens in-vivo from human food metabolites)
DEC/86/03	Faculté des Sciences, Université Libre de Bruxelles, Rhode Saint Genèse, Belgium (Development and validation of a new bacterial test (*E. coli* multitest) for the quantification of the carcinogenic activity of chemical and physical agents)
DEC/86/04	Hazleton IFT, Les Oncins, St Germain sur l'Arbresle, France (Microcapsule trapping in primates of carcinogens from human food metabolites)
DEC/86/05	Rijks Instituut voor de Volksgezondheit, Bilthoven, The Netherlands (Long-term carcinogenicity testing of environmental chemicals)

CIE/86/07	Laboratory of Carcinogenic Substances, The Oncological Research Centre, Moscow (Role of prezygotic events in increasing cancer risk in subsequent generations)
ECH/87/01	Département de Médecine du Travail et d'Hygiène du Milieu, Université de Montréal, Montréal, Québec, Canada (Study on thioethers as indicators of exposure to mutagenic and carcinogenic products)

Support of meetings

DEB/86/01	Cancer Registry of the Bas-Rhin, Faculty of Medicine, Strasbourg, France (11th meeting of Latin cancer registries, Strasbourg, 8–9 May 1986)
DEB/86/04	National Organizing Committee of the Third African Regional Conference of the International Epidemiological Association (IEA), Nairobi (IEA African Regional Conference, Nairobi, 18–23 August 1986)

Annex 6

MEETINGS AND WORKSHOPS ORGANIZED BY IARC
1 July 1985 – 30 June 1987

Sixth International Symposium on Inhaled Particles	Cambridge, UK, 2–6 September 1985
Review Board Meeting for IARC Manual Series (volume on dioxins)	Bayreuth, FRG, 20–21 September 1985
Working Group on Measurements of Aflatoxin Exposure in Humans	Lyon, 30 September–1 October 1985
Working Group on Genetic Effects in Offspring of Cancer Patients	Lyon, 2–3 October 1985
SEARCH Meeting on Pancreatic Cancer	Lyon, 3–4 October 1985
IARC Working Group on Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation	Lyon, 15–22 October 1985
Meeting of Collaborators in the International Registry of Persons Exposed to Phenoxy Acid Herbicides and Chlorophenols	Lyon, 24–25 October 1985
Working Group on Second Malignancies in Relation to Cytotoxic Therapy	Copenhagen, 29–30 October 1985
Meeting of Collaborators in the Passive Smoking Study	Lyon, 5–8 November 1985
Meeting on Silica and Lung Cancer	Lyon, 2 December 1985
An Appraisal of Long-term and Short-term Carcinogen Screening Tests	Lyon, 2–6 December 1985
Meeting on the 10th Revision of ICD and ICD-O	Washington DC, 3–5 December 1985
Meeting of the International Association of Cancer Registries	Hartford, CN, USA 10–12 December 1985
Meeting of the Technical Committee on Monitoring and Evaluating Airborne Man-made Mineral Fibres	Lyon, 16–17 December 1985
Meeting on Presentation and Discussion of the Results of the Man-made Mineral Fibres Study	Lyon, 17–18 December 1985
Working Group on Laryngeal Cancer	Lyon, 17–19 December 1985
SEARCH Meeting on Adult Brain Tumours	Lyon, 14–15 January 1986

