INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

ANNUAL REPORT 1976

LYON FRANCE

World Health Organization

SC/13/2
GC/16/2

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

ANNUAL REPORT

1976

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
LYON
1976

ISBN 92 832 1076

PRINTED IN SWITZERLAND

STAFF OF IARC

Director :	Dr J. HIGGINSON
Administrative Assistant	Mrs E. RIVIÈRE
Unit of Epidemiology and Biostatistics	
Chief	Dr C. S. MUIR
Scientists:	Dr N. E. DAY Dr O. M. JENSEN Dr J. KMET Dr R. MACLENNAN Dr J. E. H. MILNE (from March 1976) Dr L. MUENZ Mr X. NGUYEN-DINH (from October 1975) Dr R. SARACCI Dr H. TULINIUS (until September 1975) Dr A. J. TUYNS
Consultants:	Miss P. J. COOK Professor T. HEWER (February–July 1976) Dr J. SCHIFFLERS (until March 1976)
IARC Research Training Fellow	Dr C. TZVETANSKY
Technical Clerk	Mrs J. NECTOUX
Statistical Assistants:	Mr U. ARKAN (temporary) Mrs I. ARNAUD (temporary) Miss B. CHARNAY Miss B. HUBNER (temporary) Mr M. JABOULIN Mrs M. JACOB Mrs A. JOLY (temporary) Miss D. MAGNIN Miss A. RESSICAUD Mr S. SABAI (temporary) Mr M. SAEB (temporary) Mr E. TADJBAKHCHE (temporary)
Administrative Assistant	Mrs A. GESER

Secretaries:	Miss S. BASSILL Miss O. CAVOURA Mrs G. DAHANNE Mrs E. DEMARET Miss M. FALLON (temporary) Miss W. HLAHOLUK Mrs L. KEREBEL (until August 1975) Miss V. LAMPERT (until December 1975) Miss M. J. PICARD Mrs A. RIVOIRE Miss S. WHELAN

Unit of Environmental Carcinogens

Chief	Dr L. GRICIUTE
Scientists:	Mr M. CASTEGNARO Mr E. A. WALKER Mr R. WALKER
Technicians:	Mr J. C. BEREZIAT Mrs L. GARREN Miss F. LAFAVERGES Mrs B. PIGNATELLI Mr G. TOUSSAINT
Secretaries:	Mrs D. BARRET Mrs M. COURCIER Mrs C. PORTIER Miss L. SOUTHWOOD (until June 1976)
Animal Caretaker	Mrs M. LANOT

Unit of Biological Carcinogenesis

Chief	Dr G. BLAUDIN DE-THÉ
Scientists:	Dr A. GESER Dr G. LENOIR Dr N. MUÑOZ (until June 1976)
Consultants:	Dr J. P. LAMELIN Professor R. SOHIER
Visiting Scientists:	Mrs M. L. DIDIER-FICHET Dr J. M. SEIGNEURIN
Research Assistants:	Mrs M. C. BERTHELON Mrs C. DESGRANGES-BLANC Miss M. C. FAVRE Mrs M. F. LAVOUÉ Mr J. S. PELLOQUIN Mrs M. VUILLAUME
Administrative Officer	Mr P. CATTAND

Bibliographic Assistants:	Mrs M. DEBOSSU (until April 1976) Miss M. PIRON
Statistical Assistant	Miss C. BONNARDEL
Technicians:	Mrs M. COMBRISSON Mrs S. PAULY Mrs C. PICCOLI Mrs N. ROCHE Mrs M. VALIÈRE
Secretaries:	Miss C. DERIOL Miss J. DURHAM Miss J. LANGDON Mrs A. ROMANOFF Miss S. VENTON (until December 1975) Miss L. WHITBY (until May 1976)
Laboratory Aides:	Mr L. MURISET Mrs S. VEYRE
Graduate Students:	Miss O. COSTA Mrs N. THOMASSET

Unit of Chemical Carcinogenesis

Chief	Dr L. TOMATIS
Scientists:	Dr C. AGTHE (until June 1976) Dr H. BARTSCH Mr C. MALAVEILLE Dr R. MONTESANO Dr V. PONOMARKOV Mrs L. SAINT-VINCENT Mr J. D. WILBOURN
Visiting Scientists:	Dr G. MARGISON Dr T. KUROKI Dr L. ZARDI (until March 1976)
IARC Research Training Fellow	Dr A. LIKHACHEV
Bibliographic Researchers:	Mrs C. PARTENSKY Mrs I. PETERSCHMIDT
Technicians:	Mr A. BARBIN Miss H. BRESIL Mrs G. BRUN Miss O. DEBLOCK Miss C. DREVON Mrs B. EUZEBY Mrs C. GABET Mrs D. GALENDO Miss M. LAVAL Mrs N. LYANDRAT Mrs J. MARGISON (temporary) Mrs M. J. MUETTON Mrs L. PLOTON (until April 1976)

Administrative Assistants:	Mrs M. J. GHESS Mrs E. PEREZ
Library Assistant	Mrs D. MIETTON
Secretaries:	Miss A. ANDERSON Miss M. FALLON (temporary) Miss R. JOHNSON Miss P. STAFFORD-SMITH
Animal Caretaker	Mrs L. HERNANDEZ
Laboratory Aides:	Mr F. FARIA Mr J. NOGUEIRA (temporary)
Graduate Students:	Miss A. M. CAMUS Miss G. PLANCHE

Unit of Research Training and Liaison

Chief	Dr W. DAVIS
Administrative Assistant	Miss M. DELORME
Secretaries:	Mrs P. CARLE (temporary) Mrs C. DECHAUX Miss J. HAWKINS
Librarian	Mrs A. NAGY-TIBORCZ
Library Clerk	Mrs L. OSSETIAN

Interdisciplinary Programme and International Liaison

Chief	Dr C. A. LINSELL
Scientists:	Dr A. LEVIN Dr P. SIZARET Dr N. MUÑOZ (from July 1976)
Corvissiano Fellow	Dr T. YOKOTA (from January 1976)
Technicians:	Miss N. MARTEL Mrs J. SAFARI
Photographic Assistant	Mrs F. RUAULT-BURTÉ
Secretaries:	Miss S. REYNAUD Miss A. SHANNON
Laboratory Aides:	Miss C. BOUCHET Mrs M. ESSERTEL Mrs S. GIRAUD Mrs D. GRAS
Graduate Student	Miss C. DAMBUYANT

Administration and Finance

Chief	Mr W. A. PRICHARD
Personnel Assistant	Mrs A. ESCOFFIER

Translator	Mr Y. POLLET
Administrative Services Officer	Mr B. BORGSTRØM
Building Management Assistants:	Mr J. P. BONNEFOND Mr E. CATHY Mr P. CAZEAUX Mr G. THOLLY
Budget and Finance Officer	Mr T. MIRZA
Finance Officer	Mr G. W. DALSTON
Finance Clerks:	Mr C. AUGROS Mrs F. CAFFO Miss G. MARTINOD Miss M. ROMATIER
Registry Assistants:	Mrs M. GREENLAND Mrs P. MALINDINE Mrs C. WALKER (temporary)
Supplies Assistants:	Miss G. DESGURSE Mrs J. POPOFF
Documents Assistant	Mrs J. NIELSEN-KOLDING
Printing Services:	Mr J. DECHAUX Mr J. LAMY (until March 1976) Mr G. MOLLON
Secretaries:	Mrs J. BAILLY Mrs M. H. CHARRIER Mrs J. MARTINEZ Mrs R. SEXTIER
Pool:	Miss B. BAKER Mrs M. KELLY (until May 1976) Miss J. MITCHELL Miss J. PAULIN Mrs A. TROCHARD
Other Services:	Mr G. BARBERO Miss A. COOTE (until December 1975) Mr J. DIKUNDUAKILA Mrs F. FLORENTIN Mr G. MAGNIARD

CONTENTS

INTRODUCTION

The present report covers the work of the International Agency for Research on Cancer for the 12-month period ending 30 June 1976. It also marks the first decade of the Agency's existence, and it may be of interest to attempt to review and evaluate what has been achieved in 10 years.

THE FIRST DECADE

The beginning

The Agency was established in 1965 within the framework of the World Health Organization by the 18th World Health Assembly, to promote international collaboration in cancer research. Its establishment resulted from the initiative of 13 French savants who proposed to the then President of France, General Charles de Gaulle, that the nations of the world should provide a half per cent of their military budget to research in cancer.

The director, Dr John Higginson, was appointed in June 1966 and started work in offices in WHO Headquarters, Geneva. During the ensuing months, he appointed the scientific staff who were to be responsible for developing the Agency's programme.

In December 1966 the first meeting of the Fellowships Selection Committee was convened in Geneva, bringing together five senior scientists from different fields of research and different countries, to examine the first 73 applications that were received. They recommended awards of 20 Research Training Fellowships and 12 Travel Fellowships and launched the programme which has continued to the present day. The rapidity with which this programme was initiated was in large measure due to the guidance and supporting services provided by the staff of WHO Headquarters.

By 1976 a total of 183 Research Training Fellowships and 228 Travel Fellowships had been awarded.

The move to Lyon

In the Spring of 1967, a host agreement was concluded between WHO and the French Government, and in May the staff moved from Geneva to Lyon. The Agency was established in a large town house put at its disposal by Mr L. Pradel, the Mayor of Lyon, but even before this, the Mayor had made available the rooms of the Town Hall for holding meetings of the Governing and Scientific Councils and as an office for the Director.

For the next five years, the staff planned and started their many research projects under far from ideal conditions. A limited amount of laboratory space was rented, and, later,

a two-storey prefabricated building was erected on the site provided by the municipal authorities. In August 1970 the Agency was able to open its own, even if temporary, laboratories, which are still in use.

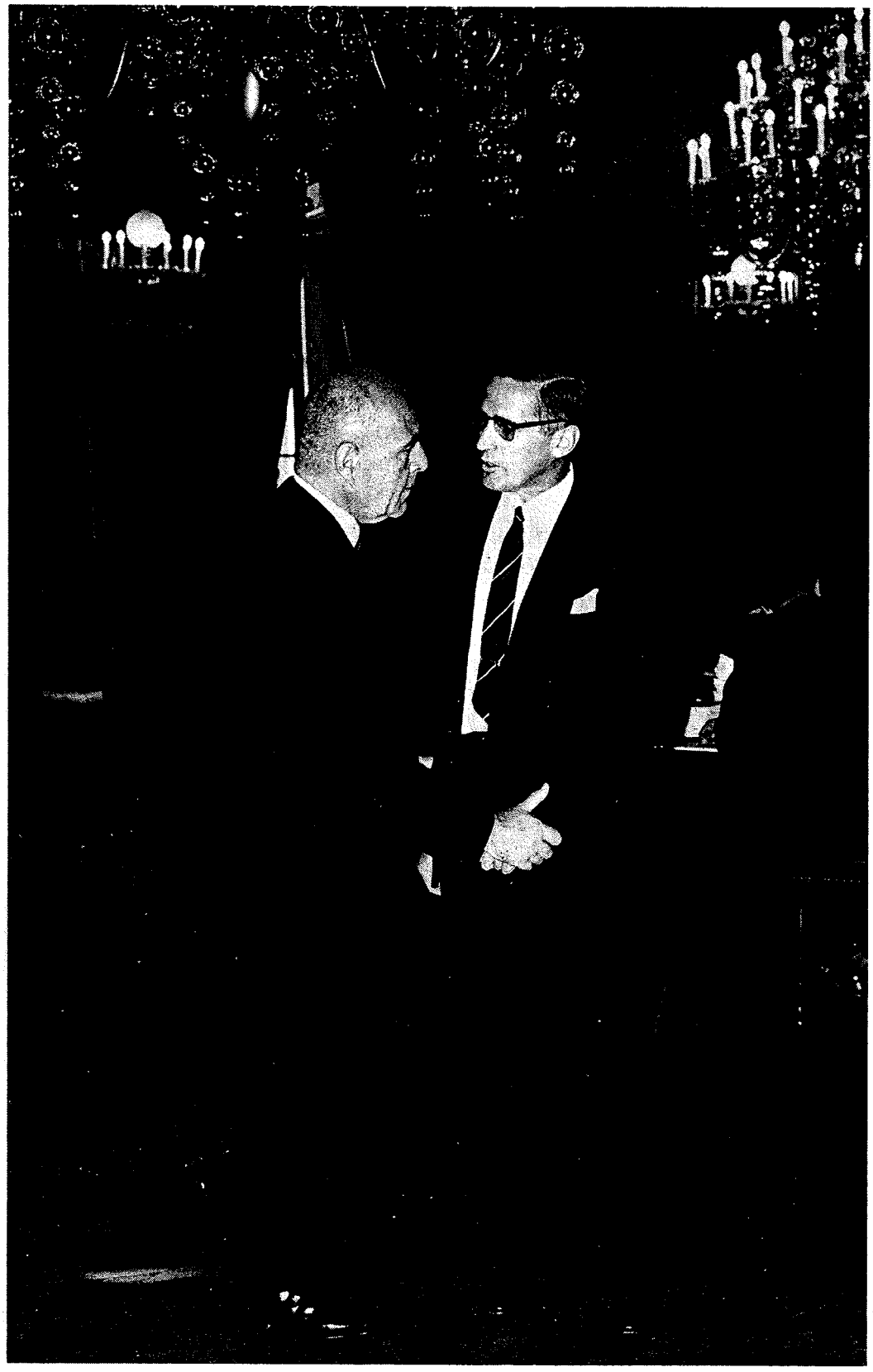
Meanwhile, close by, the 14-storey tower being built for the Agency was growing rapidly. Construction started in September 1969 on a site provided by the Municipality of Lyon. The cost of the building was shared between the Municipal Council, the Council of the Department of the Rhône and the French Government.

Inauguration

In June 1972 the late President Georges Pompidou inaugurated the new building, handing the keys to Dr M. Candau, then Director-General of WHO.

Fig. 1 The first headquarters of the Agency at 16, avenue Maréchal Foch, Lyon (1967-1972)

Fig. 2 Dr J. Higginson with Mr L. Pradel, the Mayor of Lyon, during a fellowships selection committee held in the Town Hall, Lyon (Photo by courtesy of Le Progrès, Lyon)



In November 1972, for the first time, all the staff of the Agency in Lyon began to work in the same building. The laboratory installations and most of the equipment in the building were provided from the Governing Council Special Fund and from generous loans or gifts from Australia, The Netherlands, the United Kingdom and the United States. The conditions of work in both laboratory and office were excellent, and, from 1972, the research and training programmes have developed with increasing momentum.

Viruses

By 1968, tissue culture studies were underway in a laboratory borrowed from the Virology Unit of the French National Institute of Health and Medical Research. Biopsy specimens obtained from nasopharyngeal cancer patients in Hong Kong and from Burkitt's lymphoma patients in Nairobi were established in long-term cultures. The Epstein-Barr virus had already been identified in cultures obtained from Burkitt's lymphoma patients, but it was in the Agency's borrowed laboratory that the same virus was discovered in cultures from biopsies from nasopharyngeal cancer patients.

DDT

During this same period, the first studies in chemical carcinogenesis began. In December 1967, a joint meeting of WHO and FAO issued a recommendation that the reported carcinogen DDT should be very carefully investigated, and in the following year the Agency started a multigeneration study using experimental mice housed in the laboratories of the Mérieux Institute, Lyon. That study continued until 1972, by which time 4 000 mice had been used to establish that DDT, even at doses of only 2 mg/kg, significantly increased the incidence of liver tumours in the males, whilst having no observable effect on the females.

Oesophageal cancer

1968 also saw the first steps in the studies on oesophageal cancer, especially in Northern Iran and in Brittany, France. Both of these studies are still going on. In Iran, Agency staff, in collaboration especially with the Institute of Public Health Research, Teheran, have carried out detailed surveys in the high incidence area of Gonbad in the Caspian littoral and in neighbouring areas of lower incidence. In spite of the vast amount of data collected on health diet, and cultural activities, no specific environmental factor or factors have yet been associated with the disease, except that it appears to be associated with low socio-economic status and a restricted diet. In Brittany, high consumption of alcoholic beverages and of tobacco is clearly associated with a very high risk of oesophageal cancer.

Nairobi research centre — cancer in Kenya

In this early period, the value was seen of establishing centres overseas that could lead the field studies on local problems, and in 1967 regional centres—the name was later

changed to research centres—were established in Nairobi, Singapore and Jamaica. The study of liver cancer was concentrated in Nairobi. In particular, the association between that cancer and the level of ingestion of the dietary contaminant aflatoxin was studied in the population of the Murang'a district of Kenya. In five years almost 6 000 samples of food and drinks were analysed for aflatoxin in the laboratory at the research centre, and, concurrently, all the cases of liver cancer occurring in the Murang'a district were recorded. The district fell into three natural areas of high, medium and low altitude. The level of aflatoxin contamination was significantly higher in the lowland area, and it was there, too, that more cases of liver cancer occurred. This association between aflatoxin ingestion and primary liver cancer was tested in other areas in Africa and has always held good.

Singapore research centre — nasopharyngeal cancer

In the research centre that is based in the University of Singapore, a cancer registry has been maintained since January 1968. The centre also became a focus for nasopharyngeal cancer studies, both epidemiological and immunological; the involvement of the WHO Immunology Research and Training Centre, Singapore, which started in 1969, has proved of paramount importance. The finding in 1973 of a genetic difference in the HLA antigen system of nasopharyngeal cancer patients has been more completely specified. The current results indicate the existence of a disease-susceptibility gene that is not only associated with a higher risk of contracting nasopharyngeal cancer for the bearer but also seems to be a marker of poor prognosis. The particular HLA phenotype, known as A2-BSin2, is found predominantly among Southern Chinese.

Cancer registration and descriptive epidemiology

From its inception, the Agency had seen the importance of having good quality data on cancer incidence coming from cancer registries spread throughout the world. It stressed too the need to ensure that the reported statistics were comparable between different registries. When the International Association of Cancer Registries was set up in 1969, the Agency strongly supported its aims and established a close liaison with its secretariat (1970, Vol. II). That contact has grown continuously so that now, by agreement, the Agency provides the secretariat for the Association and has accepted the responsibility for publishing the jointly prepared accumulation of cancer statistics compiled from different cancer registries—*Cancer Incidence in Five Continents,* Volume III.