Programme Committee Meeting for the 9th International Meeting on *N*-Nitroso Compounds (1–5 September 1986, Baden, Austria)	Lyon, 19–20 January 1986
IARC Working Group on Some Halogenated Hydrocarbons and Pesticide Exposures	Lyon, 4–11 February 1986
Scientific Information Retrieval Course	Lyon, 5–7 February 1986
Steering Committee for the Gambia Hepatitis Vaccination Study	Banjul, 20–21 February 1986
Working Group on Laryngeal Cancer	Obernai, France, 7 May 1986
Working Group on Second Malignancies in Cytotoxic Therapy	Vancouver, Canada, 26–28 May 1986
SEARCH Meeting on Adult Brain Tumours	Lyon, 27 May 1986
SEARCH Meeting on Childhood Brain Tumours	Lyon 28–29 May 1986
Meeting on Priorities for Epidemiological Studies on Occupational Cancer	Lyon, 29–30 May 1986
IARC Working Group on Silicia and Some Silicates	Lyon, 10–17 June 1986
Training Course for Interviewers and Meeting of Coordinators and Field Operators involved in the Cervical Cancer Study in Spain and Colombia	Gerona, Spain, 16–19 June 1986
International Course on Molecular Biology for Epidemiologists	Lyon, 15–25 July 1986
Meeting of the International Association of Cancer Registries	Budapest, 19–21 August 1986
9th International Symposium on *N*-Nitroso Compounds: Relevance to Human Cancer	Baden, Austria, 1–5 September 1986
Editorial Board Meeting for IARC Manuals Series	Lyon, 23 September 1986
Programme Committee Meeting for Meeting on Detection Methods for DNA-damaging Agents in man	Lyon, 24 September 1986
IARC Working Group on the Preamble to the *IARC Monographs*	Lyon, 29 September–2 October 1986
SEARCH Meeting on Pancreatic Cancer	Lyon, 1–3 October 1986
Course on Cancer Epidemiology in French (in collaboration with EURO and with the support of AFRO and EMRO)	Rabat, 6–19 October 1986
Review Board Meeting for IARC Manuals Series (volume on indoor air)	Essen, FRG, 21–22 October 1986
WHO-EURO/IARC International Symposium on Man-made Mineral Fibres in the Working Environment	Copenhagen, 28–29 October 1986
Editorial Board Meeting for *Cancer Incidence in Five Continents Vol. V*	Lyon, 12–14 November 1986

IARC/IPCS/IAEMS International Course on Health Monitoring of Human Populations Exposed to Mutagens and/or carcinogens	Bombay, India, 3–14 November 1986
IARC Working Group on Genetic and Related Effects: Updating of Selected IARC Monographs from volumes 1–42	Lyon, 2–9 December 1986
Working Group on Cancer Mortality Atlas of EEC Countries	Lyon, 15–18 December 1986
Working Group on Prospective Studies on Diet and Cancer	Lyon, 7–9 January 1987
IARC Scientific Council	Lyon, 12–15 January 1987
IARC Working Group on the Preamble to the *IARC Monographs*	Lyon, 16 January 1987
Working Group on Second Malignancies in Relation to Cancer Therapy	Lyon, 26–27 January 1987
Organizing Committee for the Meeting on Multigeneration Effects of Carcinogens (in conjunction with the National Cancer Institute and the N.N. Petrov Research Institute of Oncology in Leningrad, March 1988)	Lyon, 26–28 January 1987
Meeting of Clinics and Cancer Registries for the International Radiation Study to Evaluate the Risks of Radiation Exposure in Cervical Cancer Patients: European Segment	Lyon, 28–29 January 1987
European Multicentric Study Working Group on Polyvinyl Chloride	Lyon, 13 February 1987
IARC Working Group to Discuss the International Study on Cancer Risk in Research Laboratory Workers	Lyon, 10–11 February 1987
International Symposium on Cell Differentiation and Carcinogenesis – Critical Gene Expression during Carcinogenesis	Osaka, Japan, 24–26 February 1987
Working Group Meeting on Silica Exposure and Lung Cancer	Lyon, 3 March 1987
IARC Working Group for Overall Evaluation of Carcinogenicity: Updating of *IARC Monographs* in volumes 1–42	Lyon, 10–18 March 1987
Meeting on Time Trends	Lyon, 1–3 April 1987
Mid-term Follow-up Meeting on the Cervical Cancer Project	Lyon, 14–15 April 1987
IARC Governing Council	Lyon, 29–30 April 1987
IARC Fellowships Selection Committee	Lyon, 5–6 May 1987
Meeting on Laryngeal Cancer	Ponzo, Italy, 28–29 May 1987