In 1974, in collaboration with the German Cancer Research Centre, Heidelberg, a clearing house of on-going epidemiological studies was set up to maintain a computerized data bank comprised of information gathered from all over the world. The first directory compiled from these data was published in 1976.

Burkitt's lymphoma

The presence of the herpesvirus (type Epstein-Barr) in cell cultures grown from biopsy specimens taken from cases of Burkitt's lymphoma aroused increased interest when that

virus was shown to be the causal agent of infectious mononucleosis. With the collaboration of the East African Virus Research Institute, Entebbe, Uganda, and the National Cancer Institute, USA, a prospective sero-epidemiological study was planned in the West Nile District of Uganda. Starting in December 1971, the field team had, by 1976, collected more than 42 000 blood samples taken from children below 6 years of age who were at risk from Burkitt's lymphoma. The separate sera were stored for later comparison between sera from those children who developed Burkitt's lymphoma and matched controls. At the same time blood smears were taken to measure the malaria parasite loading of the children. By 1976, 14 of the children from whom samples of blood had been taken and samples stored developed Burkitt's lymphoma, and their sera were titrated for the presence of antibodies against the viral capsid antigen of the Epstein-Barr virus. The values obtained indicated virus infection at a higher level and earlier in life in those children who developed the lymphoma compared with healthy children, selected as age- and sex-matched controls. This may indicate that it is a chronic and heavy infection acquired in the neo-natal period that is linked with the development of Burkitt's lymphoma.

At the same time the malaria parasite studies have shown consistently high parasite rates in the West Nile district. This contrasts with the significantly lower parasite rate among children living on a plateau in Tanzania where Burkitt's lymphoma is absent.

Although the precise relationship between Burkitt's lymphoma, malaria and Epstein-Barr virus infection has yet to be demonstrated, the study has led to a project to suppress malaria in a child population by distribution of chloroquine tablets to see whether, along with the diminution of malaria, there will also be a diminution of Burkitt's lymphoma. Virologists elsewhere are trying to solve the considerable technical problems of preparing an anti-viral vaccine.

Chemical carcinogenesis — monographs and survey

The chemical carcinogenesis programme which started with the DDT studies in 1967 has developed in four different ways—the collection of carcinogenicity data in the series of monographs on the evaluation of carcinogenic risk of chemicals to man and the survey of chemicals being tested for carcinogenicity; the evaluation of significance for man of experimental carcinogenicity data; the long-term testing of environmental chemicals of particular socio-economic importance; and the development of rapid screening tests for potential chemical carcinogens.

The monograph series started in 1971, and the first trial volume covered a mixed group of 33 different substances. The volume was well-received, and the plans for further volumes were accelerated so that by now 11 volumes covering 272 different substances have already been published; further volumes are planned at the rate of three a year.

The survey of chemicals being tested for carcinogenicity was conceived as essential in an effort to achieve some sort of coordination of the limited facilities available, even on a world scale, for long-term testing of chemicals in experimental animals. In 1972 the Agency sent out questionnaires to 470 institutes in 62 countries asking for information on current testing programmes, and by August 1973 the first information circular was distributed in which the replies were analysed and collated. Thirty-two laboratories gave

information on the testing of 127 compounds, about half of which had been tested elsewhere before. The 1973 survey reported 523 chemicals under test and in 1974, 470. The latest survey, published this year, reported tests on 828 substances.

No absolute criteria yet exist to allow an unambiguous extrapolation to man of the results of experiments in rodents, but biochemical studies of the comparative metabolism of carcinogens in human and animal tissues have been developed in the Agency with the intention of closing the gap.

The tremendous load of substances awaiting test in rodents has prompted the efforts to find alternative test systems that are cheaper and, above all, would give results more rapidly than the two to three years required for a test in experimental animals. Among the more promising alternatives has been the test system based on the observation that a very high percentage of carcinogens are known to be mutagens and *vice versa*. The test, developed by Dr B. N. Ames, University of California, Berkeley, USA, in which mutations in *Salmonella typhimurium* strains are measured quantitatively, was modified in the Agency's laboratories in 1974 to enable volatile substances like vinyl chloride to be assayed for mutagenic action under reproducible conditions. This type of test is not proposed at this stage as an alternative to animal testing, but it can certainly provide a very useful technique for screening substances for which longer term testing seems necessary.

N-*Nitroso compounds*

In October 1969 scientists from the Agency organized a meeting in London to deal with analytical problems in the estimation of traces of nitrosamines in food and were already reporting developments in this direction from the work of a collaborating laboratory. It was not until the following year, in 1970, when the Agency was able to install laboratories in a temporary building in Lyon, that its own programme of nitrosamine analysis was started. The importance of refining the available techniques arose particularly because of the epidemiological studies on cancer of the oesophagus in Brittany and in Iran, and from both areas food and drink samples were collected for subsequent analysis.

Five years of intensive work and the organization of collaborative studies by the Agency have resulted in the availability of highly accurate and reproducible methods for analysing the volatile nitrosamines at the level of a few parts per billion.

α-*Fetoprotein*

In 1968 the Agency instigated a collaborative study for the evaluation of a serological test for cancer of the liver. It had been reported that embryonal proteins reappeared in very high concentration in the serum of patients with primary liver cancer, and a programme was set in motion to establish the specificity of this protein, later named α-fetoprotein, in the hope that it might prove a valuable diagnostic tool for the early detection of primary liver cancer. The clinical application of α-fetoprotein as a diagnostic aid has proved to be very valuable, and a great deal has been learned about the natural history of α-fetoprotein in different age groups and different populations. One outcome of all the collaborative studies has been the selection of the reference sample prepared in the Agency

as the internationally accepted reference material by the WHO Expert Committee on Biological Standardization.

Training courses

The Agency organized its first specialized training course in 1968. Thirty participants from 21 countries spent 10 days in Lyon following a course on biostatistics in cancer research. Since then nine courses have been organized covering not only epidemiology but also the use of experimental animals in cancer research and immunovirology of cancer. Courses have been given in English or French. In 1971, a regional course was held in Singapore, and in 1976 the first epidemiology course organized by the Agency in Spanish will be held in Brasilia for participants from the Latin-American countries.

Publications

The first printed report of the Agency appeared in 1968 and paved the way for the start of the Scientific Publications series in 1971. In that year, with the support and encouragement of the Publications Division of WHO Headquarters, the proceedings of a meeting on liver cancer were published by the Agency, and in the five years that followed, the Scientific Publications—of which there are now twelve—have provided authoritative data on many of the aspects of the Agency's research programmes. Most of the books contain the proceedings of meetings organized by the Agency and provide definitive reviews by eminent international contributors working in the field of environmental carcinogenesis.

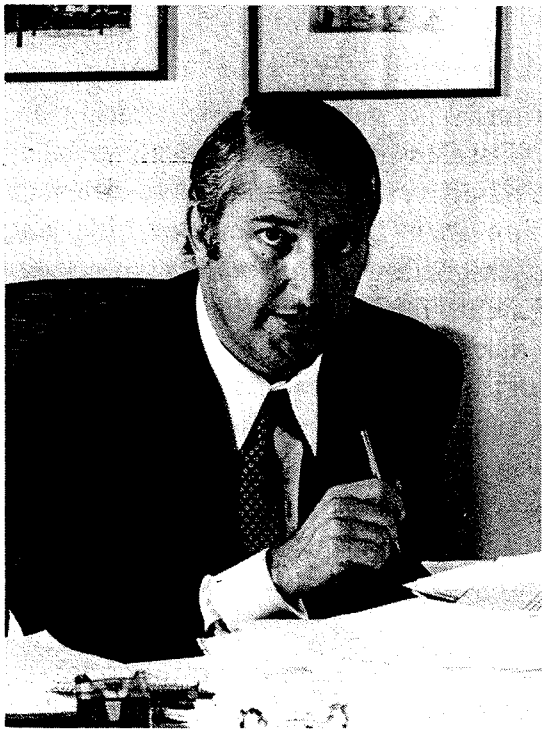
Whilst it has not been possible to give a complete inventory of ten years' development of the scientific programmes of the Agency, this backward glance can serve both as a justification for the existence of the Agency and for a clearer definition of the scientific problems that await it in the future.

GENERAL BACKGROUND TO THE CURRENT PROGRAMME

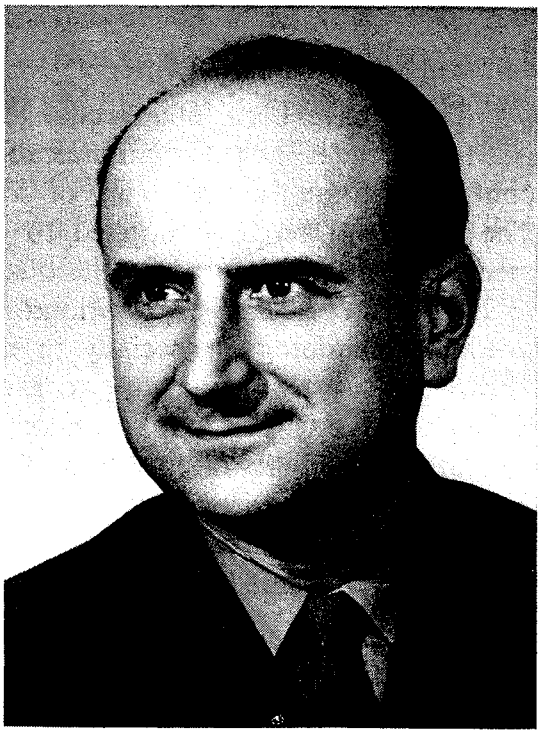
Research in cancer control has many facets, and the major national institutions between them cover almost all the fields of basic and clinical research. The task of the Agency has been to identify and develop those areas of research activity to which an international organization could contribute most effectively. Cancer epidemiology and environmental carcinogenesis have proved to be such areas, and the study of variations in cancer incidence between and within different countries lays the basis both for the identification and for the evaluation of carcinogenic risks to man. The orientation of the Agency's research toward environmental carcinogenesis was reinforced by the growing attention that governments were paying to problems of the environment, both from the health and socio-economic viewpoints. Cancer, moreover, is recognized as the most important chronic hazard of environmental pollution.

In addition, it seemed that the study of carcinogenic factors in the environment was inadequately developed at the national level, and an international research programme with a coordinated multidisciplinary laboratory and epidemiological approach would not

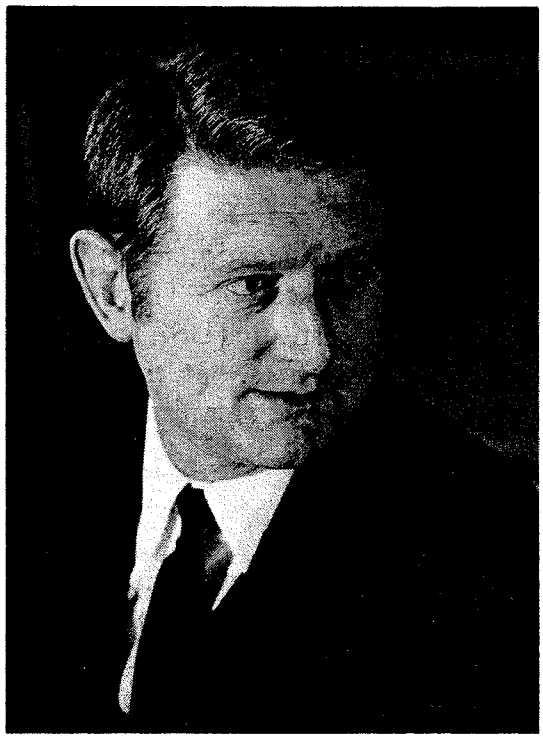
Fig. 3 New members of the Scientific Council



Professor A. Caputo



Professor S. Eckardt



Professor K. Munk

only provide valuable information to governments but would also stimulate national efforts in this direction.

Changes in patterns of disease and the significance of the chemical environment

There have been major changes in health patterns in most countries during the last five decades, as the communicable and parasitic diseases have come under increasing control. During the golden age of microbiology, the objectives of medicine were clear-cut. The results of research applied to disease prevention were obvious and exciting and medicine's role in improving the quality of life unquestioned. Unfortunately, these very successes have led to expectations from medical science far beyond the limitations of available technology, especially in relation to the diseases of later life. With present knowledge, we can only hope to retard the pathological lesions leading to degenerative diseases of the cardiovascular and cerebrovascular systems. On the other hand, there is certainly a possibility of preventing those many neoplasms that are causally related, directly or indirectly, to environmental stimuli.

The Agency's programme of research is aimed at that aspect of cancer prevention, and it has been confirmed by the decisions of the Governing Council setting out the priorities as:

(*a*) epidemiological and other comparative field studies, including the laboratory back-up necessary for investigating populations and their environments;

(*b*) work within the field of environmental carcinogenesis enabling the Agency to carry out an advisory role to responsible authorities; and

(*c*) training in manpower and in education with reference to fields of cancer epidemiology and environmental carcinogenesis.

The past ten years have confirmed the perspicacity of the founder Participating States, as there has been increasing emphasis during this period on international collaboration in cancer research, and in the field of environmental carcinogenesis the Agency's position is now firmly established.

International cancer planning

The Agency was involved with WHO Headquarters in drawing up a long-term plan for international cooperation in cancer research. This programme was based largely on the programme of the National Cancer Institute (USA) and that of the Council for Mutual Economic Assistance (COMECON) countries. It essentially covers all areas of cancer research, and it is unlikely that there will be significant changes in overall strategy in the foreseeable future.

Personnel

In June 1976, the established staff of 143 was made up of 35 scientists, 40 technical staff and 68 administrative and secretarial staff. In addition, there were 12 scientists working in the Agency as consultants or visiting fellows.

The Agency is continually being asked to provide guidance on national cancer programmes to both industrial and non-industrial states. The staff has done its best, but unfortunately this activity is limited, due to lack of manpower.

The epidemiological programme directed towards occupational carcinogenesis has been strengthened by the arrival of Drs R. Saracci and J. E. H. Milne, who are developing the study in the glass fibre industry.

There has been no significant increase in the Agency's permanent staff or programme since the budget was established in 1970. Although the budget has increased significantly in dollar terms, due to inflation this has not meant a comparable increase in activities. However, the Director has been continually conscious of maintaining the core level of expertise in the Agency's Headquarters since, if further Member States were to join the Agency, it would be possible to expand the field programmes markedly to a much greater extent through national laboratories, provided the Headquarters back-up staff is available.

Funding

During 1976, the income of the Agency totalled US $6 164 000. Of this, $4 223 000 came from the Participating States and the remainder from grants and contracts. The details of income and expenditure are given in Table 1.

The budget represents no change in real value compared with last year; the increases serve to offset the effects of inflation and exchange rate changes.

Table 1. Income and expenditure for 1976 [a]

	Amount (US $)	Percentage of total
Income		
Statutory budget	4 233 000	68.67
Extra-budgetary	1 931 000	31.33
Total	6 164 000	100.00
Expenditure		
Intramural		
Headquarters scientific staff	1 602 000	25.99
Administrative staff and office services	796 000	12.91
Building management	549 000	8.91
Laboratory research (supplies and staff salaries)	607 000	9.85
Publications and information programme	364 000	5.91
Building renovations	330 000	5.35
Other (data processing, library, organizational and scientific meetings)	161 000	2.61
Total	4 409 000	71.53
Extramural		
Contractual and collaborative research	1 312 000	21.29
Fellowships	269 000	4.36
Research centres	92 000	1.49
Duty travel	82 000	1.33
Total	1 755 000	28.47
Grand total	6 164 000	100.00

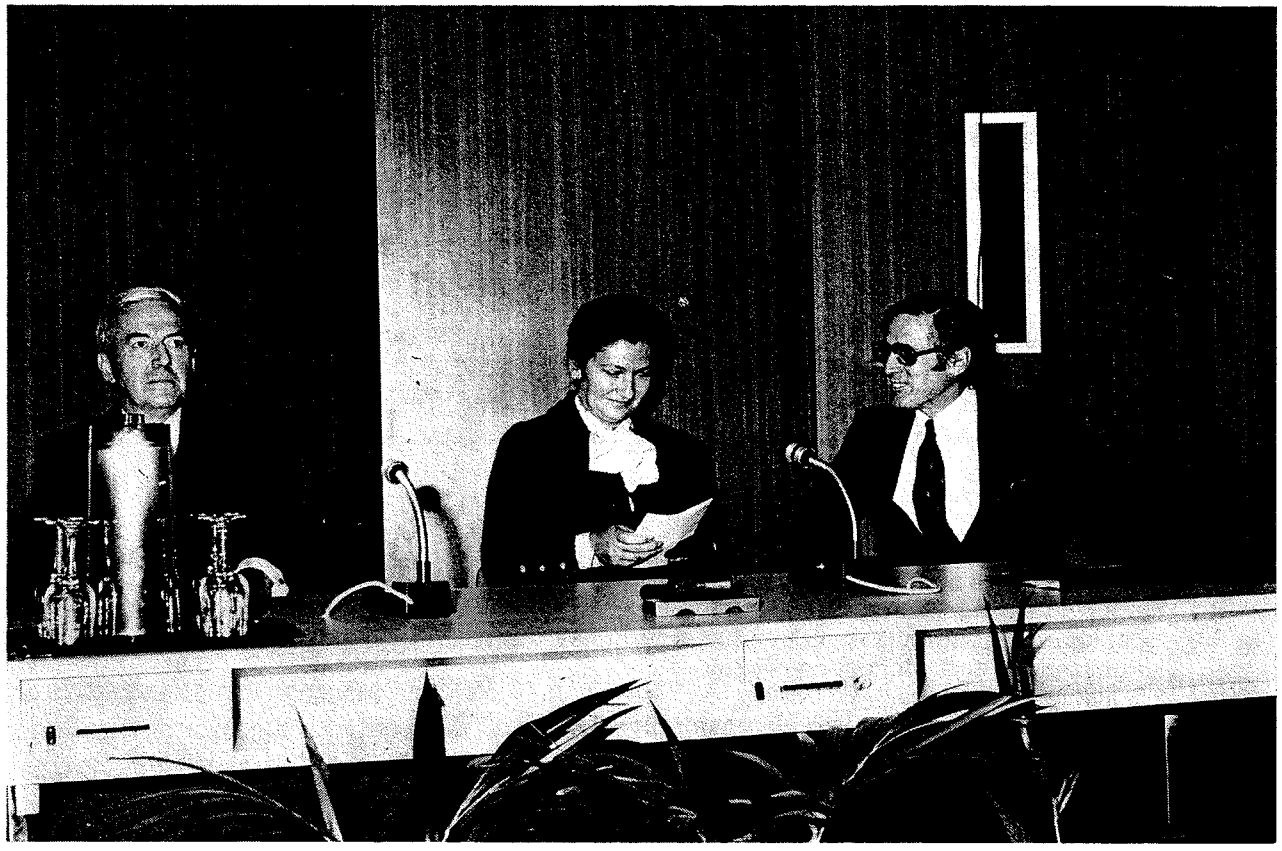
[a] All figures are estimates and based on information available in early October 1976

A joint meeting

It was with great pleasure that the Director and his staff welcomed Madame Simone Veil, French Minister of Health, when she came to the Agency in November 1975 to open the symposium on environmental pollution and carcinogenic risks. The symposium had been jointly organized by the Agency and the French National Institute of Health and Medical Research (INSERM). The one hundred and sixty participants from Europe and the United States included research scientists, environmentalists, public and occupational health officials, works medical officers, industrial management representatives and trade unionists.

Plans are already prepared for another joint meeting between the Agency and INSERM, in 1977.

Fig. 4 Madame Simone Veil, French Minister of Health, at the opening of the symposium on environmental pollution and carcinogenic risks, 3 November 1975 — left to right: Professor Pierre Denoix (French Director-General for Health), Madame Veil, Dr John Higginson



ENVIRONMENTAL CARCINOGENS — A CLARIFICATION

During its first decade, the staff of the Agency has constantly evaluated developments in the field of environmental cancer research. Among the earliest studies carried out was a further calculation of the proportion of cancers at each site that appeared to be related

directly or indirectly to environmental factors. By 1966 epidemiological data were available from many more populations than was possible hitherto, and the calculations confirmed the view that 60–90 % of all cancers were related to the environment.

The term environment, used here in its wider sense, can be divided into the 'macro or general environment', to which all individuals are exposed and which is largely conditioned by geographical and socio-economic factors, and the 'micro or personal environment', which results from the individual's cultural habits, like cigarette smoking, drinking, betel chewing, etc. Such a division is not, however, always simple, for occupational exposures may be considered to affect both the 'macro' and 'micro' environment.

Broadly speaking, change of the 'macro' environment will depend on action by 'society', by public health authorities, while the 'micro' environment is to a certain extent under the personal control of the individual.

In view of the socio-economic and health implications that might result from the banning of certain otherwise useful chemicals without sufficient reason, it is highly desirable that regulatory actions be governed, wherever possible, by scientific evaluation of available data rather than by sometimes ill-informed pressures from the general public as well as from scientific and political circles. However, today 'political oncology' must be accepted as a way of life in many countries, where groups with different interests and expertise feel justified in expressing their views.

The scientist must accept this and try to ensure the availability of accurate and carefully evaluated data on which appropriate legislation can be based. It is in this area that the Agency may play a major role in future years.

Possibilities for primary prevention

To date, it has been estimated that identified, exogenous, environmental stimuli are responsible directly or indirectly for 30–50 % of cancers in males in industrialized societies in Europe, North America and Asia. Of these, cancers due to cultural or personal environments are by far the most numerous; the micro environment created by cigarette smoking causes cancers of the lung, oesophagus and larynx. Oesophageal and liver cancers are related to excessive drinking, and cancers of the skin to excessive exposure to ultra-violet light while voluntarily sun-bathing or during open-air occupations.

Those cancers that can definitely be identified as originating from occupational exposures are relatively less numerous; it has been estimated that they vary between 1–5 % in different societies. However, these occupational cancers are exceptionally important, since not only may a carcinogenic hazard be more readily identified in such high-risk situations and preventive actions be taken, but industrial chemicals (e.g., asbestos) may also be widespread in the general environment, resulting in extensive exposure of large human populations.

In addition, there is circumstantial evidence that between one-half and two-thirds of those cancers for which etiological factors have not been established are directly or indirectly related to the environment. The data on which this hypothesis has been based have been the subject of a recent publication from the Agency [1]. However, the conclusion that approximately 80 % of all cancers are due to environmental stimuli has been the subject of considerable misinterpretation, among which the following may be cited:

(i) that this is an established fact
The evidence is in reality largely circumstantial, and the conclusion represents only the most satisfactory and logical explanation for the available data;

(ii) that it is possible to prevent 80 % of all cancers *immediately*
The exact identity of many suspected environmental stimuli is difficult to establish, especially as they cannot be measured with accuracy. This means that no immediate approaches to control are possible for these factors;

(iii) that all carcinogenic environmental factors are industrial in origin
The most important identified carcinogenic stimuli are cultural in origin;

(iv) that only direct carcinogens are important
Co-factors of hereditary and environmental origin may also be very important;

(v) that this estimate of 80 % applies to all populations
In fact, estimates will vary according to different populations and to the type of cancer common in each population. For example, the proportion of cancers in females which are of known etiology is much smaller than in males; and

(vi) that 'community' action alone will be effective in controlling cancer.

However, rates of cancer lower than those prevalent in many industrialized countries in North America and Europe are possible. Apart from cancers of the skin, males in rural Norway have only 60 % of the cancer rate observed in males in Connecticut or in other Scandinavian countries. In the United States, Seventh Day Adventists have been found to have a cancer rate about half that of the national average, and Mormons in Utah have only 60–76 % of the average national cancer rate.

The role of diet in human cancer is not clearly defined, but obesity, over-eating and dietary patterns are apparently associated with increased frequencies of certain cancers, including those of the colon and possibly that of the breast. In this context, it should be noted that many Seventh Day Adventists live on a lacto-ovo vegetarian diet and do not smoke or consume alcohol.

The role of man-made chemicals in the general environment has not been established with certainty, but in most countries rates for cancer at many sites are lower in than in urban communities, suggesting the presence of deleterious factors in urban areas, which are not necessarily of recent origin.

Planning to reduce cancer — personal responsibility

There has been a tendency to think of primary prevention as the responsibility of the community, but for many cancers some degree of personal responsibility must be recognized. Individual action is not only possible but is essential for producing any significant decrease in cancer incidence. Thus, the evidence would suggest that a male who does not smoke, who eats and drinks with moderation and who limits his exposure to sunlight may reduce his risk of developing cancer by at least 30–40 % or possibly more according to the locality

[1] Higginson, J. & Muir, C. S. (1976) *Cancer Detect. Prev.*, **1**, 79–105.

in which he lives. The corresponding figures for females are somewhat smaller. Nonetheless, such major changes in life style must be started early in life, as the later these measures are taken the less effect they have. This conclusion is not based on new data but is, rather, a conservative estimate, calculated from available epidemiological studies.

The development of a disciplined *Personal Cancer Plan*, by modification of a life style, represents the individual's responsibility. If he does not accept this, because of age or lack of education or will-power, he should at least ensure that his children have the opportunity of doing so. No one should rely on some future community action as an excuse for avoiding personal action now for himself or for his family.

Planning to reduce cancer — community responsibility

Certainly a personal cancer plan throws the onus to take action on the individual. It also places a very heavy responsibility on research workers and public health officials to investigate the nature of the environmental factors involved, to establish priorities and to ensure the dissemination of data on which the individual and communities can act. Such information will depend largely on epidemiological studies, and it is to this end that the Agency is directing its resources. The role of the research worker is especially important in relation to such complex issues as long-term medicinal drug-taking, or the effects of air pollution, and his results should be communicated carefully: it is often exceedingly difficult for the non-expert to understand all the issues involved.

The fact that the prevention of some cancers may result from personal action does not in any way reduce the obligation of governments, public health authorities, employers, etc. to ensure the greatest possible safety of the present environment. Nor does it absolve industry or trade unions from helping to establish the facts regarding occupational risks by maintaining appropriate lists of all potentially exposed individuals and appropriate documentation of exposure levels to suspected chemicals. Prudence requires that we should not unreasonably and carelessly introduce proven animal carcinogens into the human environment.

Where exposure to carcinogens occurs, appropriate legislative action should ensure that such exposures are strictly controlled, if no substitutes are available for essential chemicals. A pragmatic approach towards environmental carcinogens such as this implies that modern society represents a team and not warring factions.

A balanced approach

In the immediate future, more cancers may be preventable by personal action of an individual to control the 'micro' environment of himself and his family, and such a personal cancer plan should be featured essentially as part of the public health education in any national cancer plan. Unfortunately, there is little evidence at present that in medical schools, among doctors and public health workers the necessary self-control is well enough practised to be effectively preached.

At the same time as efforts are made to stimulate individual preventative action by educating and encouraging people to develop a personal cancer plan, there is great need to keep close watch on the introduction of new chemicals into the working and general

environment. This implies a careful collection of data on exposure and on biological effects and, where relevant, epidemiological data to ensure that these are responsibly evaluated and made available to the authorities and interested groups who should be expected to take regulatory and controlling action when necessary. Care should be taken that data are made available, therefore, in a form that is widely comprehensible. The Agency has a major role in preparing authoritative, internationally acceptable evaluations of the cancer hazards that may be involved with the use of certain chemicals and of ensuring the dissemination of their evaluations.

AN INTERNATIONAL CANCER SURVEILLANCE NETWORK

The Agency is contributing to the effort to identify new etiological factors for cancer and at the same time ensuring that the relative importance of different putative etiological agents be evaluated in terms of the society in which they are found. This is particularly important, since the Agency works in countries in all stages of industrial development, and health priorities are different in each situation. Thus, in African males today, liver cancer is still one of the most prevalent tumours, whereas tobacco-related cancers are much less common. Many other cancers common in industrial societies occur relatively rarely in other communities. It is highly desirable to follow these latter populations during the coming decade in order to determine whether or not the differences in cancer patterns change as the 'way of life' changes.

The importance of environmental factors in causing cancer in man is being recognized increasingly, resulting in strong support from both scientists and the general public for the expansion of research into environmental factors in human cancer. However, as more and more countries become increasingly industrialized, the chemical environment will become of increasing concern both because of its socio-economic importance and because of its impact on health. It is necessary that in this period the public be reassured that every possible action is being taken to protect the individual.

The present situation demands a long-term commitment to the collection of cancer data in man relative to his environment, with a view to providing, over a period of years, accurate data on which cancer control, including legislation, could be based. It would represent an extension of the present epidemiological programme of the Agency.

The research priorities in environmental carcinogenesis are two-fold: evaluation for man of cancer risks that may be already present, and the provision of a mechanism for the combined monitoring and continuing evaluation of suspected new risks.

There is, however, an absence of epidemiological data on the chronic effects in man of different environments—this lack is abundantly documented in the IARC series of *Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, and there is, therefore, a tendency at the present time to initiate *ad hoc* epidemiological studies once a report of carcinogenic action in experimental animals is published and an exposed group identified. It is not possible at present to call upon an established epidemiological mechanism for evaluating the extent of a suspected hazard.

Within industrialized states the environment tends to be relatively homogeneous, and the monitoring of cancer frequencies for the identification of possible etiological factors

may not be possible unless a group at high risk can be studied. International comparisons between industrialized and non-industrialized states may, therefore, yield valuable data.

In the past, the role of the cancer registry in environmental carcinogenesis has been insufficiently appreciated, since registration is a long-term project that is unlikely to prove effective until a sufficient body of data has been built up.

What is now proposed is essentially the creation of an international cancer surveillance network, which would represent expansion of a long-range programme already approved by the Governing and Scientific Councils of the Agency.

A limited, high-quality epidemiological network should be developed to provide reliable information on cancer risks and environment in specially selected areas. The data processing necessary for such a network is technically feasible, but there is a deficiency in the data available for input. A mechanism needs to be set up for the control of quality of data and for record linkage in both industrialized and non-industrialized countries.

Cancer morbidity data

At present, the Agency, in collaboration with the International Association of Cancer Registries, publishes standardized cancer incidence figures from approximately 80 populations collected by cancer registries in 28 countries. A number of these registries, perhaps 8–10, chosen in key environments throughout the world, need to be upgraded:

— to provide a continual inflow of data that can be used to identify changes in cancer patterns in specific population groups that might signal changes in their environments;

— to identify specific high-risk groups from several areas. The data may then be combined for several groups to provide adequate numbers to permit rapid identification of a new occupational, cultural or other hazard;

— to stimulate the establishment of record linkage systems in certain key areas.

The success of such a programme will depend in large measure on a long-term commitment being made to ensure the availability of the appropriate statistical staff at both national and international levels to ensure continual processing of the data.

Data on levels of environmental carcinogens

The establishment of a network of national laboratories, associated with the selected cancer registries, would be required to provide data on exposure levels to putative carcinogens for the general population and for specific high-risk groups. Such a programme would require international agreement on what chemicals were likely to present the greatest hazards, either because of a known human exposure or because of the suspected carcinogenic activity of a compound.

The selection of standard analytical techniques would be essential to permit valid comparisons of exposure levels in different communities, and it will also be necessary to establish 'background' levels of suspected and known chemical carcinogens in order to make comparisons between various environments, industrial and non-industrial, rural and urban, for example. These data are essential so that exposure levels in, say, atmospheric pollution, could be placed in the context of the overall chemical environment.

This part of the programme would probably be more difficult to implement, since the logistic and technical problems are considerable.

Funding of the proposed surveillance network

The proposed network of registries should reach into both industrialized and non-industrialized countries and should be selected to ensure high levels of quality control and efficiency. The network, limited to 8–10 centres, will be based on existing cancer registries, and for those in industrialized countries, the cost of operation should be borne largely by national funds. Close collaboration with national occupational cancer studies would be organized. The registries located in non-industrialized countries may require funding from external sources, since funds are unlikely to be available locally. In any case, provision of external funding assures the maintenance of quality control in such international studies.

Such a network could also contribute to other studies which might develop in fields such as congenital abnormalities or drug effects, and could complement similar national programmes within industrialized countries.

The role of the Agency

Since the primary objectives of the Agency are research on environmental carcinogens and the epidemiology of cancer, it is particularly well placed to organize such a programme calling for both laboratory and field work. Moreover, as an intergovernmental organization, it is able to organize the necessary collaboration between the scientists from both industrialized and non-industrialized countries.

The Agency already has available the staff and expertise and, through the International Association of Cancer Registries and its publication *Cancer Incidence in Five Continents*, already has the basis on which to develop such a programme. The Monographs series of the Agency has already indicated priorities of chemicals for study and the areas where information on environmental exposure is needed.

1. UNIT OF EPIDEMIOLOGY AND BIOSTATISTICS

Dr C. S. MUIR (Chief)

1. INTRODUCTION

The Agency continues its coordinating role in the field of descriptive epidemiology, contributing actively to the development of the chapter on neoplasms in the Ninth Revision of the *International Classification of Diseases, Injuries and Causes of Death* and to a related, specialized adaptation, the ICD-Oncology, as well as to the work of the International Association of Cancer Registries. Data for inclusion in the third volume of the monograph, *Cancer Incidence in Five Continents*, have been prepared for publication, and a monograph on *Cancer Registration Purposes and Techniques* nears completion. The Clearing House for On-going Research in Cancer Epidemiology has produced its first annual directory.

The search for etiological factors in oesophageal cancer continues, particularly in the Caspian littoral of Iran and in Britanny, France (see pages 125 and 35). The Unit coordinates an extensive series of studies to explore the role of alcoholic beverages in neoplasia. Work continues on the study of the familial occurrence of breast cancer in Iceland, on the frequency of 'latent' carcinoma of the prostate and on the possible association between differences in large-bowel cancer incidence in Denmark and Finland and various aspects of diet and bowel function. A study of the health effects of man-made mineral fibres, with particular reference to cancer, has been started in Europe.

The statistical section has contributed to virtually all of the research programmes of the Agency and plays an increasing role in the design of research projects.

2. DESCRIPTIVE EPIDEMIOLOGY

The aim of the programme of descriptive epidemiology is to map the occurrence of cancer throughout the world and to improve the comparability of data on incidence. The recognition of differences in cancer risks in different populations facilitates the formulation of etiological hypotheses for further study by the Agency and other bodies [1].

2.1. *International Classification of Diseases (ICD)* (Dr C. S. Muir)

In conjunction with the International Classification of Diseases Unit, WHO Headquarters, work continued on proposals for the chapter on neoplasms to be incorporated

[1] Muir, C. S. (1976) In: Fraumeni, J. F., Jr, ed., *Persons at High Risk of Cancer, An Approach to Cancer Etiology and Control*, New York, Academic Press, pp. 293–305.

in the Ninth Revision of the ICD. The draft chapter was presented and defended at the Revision Conference held in Geneva from 30 September to 6 October 1975 and will come into effect in 1979. In addition, members of the Unit staff continued to participate in a series of working parties.

(*a*) *Morphology code working party* (Dr H. Tulinius)

Further meetings were held to expand the code contained in the widely-used *Manual of Tumor Nomenclature and Coding* (MOTNAC) of the American Cancer Society. These resulted in a five-digit code, compatible with the MOTNAC code, which embodies a system permitting the user to create, within limits, his own code for terms not specifically listed; e.g., 'benign nephroblastoma' is not included, but such a diagnosis could easily be coded. Particular attention has been paid to the classification of malignant lymphomas and to use of the histological terms appearing in the 'Blue Books' *(International Histological Classification of Tumours)* prepared by the Cancer Unit of WHO Headquarters.

(*b*) *Index working party* (Mrs J. Nectoux)

All terms relating to neoplasia in the Seventh and Eighth Revisions of the ICD have been identified and examined systematically with a view to the creation of a catalogue showing the codes used for given terms or cancer sites in the Seventh, Eighth and Ninth Revisions of the ICD. In the course of this work, over 5 000 index terms have been evaluated, and obsolete descriptors have been removed.

(*c*) *Coding rules working party*

The coding rules committee met again in June 1976 to assess proposed changes in coding rules.