SEARCH Meeting of Collaborators in the Adult and Childhood Brain Tumours Study	Lyon, 1–5 June 1987
Advanced Course on Cancer Epidemiology	Heidelberg, FRG, 15–26 June 1987
IARC Working Group on Man-made Mineral Fibres and Radon	Lyon, 16–23 June 1987

Annex 7

VISITORS TO IARC
1 July 1985 – 30 June 1987

A total of 895 persons from 55 countries visited the Agency during the period under review. The following gave lectures:

Dr L. Aujame, Queens University, Kingston, Ontario, Canada
Non-expression of a major heat shock gene in murine plasmacytomas

Dr P. Bach, Toxicology Unit, The Robens Institute, University of Surrey, Guildford, UK
Relevance of animal models to analgesic-associated renal papillary necrosis in humans

Dr J.C. Barrett, Environmental Carcinogenesis Section, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
Cellular and molecular mechanisms of multistep carcinogenesis in a cell culture model

Dr J. Bertoglio, Gustave Roussy Institute, Villejuif, France
Growth factors on lymphoid cells

Dr J.S. Bertram, University of Hawaii, Basic Science Program, Honolulu, HI, USA
Cell–cell interaction and modulation of expression of neoplastic phenotypes

Dr T. Bishop, 23 Dorchester Road, Stourbridge, West Midlands, UK
Segregation and linkage analysis of breast cancer families

Dr P. Bryant, Massachusetts Institute of Technology, Cambridge, MA, USA
Level of 4-aminobiphenyl-haemoglobin adduct as a dose monitor for tobacco smoke

Dr C.T. Campbell, Imperial Cancer Research Fund Clinical Trials Service Unit, Radcliffe Infirmary, Oxford, UK
Dietary cancer survey in China

Dr J. Chen, Institute of Health, China National Centre for Preventive Medicine, Beijing
Multiple risk factor analysis of dietary constituents as a cause of cancer in China

Professor P. Correa, Louisiana State University, New Orleans, LA, USA
A review of passive smoking

Dr M. Delendi, Institute of Pathological Anatomy, University of Trieste, Italy
Fourteen years of autopsy study in the town of Trieste. Comparison between autopsies and clinical diagnoses of deaths

Dr J.A. DiPaolo, Laboratory of Biology, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, MD, USA
Progressive changes induced in human and mouse cells by HPV-16 DNA

Dr N. Drinkwater, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI, USA
Studies on molecular mechanisms of liver carcinogenesis

Dr S.W. Duffy, Department of Mathematical Sciences, University of Durham, UK
Dietary studies in Singapore

Dr L. Ehrenberg, Department of Radiobiology, Stockholm University, Stockholm
Cancerous diseases in Sweden: study of variations in mortality and incidence

Dr P. Farmer, Medical Research Council, Carshalton, UK
Monitoring for exposure to alkylating agents using GC–MS determination of their adducts with proteins and nucleic acids

Dr S. Fukushima, Department of Pathology, Nagoya City University Medical School, Nagoya, Japan
Significance of urinary nature in promotion of rat bladder carcinogenesis

Dr F. Garzòn, German Cancer Research Centre, Heidelberg, Federal Republic of Germany
Enhancement of acetoxymethanol-methylnitrosamine-induced colorectal tumours following chronic ethanol consumption in rats

Dr J. Geboers, St Raphael University Hospital, Leuven, Belgium
The role of a high salt diet in the etiology of stomach cancer

Dr M. Gérin, Department of Occupational Medicine, University of Montreal, Quebec, Canada
Occupational exposure assessment in cancer epidemiology
Determination of a specific urinary thioether for the biological monitoring of ethylene oxide exposure

Dr R. Gradini, Department of Pathology, Loyola University Medical Center, Maywood, IL, USA
Immunohistochemistry: S100 protein in differential diagnosis of histological subtypes of different cancers

Dr J. Groopman, School of Public Health, Boston University, Boston, MA, USA
Analysis of aflatoxin-DNA adducts in human urine by monoclonal anitbody affinity chromatography

Dr A. Hall, Medical Research Council Laboratories, Fajara, near Banjul, The Gambia
The Gambia hepatitis intervention study