(*d*) *ICD-Oncology (ICD-O)* (Dr H. Tulinius)

When the Agency was asked by WHO in 1968 to advise on the chapter on neoplasms for the Ninth Revision of the ICD, a classification based on three axes—topography, morphology and behaviour—was proposed. While this was judged unsuitable for mandatory international use, it was nevertheless agreed that a specialized adaptation—the ICD-Oncology or ICD-O—should be devised along these lines. The ICD-O is collapsible into the neoplasm rubrics of the Ninth Revision of the ICD.

The various working parties agreed that before its publication in a definitive form ICD-O should be tested by a variety of users. Following meetings in Paris, Washington and Leningrad, arrangements were therefore made to conduct trials in cancer registries, national vital statistics offices, hospital record rooms and pathology laboratories. The collaboration of 65 institutions in 15 countries was obtained, and over 35 000 diagnoses were coded according to the Field Trial Version of ICD-O. Although no problems occurred for 88 % of diagnoses, at a meeting held in Paris on 8–11 June the difficulties were examined and the ICD-O amended. The final version will be published in October 1976. A full list of persons who collaborated in the preparation of ICD-O is given in the preface to that publication.

(*e*) *Standing Harmonization Committee* (Dr C. S. Muir)

This committee (SHC) was created by WHO to ensure coordination between the various working parties involved in the ICD-O. At a meeting held in Le Vésinet, France, 8–11 June 1976, the SHC approved the English language version of the ICD-O, amended in the light of field trial experience, and noted with pleasure that arrangements were well advanced for versions in French, Italian, German, Japanese, Portuguese, Spanish and Russian.

2.2 *Cancer registries*

(*a*) *International Association of Cancer Registries* (Dr C. S. Muir)

In April 1973, an agreement (RA/73/016) was signed with the International Association of Cancer Registries, whereby the Agency agreed to provide a secretariat and deputy secretary for the Association. Dr C. S. Muir, Chief of the Unit of Epidemiology and Biostatistics, agreed to serve as the Deputy Secretary. This agreement has now been renewed.

The Association held a one-day business meeting in Lyon on 27 September 1975, following the meeting on Cancer Registries and Occupational Cancer [see (*e*) below]; it was attended by 40 persons.

An Association Newsletter has been created and will be published periodically.

(*b*) *Caspian Cancer Registry* (see report of the Teheran Research Centre, page 125)

(*c*) *Singapore Cancer Registry* (see report of the Singapore Research Centre, page 120)

(*d*) *Jamaica Cancer Registry* (Professor S. E. H. Brooks: RA/72/014)

The Agency continues to provide partial financial support to this registry, data from which appear in the third volume of *Cancer Incidence in Five Continents.*

(*e*) *Cancer registries and occupational cancer* (Dr C. S. Muir and Miss S. Whelan)

A workshop and training course were held in Lyon on 22–26 September 1975, which brought together workers in environmental health and in cancer registries to discuss how they could pool resources and most efficiently assess occupational cancer risk. The meeting was organized by Dr A. Englund (Sweden), Dr J. Goldsmith (USA), Mr G. Linden (USA), Dr K. Magnus (Norway), Dr C. S. Muir (IARC), Dr E. Pedersen (Norway) and Dr J. A. H. Waterhouse (UK) and was attended by 142 persons from 28 countries.

Before the workshop, participants were asked to submit statements on occupational cancer problems believed to exist in their countries and on means by which these might be investigated. Over 40 replies were received from 20 countries; and while the problems in all countries were largely similar, there appeared to be only minimal international collaboration. At the meeting, the need for such collaboration for the rapid assessment of risks was stressed. The confidentiality of records and data linkage gave rise to some concern.

The practical work of the course comprised critical examination of selected published papers, investigation of an imaginary industrial hazard and tutorial groups on the taking of occupational histories, monitoring and data analysis.

(*f*) *Cancer registries in Latin countries* (Dr C. S. Muir)

The Agency was represented at a meeting (Chairman: Professor E. Anglesio, Turin) held at the Geneva Cancer Registry (Dr G. Riotton and Mr L. Raymond) on 27–28 May 1976 on the problems of cancer registries in Latin countries. Following reports on cancer registries in France, Italy, Portugal, Spain and Switzerland, there were discussions on the education and training of registry personnel and on the problems of confidentiality which often impose constraints on the operation of cancer registries.

(*g*) *Advice to cancer registries and other bodies*

An increasing number of requests for advice and assistance are being received by the Unit, many being referred by the International Union Against Cancer and the Cancer Unit of WHO. Advice was given to the following countries:

Algeria : on exploitation of existing data on cancer frequency and on improvement of the recording system (Professor A. Yaker, Chief, Laboratory of Pathology, Mustapha Hospital, Algiers)

Burma : on the conduct of the Rangoon Cancer Registry (Dr U. Tin Aung, Department of Radiotherapy, General Hospital, Rangoon)

Fiji : on improvement of the functioning of the Fiji Cancer Registry (Medical Section of the Ministry of Health, Suva)

France : on the conduct and analysis of a study on malignant lymphomas in Bordeaux (Madame F. Bonichon, Institut Bergonié, Bordeaux); and on the creation of a cancer registry in Dijon (Dr J. Faivre, General Hospital, Dijon)

India : on the establishment of a cancer registry in Chandigarh (Professor B. K. Aikat, Director of the Postgraduate Institute of Medical Education and Research, Chandigarh)

Italy : on the creation of a cancer registry in Ferrara (Professor I. Nenci, Institute of Pathology, University of Ferrara, Ferrara)

Pakistan : on cancer registration in selected areas of Pakistan (Dr Javid A. Hashmi, Medical Research Council of Pakistan; Dr S. H. M. Zaidi, Department of Radiotherapy, and Dr N. A. Jafery, Department of Pathology, of the Jinnah Postgraduate Medical College)

Spain : on establishment of a cancer registry in Oviedo (Dr J. Sanchis and Dr A. Brugarolas, General Hospital of Asturias, Oviedo)

Switzerland : Advice continues to be given to the various cantonal cancer registries.

UK : on the planning and conduct of international studies (Dr J. A. C. Weatherall, Office of Population Censuses and Surveys, London)

In addition to the above, many enquiries about the chapter on neoplasms in the *International Classification of Diseases* and on the availability of information on the geographical distribution of various cancers were answered.

2.3 Cancer Incidence in Five Continents (Dr C. S. Muir, Miss V. Lampert and Miss S. Whelan)

The third volume of *Cancer Incidence in Five Continents*, containing incidence rates for 78 populations in 28 countries, will be published in 1976 [1].

During the past years cancer incidence data submitted to the editors for 92 populations in 34 countries have been exhaustively cross-checked by a series of operations described in detail in the publication [1]. Tables giving age-standardized incidence rates for selected four-digit rubrics have been prepared. Computer programmes have been written for the presentation of cumulated rates, the proportion of cases with histological confirmation of diagnosis, the proportion of registrations based on a death certificate only and comparison of mortality and morbidity rates in each registration area.

Extensive inquiries about variations in registry coding practices have been made: even minor variations may entail numerous 'tailor-made' amendments to computer programmes. The University of Birmingham Computer Centre has continued to make available its facilities, including a 160-character line-printer.

As the third volume also contains rates from registries which have continued to collect data according to the Seventh Revision of the ICD, tables for converting information to the Eighth from the Seventh Revision and *vice versa* have been prepared.

2.4 *Monograph on techniques of cancer registration* (Dr R. MacLennan and Dr A. J. Tuyns)

A monograph on the purposes and techniques of cancer registration is being prepared in collaboration with the International Association of Cancer Registries (Dr R. Steinitz) and the WHO Cancer Unit (Dr A. Winkler). Such information will help to improve international comparability of registry data and will serve as a guide for the new population-based cancer registries being established in many countries. As many such registries receive their reports from hospital-based registries, great care has been taken to ensure comparability with definitions of items in the WHO *Handbook for Standardized Cancer Registries* (Hospital-Based), which is primarily concerned with hospital cancer registries.

2.5 *Ratio studies*

In many regions of the world where cancer patterns are still ill defined, mortality data may be poor or non-existent and cancer registration difficult. Under these circumstances the relative frequency of the various sites of cancer seen at an institute or a pathology laboratory may form a useful indication of cancer distribution. The Agency continues to foster the collection of such material and to assist in its analysis.

(*a*) *Cameroon* (Dr O. M. Jensen and Dr A. J. Tuyns)

In collaboration with Dr P. Ravisse of the Pasteur Institute of Cameroon, 1 390 male and 1 418 female cases of cancer examined histologically in Cameroon between 1969 and 1973 were analysed. Some 30% of male cancers and 20% of female cancers were skin tumours; squamous-cell carcinomas, mainly located on the lower limbs, were dominant in both sexes. Basal-cell carcinomas were rare and almost entirely confined to the head and neck. More than 30% of all skin cancers were Kaposi's sarcomas; these were very frequent

---

[1] Waterhouse, J. A. H., Muir, C. S., Correa, P. & Powell, J., eds (1976) *Cancer Incidence in Five Continents*, Vol. 3, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 15**) (in press).

in males but occurred in only 4% of females. About 11% of all cancers in males were primary liver carcinomas; 16% of cancers in females were of the cervix uteri. Among children, 13% of all tumours were Burkitt's lymphomas, the frequencies being equal in both sexes. Regional differences were noted with regard to cancers of the skin (Kaposi's sarcomas and squamous-cell carcinomas), buccal cavity, lung and bladder; these are being explored further.

(*b*) *Indonesia* (Dr O. M. Jensen and Dr C. S. Muir)

In collaboration with Dr Soeripto of the Pathology Department of the Jogjakarta Medical School, 1 220 male and 2 102 female cases of cancer diagnosed at the department during the years 1970–1973 were analysed. This material has now been prepared for publication.

(*c*) *Cancer in Eskimos* (Dr O. M. Jensen)

Recent studies in Canada [1] and Alaska [2] suggest that the cancer pattern in Eskimos differs from that of white populations of the same countries; in particular, an excess of nasopharyngeal cancer was observed in both sexes, and a deficit of breast cancer was found among women. In view of the limited numbers of these population groups within national borders in arctic areas, the Agency is coordinating a multi-national study of cancer incidence in Eskimos in Greenland (Denmark), Canada and Alaska (USA).

2.6 *Clearing house for on-going research in cancer epidemiology* (Dr C. S. Muir, Mrs A. Nagy-Tiborcz and Mrs E. Démaret)

Many studies of cancer epidemiology take from three to five years to be completed and published. During this period, other scientists may initiate similar studies, unaware that workers are already pursuing the same problem.

The Agency and the German Cancer Research Centre, Heidelberg (Professor G. Wagner, Dr C. Köhler, Mr K. Schläfer) have thus created a Clearing House for On-Going Research in Cancer Epidemiology (RA/74/003) which operates within the framework of the International Cancer Research Data Bank Program of the National Cancer Institute of the USA and which is partially supported by that body. Scientists can refer to the Clearing House to find out about studies in their fields of interest, either to avoid duplication, or, if repetition of a study in another population seems desirable (as is frequently the case in cancer epidemiology), so that further studies can be designed in such a manner as to make the results comparable.

The topics included in the Clearing House comprise:

1. Prospective and retrospective studies
2. Occupational exposures

---

[1] Schaefer, O., Hildes, J. A., Medd, L. M. & Cameron, D. G. (1975) *Canad. med. Ass. J.*, **112**, 1399–1404.
[2] Lanier, A. P., Bender, T. R., Blot, W. J., Fraumeni, J. F. & Hurlburt, W. B., personal communication.

3. Population genetic or biochemical markers, including familial and twin studies
4. Incidence and mortality statistics
5. Relative frequency series
6. Correlation studies
7. Methodological studies
8. Sero-epidemiology and immunology relating to defined populations

The Clearing House does not solicit information on clinical trials, diagnosis or mass-screening programmes unless these include epidemiological evaluation.

To date, an address list of 4 500 persons has been created in Lyon and entered on magnetic tape in Heidelberg; 3 875 invitations to participate and 2 235 reminders have been sent out. Over 500 relevant replies reporting some 700 projects have been received; about 200 research workers replied that they had no project to report, and 300 wrote that they were not working in the field of cancer epidemiology. Projects which were considered not to fall within the scope of the Clearing House were reported by 75 workers; these were sent to the Current Cancer Research Project Analysis Center, another segment of the International Data Bank, or to the Clearing House for Controlled Therapeutic Trials in Cancer operated by UICC.

The first of a series of annual *Directories of On-Going Research in Cancer Epidemiology*, incorporating this material, has now been published.

2.7 *Lifetime cancer risks* (Dr C. S. Muir)

Although cancer might be considered to be an uncommon disease, since only three new cases are diagnosed annually for every 1 000 of population, the risk of contracting the disease over a lifetime is very high indeed—in the order of 1 in 4 to 1 in 5.

A consultant (Dr W. P. D. Logan) has examined the feasibility of expressing the risk of developing and dying from cancer over a lifetime and for specified periods of the lifespan by site, sex, geographical region and selected exposures, using the mortality data currently available from WHO and the incidence information contained in the monographs on *Cancer Incidence in Five Continents*.

A life-table model has been prepared, based either on a national or on a world population life table, the latter being theoretically more suitable for international comparisons. An example of the results obtained using the world model is given in Table 2. Further work on the risks in specific occupations and exposures is planned.

In this connection, Dr N. E. Day has proposed the use of a cumulated rate which is readily computed from the age-specific incidence and which is a close approximation of the actuarial or cumulative risk. The cumulative risk represents the chance of developing cancer over a given age span in the absence of other causes of death [1].

---

[1] Day, N. E. (1976) In: Waterhouse, I. A. H., Muir, C. S., Correa, P. & Powell, J., eds., *Cancer Incidence in Five Continents*, Vol. 3, International Agency for Research on Cancer (*IARC Scientific Publications* No. 15) (in press).

Table 2. Percentage risk of developing cancer before age 85

	Norway 1964–1966		Scotland 1964–1966	
	M	F	M	F
Stomach	4.0	2.8	2.0	1.4
Lung	1.9	0.5	6.3	1.1
Breast	—	5.1	—	4.6
Cervix uteri	—	1.6	—	1.2
Other uterus	—	1.0	—	0.8
Prostate	5.4	—	2.3	—
Leukaemia	0.7	0.6	0.4	0.3
All sites	22.8	22.6	21.8	20.1

3. ANALYTICAL EPIDEMIOLOGY

3.1 *Etiological factors in oesophageal cancer*

(*a*) *France* (Dr A. J. Tuyns and Dr O. M. Jensen)

Mortality studies. An analysis of mortality data in France for the period 1951–1971 has been published [1]. There was a continuous increase with time in the death rate from oesophageal cancer in French males but not in females.

(i) *Cohort study*

A cohort effect could be demonstrated [1], and on further study [2] this was shown to have a double wave: cohorts born between 1892 and 1901 experienced an increased mortality from oesophageal cancer, but there was no such increase for those born between 1902 and 1916. Cohorts born thereafter had an increased mortality.

A similar analysis made for lung and pancreatic cancer—sites for which an increase with time is also known to occur—failed to show any interruption in the increase. In contrast, an identical break was observed for cancer of the larynx and for alcoholic cirrhosis; this may be related to the shortage of alcoholic beverages during the Second World War, which would have prevented those born between 1902 and 1916—aged 24–38 in 1940—from acquiring their normal drinking habits and would thus break the upward trend in diseases related to alcohol consumption [2].

(ii) *Correlation studies*

The correlation described earlier between mortality from oesophageal cancer and mortality from alcoholism and liver cirrhosis, made with material from 1959–1963, was confirmed with more recent data (1967–1968) [1]. The correlation was demonstrable for the 45–64 and 75+ age groups and again appeared to be higher for alcoholism than for cirrhosis.

[1] Audigier, J. C., Tuyns, A. J. & Lambert, R. (1975) *Digestion*, **13**, 209–219.
[2] Tuyns, A. J. & Audigier, J. C. (1976) *Digestion* (in press).

(*b*) *Brittany*

Morbidity studies. A detailed description of the operational aspects of the oesophageal cancer registry conducted in Rennes (Ille-et-Vilaine), and of the results obtained, has been published [1].

The overall, average, annual, age-adjusted incidence rate established for males was 29.4 per 100,000, but in some rural areas the rates were over 60.0. Fifty per cent of the tumours were in the middle third of the oesophagus, about thirty per cent were in the upper third, and the remainder were in the lower third; most were squamous-cell carcinomas.

Case-control studies. The material collected during the case-control study in Ille-et-Vilaine has been analysed further in cooperation with the Nutrition Section of INSERM (Dr G. Péquignot).

(i) *Mean alcohol consumption*

Information concerning the mean consumption of various alcoholic beverages among the population control groups by sex and age [2] provided baseline data for comparisons among the various groups studied. It became clear, however, that the distribution of persons within a population by daily alcohol consumption is not normal but log-normal. The geometric means for the various control and study groups [3] are shown in Figure 5.

The rank order is that which would be expected. With regard to the 95 % confidence limits for each group, the lower limit is high for patients with liver cirrhosis and delirium tremens, indicating that below a certain level of alcohol intake the probability of developing one of these diseases is rather low. Although the mean consumption of oesophageal cancer patients is high, the lower limit of intake falls within the consumption of control groups. The occurrence of cases in which only a low level of alcohol consumption was noted shows that other etiological factors are involved.

(ii) *Comparison of hospital controls with population controls*

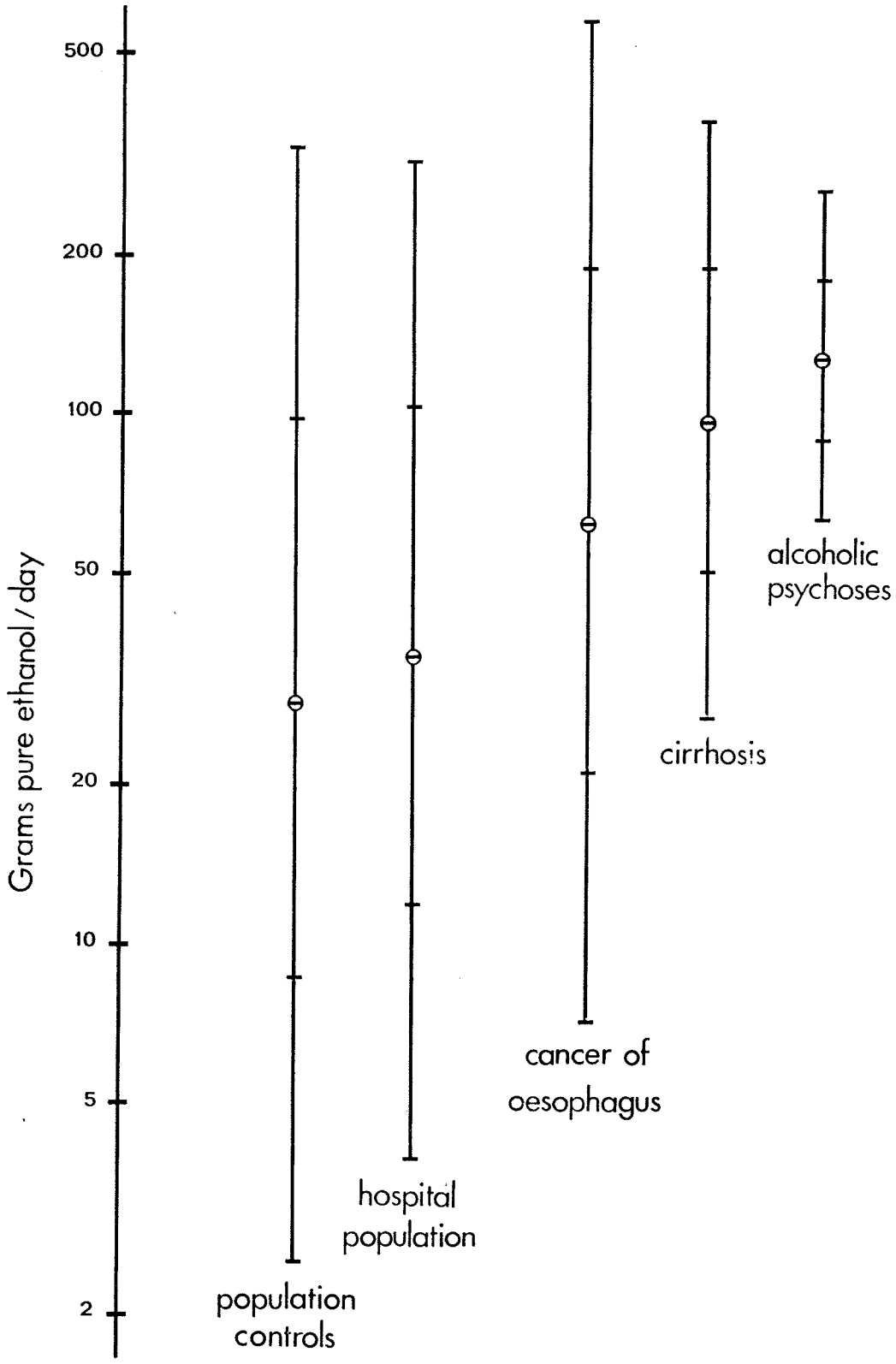
Preliminary studies indicated that population controls provide a better baseline than hospital controls for comparison with groups of patients in relation to alcohol and tobacco consumption.

The levels of consumption among hospital controls were higher than those in the population sample. These differences have been further investigated: the higher consumption of alcohol observed among hospital controls was found to be due to the inclusion among them of some cases of cancers of the buccal cavity—a neoplasm known to be related to alcohol consumption. After removal of these individuals, there was no significant difference between the two groups.

The excess proportion of smokers among hospital controls could be only partly explained by the inclusion of cases of laryngeal and bronchial cancer in the group. There was an overall, excess consumption of tobacco among hospital controls that persisted even when groups of diseases such as cardio-vascular disorders, which are known to be associated with smoking, were taken into account. This finding further illustrates the widespread noxious effects of tobacco. Since the pattern of tobacco consumption observed in the

[1] Tuyns, A. J. & Massé, G. (1975) *Ouest méd.*, **28**, 1757–1770.
[2] Tuyns, A. J., Pequignot, G., Jensen, O. M. & Pomeau, Y. (1975) *Rev. Alcool.*, **21**, 105–150.
[3] Pequignot, G. & Tuyns, A. J. (1975) *INSERM Publications* No. **54**, pp. 23–39.

Fig. 5 Average alcohol consumption in various study groups of Ille-et-Vilaine males. Log means ± 2 standard deviations



general population was consistent with available data on tobacco sales, it was concluded that the smoking data obtained from the hospital controls gave an inaccurate picture of the normal population and could therefore be misleading in comparisons examining the etiological role of this factor.

These methodological considerations are of considerable practical importance: under some circumstances the use of hospital controls might conceal the association of an agent with a cancer. Their use in the calculation of relative risks may result in an underestimation of dose levels at which carcinogenic agents are active.

(iii) *Roles of tobacco and alcohol*

The nature of the interaction of dose-response relationships for each of these two factors, independently and in combination, was investigated further. The model that provides the best fit is a multiplicative one (Table 3).

Table 3. Distribution of 200 cases of oesophageal cancer by tobacco and alcohol consumption in grams of pure alcohol or grams of tobacco

	Alcohol consumption	Tobacco consumption			
		0–9	10–19	20–29	30+
Observed	0– 40	9	10	5	5
	41– 80	34	18	15	9
	81–120	19	18	6	7
	121+	16	12	7	10
Expected (on basis of population controls)	0– 40	67.21	22.23	9.87	4.79
	41– 80	34.59	16.06	12.70	1.92
	81–120	12.08	9.92	3.56	0.63
	121+	2.41	1.36	0.38	0.48
Expected (on basis of multiplicative model[a])	0– 40	14.38	6.55	3.32	4.75
	41– 80	30.72	19.65	17.73	7.90
	81–120	17.63	19.94	8.17	4.26
	121+	15.26	11.86	3.78	14.09

[a] Relative risk for a given level of tobacco consumption multiplied by relative risk for a given level of alcohol consumption multiplied by expected numbers on the basis of population controls

(iv) *Roles of type of tobacco smoking and alcohol drinking*

Further attention was given to type of smoking: after adjustment for age and alcohol drinking and total amount of tobacco smoked, the relative risks for oesophageal cancer for smokers of commercial cigarettes and smokers of hand-rolled cigarettes were found to be 6.2 and 7.8, respectively, when compared to that for non-smokers. The risk is also increased for pipe smokers.

A similar analysis for type of alcoholic beverage was difficult to make, in view of the large number of patterns possible when five types of beverages are considered. The consumption of only one type of alcoholic beverage is rare; thus, a straight-forward calculation of the risk related to a particular type of beverage is impossible. The role played by each type of beverage will now be examined by multivariate techniques.

(*c*) *Normandy* (Dr A. J. Tuyns and Dr O. M. Jensen)

The case-control study on oesophageal cancer continues. A sample of the general population is now being interviewed as to dietary habits, including alcohol and tobacco consumption. During the first six months of 1976, the following numbers of persons were interviewed; those with:

cancer of oesophagus	51
cancer of stomach	12
cancer of colon	12
cancer of rectum	20
cancers of other parts of gastro-intestinal tract	4
cirrhosis of liver	43
delirium tremens	24
other alcohol-related diseases	4
and population controls	145

As noted previously, the types of alcoholic drinks consumed are believed to differ from those in Britanny, as does the pattern of alcohol-related disease.

Animal experiments involving the feeding of selected local alcoholic drinks have been planned and will be carried out by Professor J. Y. Le Talaer, Centre François Baclesse, Caen).

Dr A. M. Mandard, senior pathologist at the same centre examined segments of oesophagi adjacent to cancerous areas and was able to demonstrate lesions ranging from parakeratosis and cellular atypia to carcinoma *in situ*. This investigation is now being extended to other categories of patients. Several other centres in various parts of the world have been contacted with a view to enlarging this study.

(*d*) *Caspian littoral* (Dr J. Kmet) (see report of the Teheran Research Centre, page 125)

3.2 *Studies on alcohol and cancer* (Dr A. J. Tuyns and Dr O. M. Jensen)

With the support of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) of the USA, the Agency is developing a programme for elucidation of the relationship between alcohol consumption and cancer.

A review meeting on the current status of various studies composing this programme was held in Lyon on 8–10 March 1976 (see 3.1). Dr G. Pequignot, National Institute of Health and Medical Research, Le Vésinet, France, reported on the possibility of obtaining a more accurate determination of lifetime alcohol intake.

A further analysis of a cohort of patients admitted to hospital with a diagnosis of alcohol-related disease (Dr J. A. H. Waterhouse and Dr K. W. Cross, Queen Elizabeth Medical Centre, Birmingham, UK) showed a significant excess risk for cancers of the buccal cavity and pharynx and for cancers of the liver, gall-bladder and pancreas taken as a group,

In connection with the high incidence of primary liver cancer observed in Geneva [1], a case-control study on the role of alcohol in hepatic diseases is under way. The results of a pilot feasibility study were reported by Mr L. Raymond (Geneva Cancer Registry);

[1] Tuyns, A. J. & Obradovic, M. (1975) *J. nat. Cancer Inst.*, **54**, 61–64.

the drinking pattern appears to be that which was expected, namely a high wine consumption.

A proposal for a study on the cancer mortality experience of employees at a brewery in Dublin, Eire, was discussed.

An excess of stomach and rectal cancer in northern Belgium was reported by Miss L. Ramioul (School of Public Health, Brussels). The significance of this finding in terms of alcohol consumption remains to be assessed; in Belgium there is a high consumption of beer.

Agency staff members reported on various research projects more directly carried out or coordinated by the Agency [see sections 3.1 (*a*) and (*b*) and (*a*) and (*b*) below].

3.3 *Cancer among brewery-workers*

(*a*) *Denmark* (Dr O. M. Jensen)

A suggested association between the consumption of beer and cancer of the colon and rectum [1] is being studied by means of a retrospective cohort study of male Danish brewery workers with a high daily consumption of beer [2]. For comparison, a group of male Danish Temperance Society members with low or nil daily alcohol intake has been collected.

After checking for duplicates, 14 618 brewery workers and 1 606 male temperance society members were available for analysis. In collaboration with Dr J. Clemmesen (RA/75/019), the files of the Danish Cancer Registry are being used to establish morbidity in these groups; causes of death are being determined with the help of the Danish National Health Service (Dr J. Mosbech and Mr S. Sørensen: RA/75/017). At present, some 1 800 deaths have been recorded among the brewery workers, and some 600 in the temperance group. In addition to a comparison of the study groups, the cancer morbidity and mortality (all causes of death) of the two groups will be compared with the available rates for the general population of Denmark, taking into account the time of diagnosis, differences in age structure and place of residence.

(*b*) *Eire* (Dr R. MacLennan)

A substantially similar study has been started in Eire, where the health of brewery workers manufacturing another type of beer is being investigated in collaboration with the Medico-Social Research Board (Dr G. Dean: RA/76/016). Standardized mortality ratios will be calculated for all causes of death. Specific occupation within the brewery will be analysed, and beer consumption by occupation will be documented retrospectively with a view to examining a possible dose-response effect.

3.4 *Large-bowel cancer*

Incidence of cancer of the large bowel, a major cause of death in Europe and North America, shows considerable geographic variation. The Agency is coordinating an international collaborative study of this disease, giving priority to areas where there are contrasts

---

[1] Breslow, N. E. & Enstrom, J. E. (1974) *J. nat. Cancer Inst.*, **53**, 631–639.

[2] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 35.

in incidence within a relatively limited geographical region and where population-based cancer registries exist.

(*a*) *Intestinal microecology in Denmark and Finland* (Dr R. MacLennan and Dr O. M. Jensen)

A working group, chaired by Dr R. E. O. Williams (Public Health Laboratory Service, London) was held in the Regional Office for Europe of WHO, Copenhagen, on 21–23 April 1976, to consider results from the collaborative project on intestinal microecology. Following a study of random samples taken from 30 males, aged 55–64, in the spring and again in the autumn of 1975 in rural Finland (Kuopio) (Dr H. Vuori and Mr S. Kokko) and urban Denmark (Copenhagen) (Dr J. Mosbech), it was concluded that the four-fold differences in colon cancer incidence between these two areas could not be explained by current hypotheses involving faecal bacteria or faecal steroids.

The major differences were found in total dietary fibre and in consumption of milk and beer. Total fibre and milk intake were higher in Kuopio, whereas beer consumption was higher in Copenhagen (Table 4). There was less qualitative difference among the various constituents of the fibre (Drs D. Southgate, J. Cummings and W. P. T. James, Medical Research Council, Dunn Nutritional Laboratory, Cambridge, UK) in the two populations. The Finnish population sample consumed twice the amount of fibre consumed by the Danish, this high intake being associated with a greater faecal bulk. Mouth-anus transit time was, according to the single stool measurement developed by Cummings & Wiggins [1], similar in both populations.

It was decided to examine other populations, selected on the basis of differences in cancer incidence or presumed differences in the intake of dietary fibre, as soon as possible. Future studies will give greater emphasis to the methodology of surveying diet in the field.

Table 4. Comparison of intake of selected food items, faecal weight and mouth-anus transit time, combined spring and autumn, in rural Finland (Kuopio) and urban Denmark (Copenhagen)

	Kuopio			Copenhagen		
	No.	Mean	S.D. [b]	No.	Mean	S.D.
Beer (ml)	57	48.0	110.8	60	421.0	369.0
Milk (ml)	57	642.8	269.5	60	153.9	260.4
Total dietary fibre [a] (g)	13	30.9	11.3	12	17.2	5.1
Faecal weight (g)	56	192.0	91.6	62	151.6	62.9
Transit time (h)	57	39.1	12.9	62	42.1	14.7

[a] Autumn only
[b] Standard deviation

(*b*) *Faecal steroids and bacteria and large-bowel cancer mortality in Hong-Kong by socio-economic indicators* (Dr R. MacLennan and Miss D. Magnin)

This study has been made in collaboration with the Bacterial Metabolism Research Unit of the UK Public Health Laboratory Service, Colindale, London (Dr B. Drasar and

[1] Cummings, J. H. & Wiggins, H. S. (1976) *Gut*, **17**, 219–223.

Dr M. Hill) and with the University of Hong Kong (Dr C. H. Teoh-Chan). Dr N. Gibbs (St Luke's Hospital, Guildford, UK) is helping to coordinate the study.

Of three socio-economic groups in Hong Kong, the high income group was found to have a high faecal concentration of bile acids, especially the dihydroxy bile acids, compared with that in the low income group. Further, the faecal bile acids were more highly degraded, and the faecal flora contained bacteroids and fewer *Eubacteriales*. Very few of the *Clostridia* that can dehydrogenate the steroid nucleus were isolated. An epidemiological study based on street blocks indicated that the high income group also has a higher incidence of cancer of the large bowel and of the breast. These results support the faecal steroids hypothesis [1].

(*c*) *Large-bowel pathology in autopsy series* (Dr R. MacLennan)

The large bowels from a series of autopsies are being examined in a comparable manner in Denmark, Finland, Norway, The Netherlands and the UK [2]. More recently, autopsy samples in Krakow, Poland, where large-bowel cancer rates are low but rising, have been included (Dr A. Urban).

3.5 *Cancer of the oral cavity, pharynx, larynx and lung in North Thailand* (Dr R. MacLennan)

A re-analysis of the data from this case-control study [3] is being undertaken (Dr N. Breslow, University of Washington, Seattle, Wash., USA) to examine more specifically the presumed site of origin of the tumour in relation to smoking and chewing habits.

A collaborative medico-anthropological study is being carried out with the assistance of Miss C. Mougne (School of Oriental and African Studies, London) who has done field work in a rural area near Chiang-Mai in North Thailand. More information is being collected on the types of cigars and smoking and chewing habits of the 'normal' population.

Analysis of smoke undertaken by Drs C. J. Dahl and P. Scheelings (Australian Government Analytical Laboratories, Melbourne) show that the large 'khii yoo' cigars smoked in North Thailand produce an acid smoke and half as much tar as the smaller, more pungent 'burii' cigars, which have an alkaline smoke. Among women, the 'khii yoo' smoking habit is associated with lung cancer. On a weight for weight basis the nicotine and tar contents of 'burii' cigars were 5–10 times greater than these of Australian cigarettes.

3.6 *Lung cancer*

(*a*) *Etiological factors in lung cancer in Singapore Chinese* (Dr R. MacLennan and Dr N. E. Day)

As described in previous annual reports [3], Chinese females in Singapore, especially the Cantonese, have high rates of lung cancer which can only partly be attributed to smoking. The number of female cases in the case-control study [3] for whom histological type was

[1] Crowther, J. S., Drasar, B. S., Hill, M. J., MacLennan, R., Magnin, D., Peach, S. & Teoh-Chan, C. H. (1976) *Brit. J. Cancer* (in press).

[2] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 37.

[3] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 38.

available was insufficient to undertake an analysis by smoking history. Thus, a search was made of hospital records for female lung cancer cases which had been histologically typed in another study (see 4.3); those in which there was a statement about smoking, obtained prior to histology, were analysed together with data from the case-control study.

Table 5 lists histological types of lung cancer by dialect and smoking history for this joint material. As in the larger histopathological survey (see 4.3), Cantonese females have a relatively high proportion of adenocarcinoma which does not appear to be associated with smoking. Thus, the low risk attributable to smoking in Cantonese females with lung cancer may be due in part to high rates of adenocarcinoma. This possibility is being explored further in a study in Hong Kong [see section (*b*)].

Table 5. Histological types of lung cancer by dialect and smoking history in Singapore Chinese females, 1968–1973

Histological type	Cantonese		Non-Cantonese	
	No.	Percent smokers	No.	Percent smokers
Epidermoid carcinoma	5	80	7	85.7
Small-cell carcinoma	7	85.7	6	83.3
Adenocarcinoma	16	31.3	9	22.2
Large-cell carcinoma	6	66.7	2	0
Other types	4	75	2	0
Not typed [a]	16	62.5	23	30.4
Totals	54	59.3	49	40.8

[a] 33 were inadequate biopsies; 1 could not be fitted into the classification; and for 5 slides could not be found.