Dr J. Hall, Imperial Cancer Research Fund, Clare Hall Laboratories, Potters Bar, UK
Complementation of a mammalian DNA repair defect by expression of a cloned bacterial gene

Dr L. Hardell, Department of Oncology, University of Umeå, Sweden
Epidemiological investigations on primary liver cancer with special regard to organic solvents and porphyria

Dr Y. Hayashi, National Institute of Hygienic Sciences, Tokyo
Risk evaluation of tumour-inducing agents

Dr K. Hemminki, Institute of Occupational Health, Helsinki
Use of carcinogenic potency data from animals in the design of epidemiologic studies

Dr M. Hill, Centre for Applied Microbiology, Salisbury, UK
Bacteria, *N*-ntrioso compounds and human cancer

Dr Y. Hinuma, Institute for Virus Research, Kyoto University, Japan
Natural history of adult T-cell leukaemia associated with a retrovirus

Dr J. Jarvisalo, World Health Organization, Regional Office for Europe, Copenhagen
A follow-up of biochemical markers in asbestosis patients

Dr C. Junien, Research Group, Prenatal Biology, U73, Château de Longchamp, Paris
11 p chromosome rearrangement and predisposition to nephroblastoma

Dr R. Klann, University of Texas System Cancer Center, Smithville, TX, USA
Characterization of cell lines derived from SENCAR mouse epidermis during two-stage carcinogenesis

Dr A.G. Knudson, Fox Chase Cancer Center, Institute for Cancer Research, American Oncological Hospital, Philadelphia, PA, USA
Visiting Professor Award from the General Motors Cancer Research Foundation: Genetics and human cancer

Dr M. Kodama, Aichi Cancer Center Research Institute, Nagoya, Japan
Hormonal aspects of environmental carcinogenesis with special reference to the presence of diet-hormone linkage

Dr. G. Kreibich, New York University, NY, USA
Mechanisms for the insertion of proteins into biological membranes: biosynthesis and membrane disposition of cytochrome P450

Professor G.R.F. Krueger, Pathology Institute, University of Cologne, Federal Republic of Germany
Experimental studies of T-cell lymphoma disease

Professor Z. Kulcar, Chronic Disease Service, Institute of Public Health of SR Croatia, Zagreb
Cancer prevention and control in Yugoslavia

Dr G.A. Kune, University of Melbourne, Australia
The Melbourne colorectal cancer study. Results up to 1987

Dr M. Leppert, Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT, USA
Human gene mapping and diseases: heterogenicity for X-linked spastic paraplegia

Dr R. Loope, Academy of Sciences of the Estonian SSR, Tallinn, USSR
Fluorescence methods in studies of the regularity of carcinogen metabolism

Professor C. Maltoni, F. Addarii Institute of Oncology, Bologna, Italy
Results of recent experimental research on the carcinogenicity of natural and modified asbestos

Professor M. Marmot, Department of Community Medicine, University College, London
Differentials in mortality

Dr A.P. Maskens, European Organization for Cooperation in Cancer Prevention Studies, Brussels
Activities of the European Organization for Cooperation in Cancer Prevention Studies

Professor A.B. Miller, Epidemiology Unit, National Cancer Institute of Canada, Faculty of Medicine, Toronto, Ontario, Canada
Radiation induction of breast cancer: the Canadian study of cancer following multiple thoraxscopies

Dr F. Mitelman, Department of Clinical Genetics, University Hospital, Lund, Sweden
Chromosomes and cancer

Dr S. Monfardini, Oncological Reference Centre, Aviano, Italy
AIDS-related cancer

Dr A.W. Murray, The Flinders University of Southern Australia, Bedford Park, SA, Australia
Molecular and cellular mechanisms of action of phorbol ester tumour promoters

Dr R.F. Newbold, Chester Beatty Laboratories, The Institute of Cancer Research, Royal Cancer Hospital, London
Fibroblast transformation as a model for multistage carcinogenesis

Dr P. Paoletti, CNR Institute of Clinical Physiology, Pisa, Italy
Prospective follow-up of a population living near a thermal electricity plant