(*b*) *Histological types of lung cancer in Hong Kong* (Dr R. MacLennan)

With Dr W. C. Chan (University of Hong Kong), an analysis has been made of pathological material obtained during the period 1960–1972.

Age-adjusted mortality per 100,000 per annum from lung cancer rose rapidly in both males and females in Hong Kong between 1960 and 1972, from 21.7 to 39.6 in males and from 11.4 to 19.7 in females. Among males, the relative frequency of squamous carcinoma in bronchial biopsies increased but not that in lung parenchymal biopsies, resections or autopsies. There was a concomitant decline in the frequency of small-cell anaplastic carcinoma. Taking into account all sources of material, the Kreyberg ratio did not increase despite a two-fold rise in mortality from lung cancer. In females, there was a 60% increase in mortality but again no increase in the Kreyberg ratio. Despite the high mortality from lung cancer, adenocarcinoma was the most common histological type in females. It is thought that cigarette smoking might produce a different pattern of histological types among Hong Kong Chinese than that seen in the Occident, or that additional etiological factors may be operating. A case-control study is being undertaken by the Department of Community Medicine of the University of Hong Kong (Professor M. Colbourne), using a protocol comparable to that used in Singapore, to relate histological type of lung cancer in women to smoking history.

3.7 *Carcinoma of the prostate* (Dr H. Tulinius, Miss D. Magnin and Mrs L. Kerebel)

The study coordinated by the Agency of 'latent' carcinoma of the prostate in populations of contrasting incidence rates of clinical prostatic carcinoma has continued, in collaboration with Professor G. Dhom (Federal Republic of Germany), Dr W. C. Chan (Hong Kong), Professor B. Gellei (Israel), Dr B. Sparke (Jamaica), Dr Lee Yoke Sun (Singapore), Dr S. Lundberg (Sweden) and Dr R. A. B. Drury (Uganda). Statistical analysis is being carried out by Dr N. Breslow (University of Washington, Seattle, Wash., USA).

In this study, prostate sections from consecutive necropsies in the age groups 45–54, 55–64, 65–74 and 75+ were prepared according to an agreed protocol. The quota of 50 cases per age group was not achieved in all areas, since it proved impossible to collect sufficient numbers in the oldest age groups in Uganda. A total of 1 327 cases were admitted to the study.

Each pathologist evaluated his own material for the presence of latent carcinoma and, if one was present, for histological type, differentiation and invasion. The study coordinator used a table of random permutations to select another pathologist from one of the six other areas to review the slides for the case. This second pathologist was unaware of the origin of the material. A major source of disagreement related to the presence or absence of very small neoplasms; re-evaluation reduced the number of serious disagreements by nearly two-thirds. In all, 284 of the prostates, or 22%, were held to contain carcinomas, and 43, or 3%, were agreed to be doubtful. Area and age had highly significant effects ($P<0.0001$); the statistical significance of the pathologist effect was somewhat less ($P<0.01$) but was nevertheless clearly present.

In all areas except Singapore and Israel there was a continuous increase in the frequency of latent carcinoma of the prostate with age. Both Asian areas had significantly lower frequencies than did the European areas and Jamaica; however, only Singapore had a significantly lower frequency than Israel and Uganda. Sweden could be distinguished from Israel and Uganda, but Jamaica and the Federal Republic of Germany could not. In general terms, the frequency of latent carcinoma paralleled that of the invasive form of the disease. Further analysis of this complex study continues.

3.8 *Industrial exposure*

(*a*) *Health risks from man-made mineral fibres* (Dr R. Saracci and Dr J. Milne)

Following demonstration [1, 2, 3] that certain sizes of man-made mineral fibres produce neoplastic changes in the pleural and peritoneal cavities of experimental animals, the question arose of whether a possible carcinogenic hazard from these fibres existed for workers in the industry.

Realizing that the problem necessitated full investigation, firms involved in the production of man-made mineral fibres decided to approach the Agency with regard to

---

[1] Stanton, M. F. & Wrench, C. (1972) *J. nat. Cancer Inst.*, **48**, 797–821.
[2] Wagner, J. C., Berry, G. & Timbrell, V. (1973) *Brit. J. Cancer*, **28**, 173–185.
[3] Pott, F. & Friederichs, K. H. (1972) *Naturwissenschaften*, **59**, 318.

epidemiological studies into possible risk, and to ask the Medical Research Council of the United Kingdom and the Institute of Occupational Medicine, Edinburgh, Scotland, to carry out animal and environmental studies, respectively. To ensure scientific independence, these research projects are coordinated by an *ad hoc* Scientific and Technical Committee, at present composed of six members representing the three participating institutes, and two technical representatives of the Joint European Medical Research Board of the glass fibre and mineral wool industries. Organized labour has been asked to provide representation.

A feasibility study presently underway involves determination, by visits, of the situation at each factory with respect to number of exposed workers, type of fibres produced, manufacturing methods, storage, time since production began, availability and quality of personnel records, environmental measurements and facilities for follow-up. Once this information is obtained, it will be possible to decide on the appropriate type of study. Close contact is maintained with the other collaborating institutes.

(*b*) *General*

Discussions were held with Dr J. Clemmesen (Danish Cancer Registry, Copenhagen) on the feasibility of a study for the registration of industrial exposures to be undertaken for the European Economic Community.

4. BIOSTATISTICS (Dr N. E. Day and Dr L. Muenz)

During this past year, Dr E. Schifflers (Centre de Calcul, Catholic University of Louvain, Belgium) was appointed as a consultant for six months to review and improve the Agency's computing capability. In consequence, there has been a general improvement in the level of computer support. A report is awaited on plans for the evolution of the computing configuration. In addition, Dr Schifflers, with Mr S. Sabai, has begun a study of the role of the computer in cancer registration.

Apart from providing a general statistical service to the Agency, the biostatistics section has been involved particularly in the projects described below.

4.1 *Oesophageal cancer in the Caspian littoral of Iran* (Dr N. E. Day)

The case-control trial begun last year has been completed. Processing of the data in Lyon is nearly finished, and the full analysis is expected in the coming months. A preliminary analysis has been performed (see page 125).

4.2 *Immunogenetics* (Dr N. E. Day)

(*a*) *Nasopharyngeal carcinoma*

In collaboration with Dr M. Simons (WHO Immunology Research and Training Centre, Singapore), the association of the HLA phenotype A2-BSin2 with risk for nasopharyngeal cancer has now firmly been established [1]. Preliminary results indicated that the HLA locus D antigen Sin2a may be associated with a considerably higher risk.

---

[1] Simons, M. J., Wee, G. B., Chan, S. H., Shanmugaratnam, K., Day, N. E. & de-Thé, G. (1976) *J. nat. Cancer Inst.* (in press).

(*b*) *Methodology*

Associations between disease and specific histo-compatibility antigens often depend on the existence of genetic disequilibrium between alleles at neighbouring loci and do not usually represent the full association of disease risk with the histo-compatibility region. To investigate this full association, joint segregation of HLA haplotypes and disease can be exploited in families with multiple occurrences of the disease. Classical linkage analysis is inappropriate in this situation, since only those with the disease carry information (It is not known whether those without the disease were exposed to the necessary agent, or if other genetic factors may play a role). The necessary statistical methodology has been developed, and its efficacy has been demonstrated in published data on juvenile diabetes [1]. A study is being planned to investigate joint segregation of HLA haplotypes and various forms of cancer.

4.3 *Lung cancer in Singapore* (Dr N. E. Day)

Further work was done in collaboration with the Department of Pathology, University of Singapore, on specific histologic types of lung cancer. Various techniques were used to eliminate bias, after which age-standardized incidence rates were calculated for different histologic types by dialect group [2] (see 3.6).

4.4 *Breast cancer*

(*a*) *Familial study in Iceland* (Dr N. E. Day and Dr H. Tulinius)

A risk surface, taking account of age, year of birth and age at first pregnancy, has been constructed, which is applicable to all Icelandic women born since 1840. This risk surface demonstrates that the rise in incidence of breast cancer in Iceland over the past 100 years cannot be accounted for by factors in a woman's reproductive history [3]. Data collection and checking for the study of familial risk have now been completed, and the results are being prepared for publication. This work has been undertaken in collaboration with Professor O. Bjarnason of the Icelandic Cancer Registry (RA/73/004).

(*b*) *Lausanne* (Dr L. Muenz and Mrs A. Joly)

In cooperation with the Ludwig Cancer Research Institute of Lausanne, the Biostatistics section is managing and analysing data from a breast cancer clinical trial. Nearly 300 patients are now being followed, some for more than three years. Patients are randomized between those receiving X-ray therapy and those with no further treatment; all have received a radical mastectomy. To date, 28 patients have had either a local or a distant metastasis, 14 in each group; there is slight evidence that distant metastases appear sooner in those receiving X-rays. The study will also show whether the degree of radiation-induced lymphopenia is a prognostic factor. The evidence, to date, is inconclusive.

Stored serum samples will shortly be analysed for immunoglobulin levels as part of a larger effort to select subsets of patients likely to profit from one or the other treatment.

---

[1] Day, N. E. & Simons, M. J. (1976) *Tissue Antigens* (in press).
[2] Law, C. H., Day, N. E. & Shanmugaratnam, K. (1976) *Int. J. Cancer*, **17**, 304–309.
[3] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 39.

(*c*) *Geneva Cancer Registry* (Dr L. Muenz)

An analysis has been made of the TNM and End Results Group breast cancer staging systems, using records of 389 patients followed up to 20 years. The data has been supplied by the Geneva Cancer Registry, and each patient was staged according to both systems. One system is considered to be superior to the other if a higher proportion of correct responses can be given to the question: will the patient be alive six months (or 12 or 18, etc.) after diagnosis ? Preliminary conclusions indicate the statistically significant superiority of the TNM system. However, the influence of covariates such as age or tumour histology has not yet been considered and may result in one system being more useful for some patients than for others. Also, since the criterion employed is a function of time, one system may be superior at a two-year interval, say, and inferior after four years, with respect to the question posed above.

(*d*) *Breast cancer in Tunisia* (Dr L. Muenz)

Advice has been given to Dr N. Mourali of the Salah Azaiz Institute regarding epidemiology and clinical presentation of breast cancer in Tunisia, and in particular for analysis orientated towards a description of cases classified as 'poussée évolutive'.

4.4 *α-Fetoprotein bioassays* (Dr L. Muenz)

Analysis of the second cooperative α-fetoprotein (AFP) experiment has nearly been completed. The proportion of assays which are incapable of providing a useful AFP concentration (because of non-parallelity, non-linearity or inadequate range on the precipitation scale) is a smoothly decreasing function of the 'true' AFP concentration. The use in those assays which pass the tests of statistical validity of the proposed 72/225 standard results in a coefficient of variation in AFP concentration of about one half that obtained by use of local laboratory standards. In either case, however, the variations are relatively small. Between-assay variability must now be partitioned into within- and between-laboratory components. These analyses have involved the use of cubic splines, a technique which has not hitherto been widely exploited in studies of dose-response data.

5. MISCELLANEOUS

In addition to the teaching courses organized by the Agency, members of the Unit have participated in a variety of educational seminars. Several members of the staff are on the editorial boards of international cancer journals and are frequently asked to review scientific papers. Acceptance by staff members of chairmanships of sessions at carefully selected international meetings had led to increased awareness of the programmes and policies of the Agency.

2. UNIT OF ENVIRONMENTAL CARCINOGENS

Dr L. GRICIUTE (Chief)

1. INTRODUCTION

The Unit of Environmental Carcinogens has continued to elaborate approaches for measuring the distribution of chemical carcinogens in the human environment.

In the Unit's analytical laboratory, a method for the identification of trace levels of volatile *N*-nitrosamines has been improved, and collaboration with other laboratories (particularly the Institute of Experimental Toxicology and Chemotherapy, Heidelberg) on analytical methods has been continued and developed. A new technique for the detection and measurement of nitrosamines, the Thermal Energy Analyzer, is being used. Wide international collaboration has been continued: stage IV of the cooperative studies on methods of analysis for volatile *N*-nitrosamines has begun, and others are planned. It is gratifying to note that the number of laboratories collaborating in these studies is increasing.

New data on the analysis and formation of *N*-nitrosamines were discussed during the IVth international symposium on this subject, organized by the Unit in collaboration with the Estonian Institute of Clinical and Experimental Medicine in Tallinn (USSR).

Scientists concerned with the measurement of chemical carcinogens have expressed the need for standardization and evaluation of analytical methods to enable them to compare data presented by different laboratories in different countries. The Unit of Environmental Carcinogens has therefore undertaken, with the guidance of an editorial board, the preparation of a *Manual of Selected Analytical Methods for Environmental Carcinogens*.

The Unit maintains close collaboration with epidemiologists in the Agency by joint planning in the selection of samples and areas of sampling and by providing relevant environmental data. An initial survey of Iranian foods from areas with high and low rates of morbidity from oesophageal cancer has been completed; and analyses of alcoholic beverages for *N*-nitrosamines, polycyclic aromatic hydrocarbons and certain mycotoxins are now being undertaken.

The Unit also collaborates with the Unit of Chemical Carcinogens by undertaking fractional extraction of various samples of common interest for mutagenicity testing.

Studies on the relationship between asbestos and cancer are continuing. Data obtained at the Medical Research Council Pneumoconiosis Unit (Penarth, UK) provide the basis for a trial in which the intake of asbestos fibres from the air (non-occupational exposures) will be correlated with morbidity from mesotheliomas, lung cancer and cerebral haemorrhages.

2. QUANTITATIVE DATA ON ENVIRONMENTAL CARCINOGENS

2.1 *Studies on* N-*nitroso compounds* (Mr E. A. Walker)

(*a*) *Analytical method for screening*

(i) A method for the screening of nitrosamines in foodstuffs has been published [1]. The specificity of the method has been improved by the use of specific-ion monitoring, in gas chromatography coupled with mass spectrometry, to identify the nitramines obtained in the method; this step takes advantage of the detailed clean-up used and minimizes the number of samples requiring confirmatory analysis of nitrosamines by mass spectrometry. The technique is also applicable to samples of alcoholic beverages. A modified oxidation procedure, which further improves the method, has recently been introduced; details will be submitted for publication.

During the screening analysis, a peak which could not be separated from dimethylnitramine by gas chromatography but which appeared in a different fraction during analysis gave rise to speculation that the compound was a nitramine arising from the oxidation of a nitrosamine-like compound. It has been possible with mass spectrometry to demonstrate that this compound is not a nitramine, thereby dismissing further speculation.

(ii) Problems are still being encountered in the development of an analytical method involving reduction of nitrosamines to the corresponding hydrazines and subsequent derivatization as trifluoroacetyl acetonates. The method works extremely well for nitrosodimethylamine, giving 100% recoveries; but poor yields are still being obtained for other volatile nitrosamines. The problem remains under investigation.

(iii) A Thermal Energy Analyzer capable of highly selective detection of *N*-nitroso compounds has been received on loan from the National Cancer Institute (USA) for evaluation and analysis of nitrosamines. Evaluation tests have proved satisfactory, and the instrument is being used for the analysis of foods and alcoholic beverages, resulting in a considerable economy in time and materials such as solvents.

(iv) The mass spectrometer has been used not only for positive identification of nitrosamines in environmental samples, but also for the identification of a number of hydroxynitrosamines prior to testing for mutagenicity.

(*b*) *Analyses of foods*

Institute of Experimental Toxicology and Chemotherapy, Heidelberg, Federal Republic of Germany (RA/70/026)
Principal investigator: Dr R. Preussmann

(i) *Volatile nitrosamines*

During the past year, the study involving screening of food samples from Iran for their volatile nitrosamine content was terminated. Two complementary analytical techniques were used: nitrogen-specific determination of intact nitrosamines by a modified Hall electrolytic conductivity detector and ion-specific detection in a gas chromatograph-mass

[1] Walker, E. A., Castegnaro, M. & Pignatelli, B. (1975) *Analyst*, **100**, 817–821.

spectrometer at low resolution after formation of heptafluorobutyramides from nitrosamines. A total of 146 samples were analysed: seven wheat and rice samples were found to contain *N*-nitrosodimethylamine (NDMA), and three contained *N*-nitrosodiethylamine (NDEA); two of the latter were foods for direct consumption. The concentrations were in the range of 2–10 μg/kg (Table 6). These results have not yet been confirmed by high-resolution mass spectrometry.

Strong support for the reliability of the above technique was given by a MS 902 high-resolution mass spectrometer coupled to a gas chromatograph. A series of foods from the Federal Republic of Germany for which positive results were obtained with the screening techniques were subjected to gas chromatograph-high-resolution mass spectrometric measurement. In virtually all cases in which both detection systems had indicated a content of NDMA, NDEA, *N*-nitrosopyrrolidine or *N*-nitrosopiperidine, confirmation was obtained by gas chromatography-mass spectrometry at a resolution of over 10 000. These results are described in detail elsewhere [1, 2].

Table 6. Food samples from Iran in which nitrosamines have been found by two different methods

Sample	% Recovery of ^{14}C-NDMA	% Recovery of nitrosodi-*n*-propylamine		μg/kg nitrosamines found by Hall *and* HFB methods (corrected values)
		Hall	HFB	
Wheat	104	89	82	NDMA 7
Wheat	58	82	53	NDMA 2
Wheat	90	61	59	NDMA 7
Bread	79	54	54	NDEA 2
Fava beans	92	70	60	NDEA 3
Rice champa	63	46	67	NDMA 10
Rice champa	65	68	100	NDMA 5
Rice champa	100	97	91	NDMA 5
Rice champa	63	44	53	NDMA 5
Dough	99	86	62	NDEA 3

Hall — Hall electrolytic conductivity detector
HFB — heptafluorobutyramide method
NDMA — nitrosodimethylamine
NDEA — nitrosodiethylamine

(ii) *Non-volatile nitrosamines*

Analysis of non-volatile *N*-nitroso compounds continues: ^{14}C-labelled *N*-nitrososarcosine and *N*-nitrosoproline have been prepared and used for the development of an isolation procedure for quantitative determinations of *N*-nitrososarcosine, *N*-nitrosoproline and *N*-nitrosohydroxyproline in foods. A combination of solvent extraction, liquid-liquid partition and column chromatography has been found to give promising results. Final determination of the compounds under investigation is performed by gas chromatographic separation of the trimethylsilyl derivatives, followed by mass fragmentography. *N*-Nitroso-

[1] Eisenbrand, G., Rappardt, E. V., Zappe, R. & Preussmann, R. (1976) In: Walker, E. A., Bogovski, P. & Griciute, L., eds, *Environmental* N-*Nitroso Compounds — Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 14**) (in press).

[2] Rappardt, E. V., Eisenbrand, G. & Preussmann, R. (1976) *J. Chromat.* (in press).

3-hydroxypyrrolidine was included in these studies, and it is hoped that this non-volatile nitrosamine may be detected by the same procedure.

(*c*) *Collaborative analytical network*

(i) *Cooperative studies*

An important activity of the network continues to be the organization of cooperative analytical studies. The word 'cooperative' has been adopted to differentiate these studies from collaborative ones which, by normal definition, are studies of a single method. The studies carried out by the Agency are designed to evaluate and compare all methods of analysis for nitrosamines. A detailed report of statistical evaluations of studies carried out to date has been presented [1].

As anticipated in the preceding Annual Report [2], a cooperative study for analysis of nitrosamines in a spiced meat is now in progress (stage IV of the cooperative studies). A preliminary study for the analysis of three non-volatile nitrosamines (nitrososarcosine, nitrosoproline and nitrosohydroxyproline) is now being organized and will constitute a first attempt to establish the levels of non-volatile nitrosamines. A further study is planned for the analysis of nitrosamines in bread.

Priorities for these studies were decided by the European Sub-Committee on the Guidance of Collaborative Studies on Analytical Methods of *N*-Nitrosamines. The last meeting of this committee was held in Lyon on 29–30 March 1976 (*IARC Internal Technical Report* No. 76/001).

(ii) *Fourth biennial meeting on analysis and formation of* N-*nitroso compounds in the environment*

The fourth biennial meeting on analysis and formation of *N*-nitroso compounds in the environment was held at Tallinn on 29 September–4 October 1975. Eighty-one participants from sixteen countries attended, and fifty-two papers were presented. The conference covered three aspects: analysis of *N*-nitroso compounds, their formation and their occurrence in the environment. The proceedings are in press [3].

(*d*) *Catalytic and inhibiting effects of phenolic compounds on nitrosamine formation*

The dependence of nitrosodiethylamine formation on pH and relative concentration of gallic acid has previously been demonstrated in model experiments [4, 5]; these have been extended to show the existence of an optimum concentration of gallic acid for maximum formation of nitrosodiethylamine at pH 4 (Fig. 6). Since the reaction time could be an important factor from the point of view of *in vivo* formation, the relationship between reaction time and nitrosamine formation has been investigated in the presence of two different concentrations of gallic acid at pH 4. The results are shown in Figure 7. While

---

[1] Walker, E. A. & Castegnaro, M. (1976) In: Walker, E. A., Bogovski, P. & Griciute, L., eds, *Environmental N-Nitroso Compounds — Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 14**) (in press).

[2] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 47.

[3] Walker, E. A., Bogovski, P. & Griciute, L., eds (1976) *Environmental N-Nitroso Compounds — Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 14**) (in press).

[4] Walker, E. A., Pignatelli, B. & Castegnaro, M. (1975) *Nature (Lond.)*, **258**, 176.

[5] Pignatelli, B., Castegnaro, M. & Walker, E. A. (1976) In: Walker, E. A., Bogovski, P. & Griciute, L., eds, *Environmental N-Nitroso Compounds — Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 14**) (in press).

Fig. 6 Formation of nitrosodiethylamine at pH 4 in relation to concentration of gallic acid

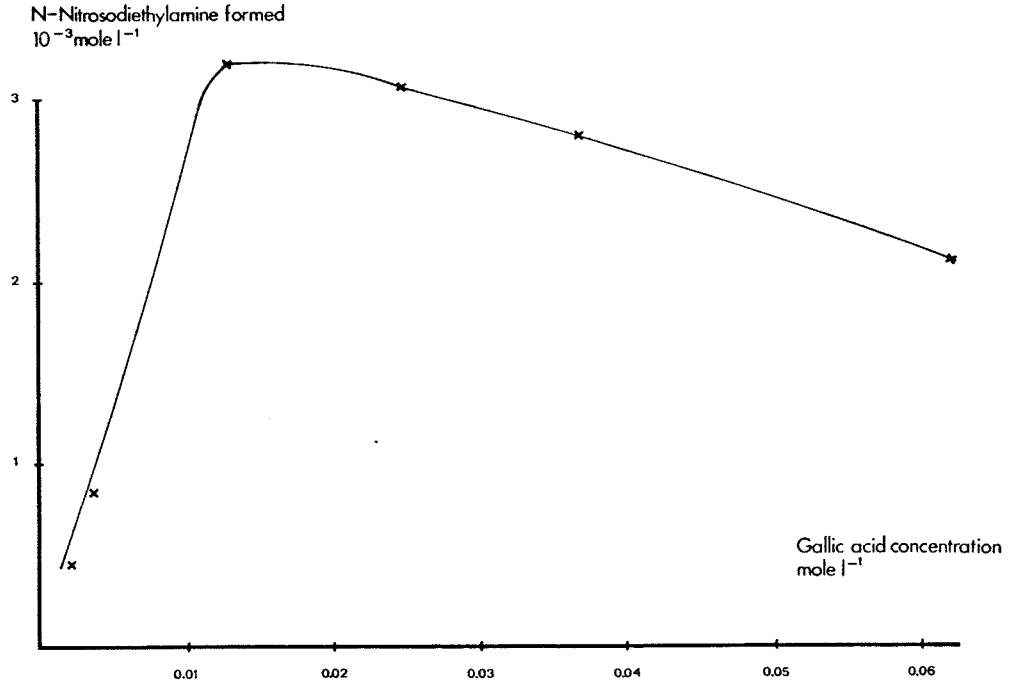
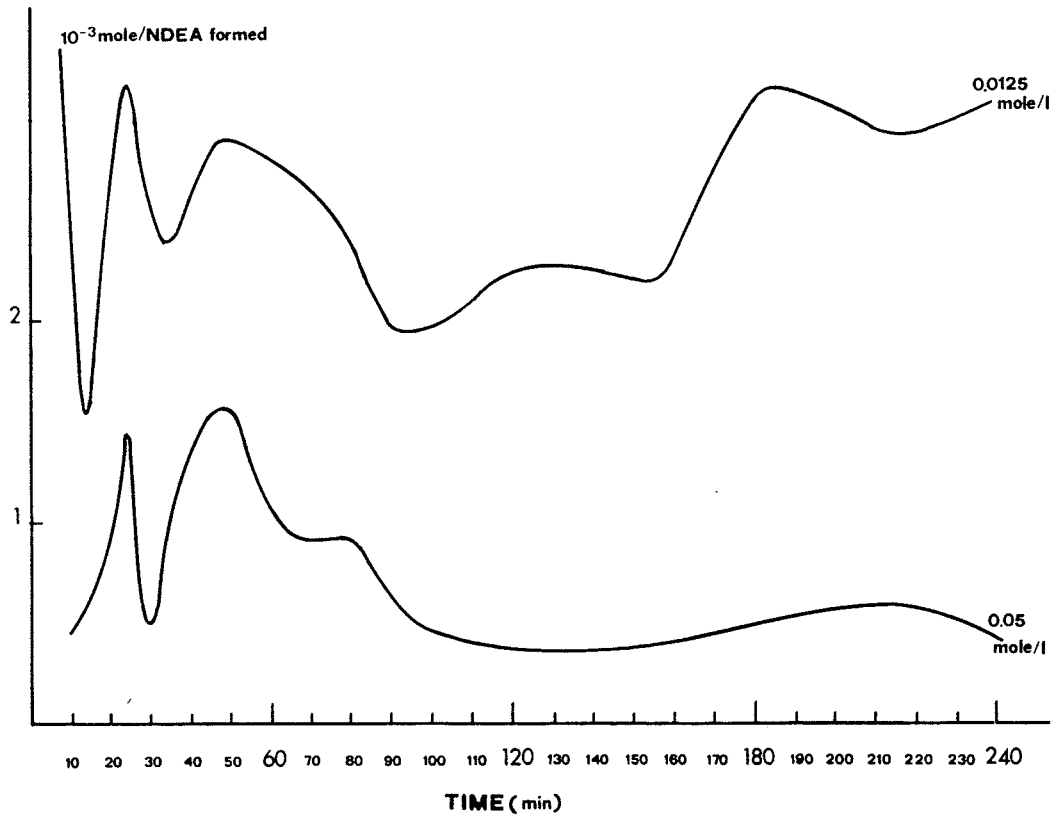


Fig. 7 Formation of nitrosodiethylamine in relation to time



these results need further study, they indicate not only that initial formation is rapid, but also that the reaction is more complex than it first appeared: there is a competition between gallic acid and amine for nitrite and a catalytic effect of the acid; in addition, there is evidently at least one further reaction leading to removal or destruction of the nitrosamine, presumably by other reaction products. This is indicated by the lower curve in Figure 6, which is a plot of the amount of nitrosodiethylamine formed in relation to time.

In view of the evident complexity of the reaction, a study is being undertaken of the role of phenol (hydroxybenzene), the simplest member of this class of compounds, and of its *C*-nitroso derivatives, which are products of nitrosation of the phenol. An initial study has shown that 4-nitrosophenol is a more powerful catalyst than is the parent phenol. Thus, it is possible that catalysis by phenol may result from its C-nitrosation during the reaction. Since nitrosamines are not formed from amine and the *C*-nitroso compound in the absence of nitrite, the effect is not a simple trans-nitrosation. A study has also been carried out to examine the effect of ethyl alcohol on nitrosamine formation. In this case a simple catalytic effect was observed. Discussions have been undertaken with Dr Knowles of the Ministry of Agriculture, Fisheries & Food, UK, for collaborative studies on this topic.

2.2 *Manual of selected methods of analysis for environmental carcinogens* (Dr L. Griciute)

The need for standardization of methods of analysis for environmental carcinogens has been recognized by all the workers in this field; thus, the Unit is planning to edit a series of manuals to cover the most important chemicals for which methods of analysis are available. The first meeting of the editorial board (Chairman: Professor H. Egan, Laboratory of the Government Chemist, London) was held on 18–19 June 1975 (*IARC Internal Technical Report* No. 75/002). The aims of the manual, criteria for the selection of methods to be included, collaboration with other bodies and priorities, contents and format of each volume were discussed. The relative priorities for publication were established in relation to the practical importance of substances and to the state of advancement of the analytical methodology:

Priority 1: Mycotoxins; *N*-nitroso compounds

Priority 2: Polycyclic aromatic hydrocarbons; vinyl chloride and related compounds

Priority 3: Aromatic amines; diethylstilboestrol and other synthetic hormones

Priority 4: Asbestos; direct alkylating agents; certain plant constituents; urethane

Chairmen have been designated for the review boards of the first four volumes; they are all members of the editorial board. Professor R. Preussmann, chairman of the *N*-nitrosamines review board, designated the members of his board shortly after the editorial board meeting. The authors and reviewers of this volume were designated in September at the meeting of the European Sub-Committee on the Guidance of Collaborative Studies on Analytical Methods of *N*-Nitrosamines (Tallinn, 30 September 1975).

2.3 *Carcinogens in alcoholic beverages* (Mr E. A. Walker, Mr M. Castegnaro and Mr G. Toussaint)

(*a*) *Nitrosamines*

Previous work on alcoholic beverages was carried out on samples which were not collected in specific relation to cancer incidence. As certain chemical carcinogens have been shown to be present in such samples, particularly in those produced on farms in the regions of Brittany and Normandy in France where the incidence of oesophageal cancer is higher than elsewhere in the country, an extensive survey of alcoholic beverages generally consumed in the area is being made in selected areas of high and low cancer incidence in an effort to assess relative intakes of chemical carcinogens. Preliminary studies, using the Thermal Energy Analyzer (TEA), have confirmed the presence of nitrosodimethylamine found in the previous work and show the presence in several samples of other nitrosamines, including nitrosodiethyl- and nitrosodipropylamines, at the 1–2 μg/kg level. The presence of these three nitrosamines has been corroborated in the same samples by Dr Fine (Thermo Electron Research Center, Waltham, Mass., USA), using high-pressure liquid chromatography coupled with TEA. The results were confirmed by high-resolution mass spectrometry. Another peak, which was also observed with gas chromatography, is consistent with the presence of about 1 μg/kg nitrosomethylvinylamine. Although the evidence obtained by mass spectrometry tends to support this, in view of its very low concentration and of its labile nature, the identity of this compound requires further confirmation. It is, however, interesting to note that the suspected nitrosamine occurred only when other nitrosamines were present in a sample.

Eighteen samples of African maize beer collected by Dr van Rensburg in the course of epidemiological studies on cancer of the oesophagus in the Transkei have been analysed. Nitrosodimethylamine has been detected in three of the samples at levels of 1.0, 1.5 and 7.5 μg/kg.

(*b*) *Polycyclic aromatic hydrocarbons*

The presence of polycyclic aromatic hydrocarbons in samples of apple brandy has been demonstrated by a simple thin-layer chromatographic technique [1]. A typical sample has been examined in detail by Dr Grimmer (University of Hamburg) by gas chromatography. The results are shown in Table 7.

2.4 *Aflatoxins in olive oil* (Mr E. A. Walker and Mr G. Toussaint)

In view of a high incidence of primary liver cancer which has been observed in Greece [2], the Agency was asked by Professor Trichopoulos (Department of Hygiene & Epidemiology, University of Athens) to examine a number of olive oils, particularly unrefined products, for aflatoxins. Since no specific method was available for the analysis of olive oil, a standard method of analysis using thin-layer chromatography was adapted for this purpose. The results indicated low levels of aflatoxins (Table 8); however, small differences in concentration could not be specified, and the results shown are mostly in the range of 5–10 μg/kg.

---

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 50.
[2] Trichopoulos, D., Violakis, M., Sparros, L. & Xirouchaki, E. (1975) *Lancet*, **ii**, 1038.

To obtain more precise analytical data a method has been developed that employs semi-preparative high-performance liquid chromatography for the clean up and normal analytical high-performance liquid chromatography for the analysis. As can be seen from the results in Table 8, the method is more precise than is the thin-layer chromatographic method with which only B_1 is detected, since G_1, B_2 and G_2 are masked at this level by background interference. Repetitive analysis of four spiked samples showed that precision was better than $\pm$ 10% at the 5 μg/kg level. The method also allowed relatively precise determination of aflatoxins B_1, G_1, B_2, G_2 at the 1 μg/kg level.

Table 7. Polycyclic aromatic hydrocarbons (PAH) detected in apple brandy by gas chromatography

PAH [a]	μg/kg
Fluoranthene	27.3
Pyrene	19.0
Benzo[*a*]fluorene	1.71
Benzofluorenes (*b*, *c*)	0.99
Benzo[*c*]phenanthrene	2.18
Benzo[*ghi*]fluoranthene	1.04
Benzo[*a*]anthracene	4.57
Chrysene	5.30
Benzofluoranthenes (*b*, *k*, *j*)	3.50
Benzo[*e*]pyrene	1.95
Benzo[*a*]pyrene	1.79
Perylene	0.57
Dibenz[*a*, *j*]anthracene	0.26
Indeno[1, 2, 3-*cd*]pyrene	0.65
Benzo[*ghi*]perylene	0.75

[a] Several unknown PAH were also detected.

Table 8. Comparative analyses for aflatoxins in olive oil by thin-layer chromatography (TLC) and by high-performance liquid chromatography (HPLC)

Sample type	TLC (μg/l) B_1	HPLC (μg/l) B_1	G_1	B_2	G_2
crude farm	5–10	7.5	–	–	–
crude farm	5–10	5	–	–	–
crude farm	5–10	5	–	–	–
crude farm	5–10	3	–	2	–
crude farm	5–10	6.5	2.5	5.5	5
crude farm	5–10	4	1	2	2
crude farm	$\gtrsim$ 5	3	–	2	–
crude farm	$\gtrsim$ 5	3	2.5	2	2
crude farm	$\gtrsim$ 5	2.5	–	–	–
commercial	< 5	1	–	1	–
commercial	< 5	1	–	1	–
commercial	5–10	5	1	1	1
commercial	5	3	1	1	1
crude farm	5	–	–	–	–
crude farm	trace	1	–	1	–

3. ENVIRONMENTAL STUDIES IN IRAN (Mr E. A. Walker and Mr M. Castegnaro)

(*a*) Bread, wheat and tea samples taken in areas of Iran in which there is a high incidence of oesophageal cancer have been extracted with solvents of various polarities (cyclohexane, methylene chloride, ethanol and water); the residues were used by the Unit of Chemical Carcinogenesis for mutagenicity testing.

(*b*) Following reports of opium addiction in Iran, two samples, one of residue and the other of a dried aqueous extract of the carbonized residue from opium pipes, have been analysed for nitrosamines and for polycyclic aromatic hydrocarbons. Although the total quantity of each sample was less than 200 mg, polycyclic aromatic hydrocarbons were detectable, suggesting probable levels of about 50 mg/kg. No volatile nitrosamines were found in either sample, but evidence of the presence of non-volatile *N*-nitroso compounds was obtained by using the TEA detector in the direct injection mode. Aliquots of extracts made with two different solvents were tested for mutagenicity in the Unit of Chemical Carcinogenesis.

(*c*) A comparative study of nitrite and nitrate in saliva in areas of high and low cancer incidence in Iran is being planned. Two collaborators, Dr Eisenbrand (Institute of Toxicology and Chemotherapy, Heidelberg) and Mr Telling (Unilever Research Laboratories, Sharnbrook, UK), are studying field kits used to test water supplies in order to evaluate their applicability for analyses of saliva.

4. STUDIES ON THE RELATIONSHIP BETWEEN ASBESTOS AND CANCER

4.1 *Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, United Kingdom* (RA/70/014)

Principal investigators: Dr J. C. Gilson and Dr J. C. Wagner

(*a*) *Physics and chemistry*

(i) *Standardization of methods for monitoring dust counts in the asbestos industry* (Mr W. H. Walton, Institute of Occupational Medicine, Edinburgh, UK)

The international meeting on this topic has been delayed and will now be convened for early in 1977. More information has been collected, and the methods used in different countries have been studied; these data will be of assistance in planning the comparative studies that will be required for standardization of the counting of fibres in membrane filter samples.