Dr L. Parada, Pasteur Institute, Paris
The effect of *myc* and P53 on primary rat embryo fibroblasts

Dr N. Pearce, Occupational Health Studies Program, Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, NC, USA
New Zealand case-control studies of farming and cancer

Professor O. Pelkonen, Department of Pharmacology, University of Oulu, Finland
Cytochrome P450 phenotype and hypersusceptibility to carcinogens

Dr H. Pézerat, Surface Activity and Structure Laboratory, Pierre et Marie Curie University, Paris
Inorganic carcinogenesis: role of surface activity of solids and ionic activity in solution — examples of possible initiation mechanisms

Dr V. Préat, Laboratory of Biotoxicology, Catholic University of Louvain, Brussels
Biological modulation of hepatocarcinogenesis

Dr M. Presta, General Pathology and Basic Biomedical Sciences, Faculty of Medicine and Surgery, Brescia, Italy
Human basic fibroblast growth factor: an angiogenesis factor

Professor H. Remmer, Institute of Toxicology, University of Tübingen, Federal Republic of Germany
Passive smoking: a challenge for toxicology and preventive medicine

Dr C.A. Rohde, Johns Hopkins University, Baltimore, MD, USA
Confounding and interaction in epidemiological studies

Dr D. Rose, Division of Nutrition and Endocrinology, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA
Endocrinological and epidemiological investigations of breast cancer etiology

Dr J. Rubinstein, Cellular Genetics Unit, Pasteur Institute, Paris
Construction of transgeneic mice using recombinant retroviruses

Dr M.A. Siddiqi, University of Kashmir, Srinagar, India
Oesophageal cancer in Kashmir and an assessment of possible dietary and lifestyle risk factors

Dr J. Siemiatycki, Armand Frappier Institute, Laval, Quebec, Canada
Associations between several sites of cancer and several occupational exposures (nickel-chromium, organic dusts, petroleum-based liquids) from a case-control study in Montreal

Dr N. Simberg, University of California, San Francisco, CA, USA
Studies on the endocrinology of breast cancer

Dr H.F. Stich, Environmental Carcinogenesis Unit, British Columbia Cancer Research Centre, Vancouver, BC, Canada
Intervention studies with β-carotene in betel-quid chewers

Dr S. Sukumar, Frederick Cancer Research Facility, National Cancer Institute, Frederick, MD, USA
Oncogenes in chemical carcinogenesis

Professor C.C. Tan, Institute of Genetics, Fudan University, Shanghai, China
Research progress of genetic toxicology in China

Dr P. Toniolo, Institute of Environmental Medicine, Laboratory of Biostatistics and Epidemiology, New York University Medical Center, New York, NY, USA
Hormones and breast cancer: an international case-control study

Dr J.M. Vasiliev, All-Union Cancer Research Center, The USSR Academy of Medical Sciences, Moscow
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Dr K. Wakabayashi and Dr M. Tsuda, National Cancer Centre Research Institute, Tokyo
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Dr P.G. Watanabe, Mammalian and Environmental Toxicology Research Laboratory, The Dow Chemical Company, Midland, MI, USA
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Professor Y. Zeng, Institute of Virology, Chinese Academy of Preventive Medicine, Beijing
Early diagnosis of nasopharyngeal carcinoma and Epstein–Barr virus inducers

Dr B.C. Zook, Hanover Medical School, Hanover, Federal Republic of Germany
Chronic pathological changes in beagles induced by neutron or photon irradiations

Annex 8

INTERNAL TECHNICAL REPORTS, 1985 – 1987

IARC Internal Technical Report No.	
86/001	Evaluation of Methods for Assessing Human Health Hazards from Drinking-water
86/002	Report on the IARC Research Training Fellowships Programme (1966–1984)
86/003	CANREG — Cancer Registration Using a Microcomputer
86/004	Priorities in Occupational Cancer Epidemiology
86/005	Rank Orders, Sex Ratios and Other Numerical and Descriptive Data, Based on Volume IV of *Cancer Incidence in Five Continents*
87/001	*IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* — Preamble

Annex 9

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