(ii) *Asbestos in tissue* (Dr F. D. Pooley, Department of Mineral Exploitation, University College, Cardiff, UK)

Dr Pooley has extended the scope of his studies by the use of a transmission electron microscope fitted with energy dispersive X-ray analytical equipment. He is now able to identify the mineral dusts present in samples prepared from lung tissue, to quantify the various types of mineral present and to assess the number of particles of each type of

material. This will be of particular value in the study of cases of diffuse pleural mesothelioma, in which it is hoped to examine the mineral content of the lungs of all cases seen in the United Kingdom over the period of a year. At present, Dr Pooley is undertaking a preliminary study to test various methods of sampling lung tissue from human cases of asbestosis with or without mesothelioma.

(iii) *Characterization and identification of mineral fibres*

Dr V. Timbrell is continuing to develop methods for the characterization and identification of mineral fibres in dust samples and in tissues. Techniques for the characterization of fibre size include a convection-free centrifuge cell and magnetic alignment. Examination by light scattering of liquid suspensions of fibre is being used to correlate particle size parameters to light scattering indices. High-gradient magnetic field separation of asbestos has also been investigated. A microscope-based light scattering system that will be used to derive particle size information from the light scattering of single fibres and for the examination of membrane-filter asbestos dust samples collected in the field has also been developed.

Dr R. Davies is analysing lung tissue, after chemical digestion of the organic matter, for asbestos fibres by the Timbrell laser-light scattering method. It is hoped that use of this technique will provide quantitative and qualitative information on the asbestos present, including the geographical source of the asbestos encountered and indices of fibre size.

(*b*) *Experimental pathology*

(i) *Animal studies*

Inhalation and intrapleural studies are being planned to compare the biological effects of the standard UICC asbestos samples with those of various types of man-made fibres, including fibre-glass, slag wool and rock wool. These findings will be part of a study of the man-made fibre industry in Europe being carried out in collaboration with the Unit of Epidemiology and Biostatistics.

Studies in which animals were exposed to asbestos and then to cigarette smoke have shown fewer tumors than were seen in previous studies with asbestos alone.

Feeding experiments in which animals were given 100 mg daily of either crocidolite or chrysotile UICC samples in malted-milk powder for 100 days produced no peritoneal mesotheliomas in animals which were allowed to survive their normal lifespan.

(ii) *Immunology*

The study of men at high risk after exposure to crocidolite asbestos in a dockyard in the United Kingdom continues. A third survey of the initial group has recently been completed, and the results are being assessed. Further tests have been added, including a study of the surface membrane immuno-globulin-bearing cells. The HLA grouping has been carried out in collaboration with Dr C. Darke of the National Blood Transfusion Centre of Wales.

(*c*) *Pathology*

It is hoped to be able to study all the mesotheliomas of the pleura that occur in the United Kingdom over the period of a year. A Mesothelioma Panel has been established under the chairmanship of Dr J. S. P. Jones (University of Nottingham). Pathologists will

be requested to send him part of the tumour and a mid-saggital slice of the least affected lung. The lung tissue will be prepared for assessment of mineral content, including asbestos, at the MRC Pneumoconiosis Unit in Penarth by Dr F. Pooley and Dr V. Timbrell. Control material will also be studied. These data will be correlated with an epidemiological investigation in which the environmental and occupational histories of the mesothelioma cases and controls will be obtained. All these results will be analysed, and it is hoped that a closer understanding of the association between mesotheliomas and asbestos exposure will be obtained.

Dr Wagner visited Professor I. Webster at the South African Medical Research Council, National Research Institute for Occupational Diseases, Johannesburg, to study peritoneal mesothelioma cases from the Northern Transvaal crocidolite mines. Material for analysis by Dr Pooley was obtained, and arrangements were made for obtaining lung tissue from further cases, if they occurred.

Further biopsy material has been obtained from Professor I. Baris, and at least three pleural mesotheliomas have been diagnosed. It is not known, however, how many biopsies are available, and arrangements are being made for one of Professor Baris' assistants to bring all the available material to the MRC Pneumoconiosis Unit.

Histological material from French naval dockyards has been discussed with Dr J. Bignon (Laennec Hospital, Paris). It is hoped that the lung tissue available will be studied by Dr Sebastien in collaboration with Dr Pooley.

(*d*) *Epidemiology*

Collection of data in the detailed study of men exposed to asbestos in dockyards in the United Kingdom (Sgn Cdr P. G. Harries) has been completed, and preliminary reports have been submitted to the Ministry of Defence (Navy) and to the Medical Research Council. These reports are being prepared for publication. The major X-ray reading exercise has shown that in order to extract all necessary information for assessing response to asbestos exposure each radiograph must be read to the full ILO/UICC 1971 classification by at least three readers.

There is evidence of an interaction between cigarette smoking and exposure to asbestos in the development of pleural thickening and of pulmonary fibrosis. The separate nine-year follow-up of a group of dockyard workers at Devonport who had heavy exposure to asbestos before 1966 has shown that radiographic and lung functional changes occurred subsequently, despite cessation of asbestos exposure.

The results of the study of chrysotile asbestos workers in North Vermont (Professor G. M. Green, Burlington) have been published and confirm earlier reports from the Quebec chrysotile mining industry.

The mortality and repeat morbidity studies of asbestos cement workers in New Orleans (Professor H. Weill) are continuing.

4.2 *Institute of Experimental and Clinical Medicine, Tallinn, Estonian SSR* (RA/74/011)
Principal investigator: Dr A. Vôsamäe

The role of chrysotile asbestos dust in respiratory tract carcinogenesis in Syrian golden hamsters has been studied by comparing the effect of intratracheal instillation of asbestos

with oral or subcutaneous administration of nitrosodiethylamine (NDEA) in long-term experiments. Solutions of NDEA in distilled water were given

(*a*) orally (through a gastric tube) as 0.4 ml of a 0.4% solution twice weekly for 20 weeks (single dose/animal was 1.5 mg, total dose was 60 mg); or

(*b*) subcutaneously as 3.5 mg NDEA in 0.2 ml water injected once weekly for 12 weeks (total dose, 42 mg).

Canadian chrysotile asbestos dust (a standard UICC sample) was administered intratracheally in a dose of 1 mg suspended in 0.5 ml polyglucin (a standard synthetic blood plasma substitute) once weekly for 6 weeks (total dose, 6 mg). The dust applications were started one month after the commencement of NDEA treatment.

Syrian golden hamsters were obtained from the Laboratory Animal Production Farm 'Rappolowo' of the Academy of Medical Sciences of the USSR. The animals were 3 to 4 months old at the beginning of the treatment; they were separated by sex and housed in sets of 5 to 6 in autoclaved, stainless-steel cages. A total of 355 hamsters were divided into 6 groups of approximately equal numbers of male and female animals. The test substances, routes of administration, initial numbers of hamsters and numbers of animals investigated histologically are presented in Table 9.

The experiment was terminated after 24 months. Animals were allowed to die or were killed when moribund. Animals treated with NDEA or NDEA plus asbestos dust had a much higher mortality than did asbestos dust-treated and polyglucin-treated controls: for groups 1 to 4, the experiment was terminated at 8–10 months, for group 5 at 21 months and for group 6a at 24 months. The first tumour, a tracheal papilloma, was found in a hamster in group 4, which died 1.5 months after the beginning of subcutaneous injections of NDEA and immediately after the 3rd intratracheal instillation of asbestos dust suspensions.

NDEA exerted a marked carcinogenic effect on the upper respiratory tract (trachea, and to lesser extent, laryngeal mucosa) of the hamsters. In animals in groups 1 to 4, multiple tracheal papillomas occurred, and no significant inter-group differences in their frequency or histological characteristics could be found. The incidence of pulmonary tumours was significantly greater in hamsters given NDEA and asbestos dust (groups 3 and 4) than in those to whom only NDEA was administered (groups 1 and 2). Most of the benign pulmonary tumours were peripheral pulmonary adenomas (adenomas of the bronchiolar region) occurring singly or severally. Most of the malignant lung tumours were bronchial or brionchiolar mucus-producing adenocarcinomas. In addition, pretumorous epithelial lesions were seen in all NDEA-treated animals.

No lung tumours developed in animals treated with asbestos dust only (group 5), and single tracheal or laryngeal papillomas were found in only a very few cases. No respiratory tract tumours were found in animals of the two control groups (groups 6a and b). Liver tumours (hepatomas and hepatocellular carcinomas) occurred with low to moderate frequency in those animals treated with NDEA that died in the late stages of the experiment.

The present data show that Canadian chrysotile asbestos dust, instilled repeatedly into the tracheas of NDEA-treated hamsters, increases the neoplastic response of the lower respiratory tract.

Table 9. Effects of nitrosodiethylamine (NDEA) and asbestos dust on hamsters

Group no.	Test substance and route of administration	Initial no. of animals (M, F) [a]	No. of animals investigated histologically	Effective no. of animals	No. of animals with tumours									
					laryngeal		tracheal		pulmonary		liver		other tumours [b]	
					benign	malignant	benign	malignant	benign	malignant	benign	malignant	benign	malignant
1	NDEA orally	50 (25, 25)	50	50	—	—	44	—	1	—	3	7	1	—
2	NDEA subcutaneously	50 (28, 22)	50	47	13	—	42	—	2	1	3	1	1	—
3	NDEA orally and asbestos dust in polyglucin intratracheally	60 (30, 30)	56	52	4	—	34	—	15	6	3	1	5	—
4	NDEA subcutaneously and asbestos dust in polyglucin intratracheally	60 (30, 30)	58	51	6	—	31	—	5	9	1	—	3	—
5	Asbestos dust in polyglucin intratracheally	50 (28, 22)	50	50	1	—	2	—	—	—	1	—	5	2
6	Controls:													
	(*a*) Polyglucin intratracheally	30 (15, 15)	30	30	—	—	—	—	—	—	—	—	—	—
	(*b*) No intratracheal or other treatments	55 (22, 33)	55	55	—	—	—	—	—	—	—	—	8	5

[a] Numbers in parentheses refer to male and female animals.
[b] Except tumours of nasal cavity

5. BIOLOGICAL EXPERIMENT TO TEST THE POSSIBLE CARCINOGENICITY OF DAPSONE

(Dr L. Griciute)

Long-term testing of the possible carcinogenicity of the anti-leprosy drug dapsone has been continued. BDIV rats and C57BL mice received dapsone *in utero* and during suckling, by administration to pregnant and lactating females, and from seven weeks of age by peroral intubation of the drug in water. A dose of 100 mg/kg bw was administered five times weekly. Up to May 1976, 138 of 174 rats receiving dapsone and 42 of 55 positive controls receiving injections of benzo[*a*]pyrene were still alive; 65 of 70 mice receiving dapsone, 64 of 67 which received it only *in utero* and during suckling and 67 of 70 positive controls which received injections of urethane were still alive. Only a few tumours were observed in both experimental and positive control groups.

3. UNIT OF BIOLOGICAL CARCINOGENESIS

Dr G. BLAUDIN DE-THÉ (Chief)

1. INTRODUCTION

The main emphasis in the programme for study of the etiology of Burkitt's lymphoma was the detection of cases among the population which had been bled previously. The low incidence reported last year was followed by a very high incidence during the second part of 1975: up to June 1976, twelve cases of Burkitt's lymphoma had been detected in the previously bled cohort of children. Testing of the first ten pairs of lymphoma sera with matched controls indicated that children who later developed Burkitt's lymphoma were marked a long time before developing the disease by elevated titre of antibodies against viral capsid antigen of the Epstein–Barr virus. If confirmed, this finding would favour one of the hypotheses which was put forward in the prospective study, namely that heavy chronic Epstein–Barr viral infection is a risk factor for the development of Burkitt's lymphoma. The hypothesis that Epstein–Barr viral infection in the neo-natal period of life might represent a risk factor for Burkitt's lymphoma is being tested in the West Nile district of Uganda.

It is obvious, however, that the ubiquitous Epstein–Barr virus infection cannot be the only cause of the high but geographically restricted incidence of Burkitt's lymphoma; thus, a search for co-factors is being built into the programme. Results of malaria surveys in both the West Nile District of Uganda and in the North Mara District of Tanzania have shown that parasitaemia and presence of malaria antibodies parallel the risk for Burkitt's lymphoma. To investigate whether hyperendemic malaria is an essential factor in the development of Burkitt's lymphoma, an intervention trial is to be carried out in the North Mara District of Tanzania. Feasibility studies were made during the period under review. Another co-factor might involve genetic susceptibility to some oncogenic factor, since family clustering of Burkitt's lymphoma indicates that some families have an unusually high risk for Epstein–Barr virus-associated diseases (Burkitt's lymphoma and nasopharyngeal carcinoma).

In the framework of the nasopharyngeal carcinoma etiology programme, the study of genetic markers associated with this tumour has made it possible to propose the hypothesis of a disease-susceptibility gene. Immunogenetic studies in Singapore indicate that the presence of a mixed-lymphocyte reaction gene is a genetic marker for nasopharyngeal carcinoma risk; this gene is probably closer to the disease-susceptibility gene than the previously described HLA genes. Collaborative studies in Hong Kong, Singapore and Tunis and by a network of collaborating laboratories further extended the *in vitro* cell-mediated immunity studies reported last year, by a coordinated effort to use the same

antigenic preparation in the various laboratories. Cold lymphocytotoxic antibodies, circulating immune complexes and small, smooth-muscle antibodies were found in nasopharyngeal carcinoma patients from different geographical areas. A new serological test to be adapted to field conditions is being developed in collaboration with national laboratories, using a partially purified Epstein–Barr virus nuclear antigen. Such a test could become very useful in the epidemiology of Epstein–Barr virus-associated disease.

The international reference programme on oncogenic herpes viruses was pursued with the help of consultants, and two newsletters were produced and distributed during the review period.

Projects on genital cancers and on the relationship between prenatal events and childhood cancer were pursued according to plan. The former should reach completion by the end of 1976.

2. ETIOLOGICAL STUDIES OF BURKITT'S LYMPHOMA (Dr A. Geser)

2.1 *Prospective study of Burkitt's lymphoma in the West Nile District, Uganda*

East African Virus Research Institute, Entebbe, Uganda
Principal investigator: Dr P. M. Tukei

Burkitt's Lymphoma Field Station, West Nile District, Uganda
Principal investigator: Dr D. P. Beri

Kuluva Hospital, Arua, Uganda
Principal investigator: Dr E. H. Williams

(*a*) *Case detection*

The search for new cases of Burkitt's lymphoma continued throughout the entire West Nile District and, in particular, in the cohort of 45 235 children who were bled in the initial survey between 1972 and 1974. The low incidence of this tumour seen in 1974 (only six cases), which was reported in the last Annual Report [1], was followed by a peak incidence in 1975, when 19 cases were detected in the West Nile District. Six of these cases had been bled in the serum survey; the total number of pre-bled cases now stands at 12. Ten of these sera have been tested for Epstein–Barr virus antibody activities, and the results are discussed in section (*b*) below.

No explanation has been found for the drastic variation in incidence of Burkitt's lymphoma in the West Nile during 1974 and 1975. The incidence of malaria infection remained at the same hyperendemic levels from 1972 to 1975; and the intensity of malaria treatment did not vary in such a way as to explain the observed changes in Burkitt's lymphoma incidence. Rain has been abundant in the West Nile during recent years, and there is no indication that the 'outbreak' of lymphomas in 1975 was preceded by unusually severe malnutrition in the area.

---

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 63.

(*b*) *Testing of 'pre-Burkitt's lymphoma' sera* (Mrs M. F. Lavoué)

As mentioned above, twelve sets of pre- and post-Burkitt's lymphoma sera are now available. For two of the cases, only small quantities (less than 0.1 ml) of pre-Burkitt's lymphoma sera were available, and these will be kept for testing at the end of the study. The remaining ten pre-Burkitt's lymphoma sera were tested simultaneously and under code by Dr W. Henle in Philadelphia and in the Agency laboratories. The ranking correspondence between the two sets was very good in the analysis of data on sera from cases, controls and the general population [obtained from the in-depth studies described in (*c*) below].

The results were analysed by comparing antibody levels in pre-Burkitt's lymphoma sera with those found in five controls which were bled in the main study in the same areas and at the same time as the Burkitt's lymphoma patients. The figures presented below refer to Agency data.

Figure 8 shows the Epstein–Barr virus reactivities before tumour development in ten pairs—each pair composed of a Burkitt's lymphoma patient and a control. The figure shows that pre-lymphoma sera differ from those of matched controls taken at the time of the main bleeding, mostly with respect to viral capsid antigen titres, which were higher in the pre-lymphoma sera than in the matched control sera in about half of the cases. Antibody reactivities for early antigen were not significantly different in the two groups. With regard to Epstein–Barr virus nuclear antigen and complement-fixing antigen, a few sera showed lower reactivities than matched control sera.

Figure 9 shows the viral capsid antigen titres of pre-Burkitt's lymphoma sera and those of neighbours taken at the time of the main bleeding. The figure also shows the time interval between the initial bleeding and the development of Burkitt's lymphoma. Pre-Burkitt's lymphoma sera tended to have a higher viral capsid antigen titre than sera from matched controls, and the level of viral capsid antigen antibodies in the sera decreased with increasing age at the time of the main bleeding.

The same phenomenon is apparent in Figure 10, in which the viral capsid antigen titres of pre-Burkitt's lymphoma sera are compared with the geometric mean titre of the corresponding age groups in the general population. Pre-Burkitt's lymphoma sera from very young children showed significantly higher titres than those from older lymphoma cases; this trend seems to parallel the decline in viral capsid antigen titres among the general population between the ages of two and fifteen years.

Pre-Burkitt's lymphoma viral capsid antigen titres for each case and the titres of the corresponding five matched controls from the main bleeding are given in Table 10. The penultimate column gives the rank of the case among the six values; the final column gives the proportion of the general population (see also Fig. 10) of the same age and sex with titres greater than that of the corresponding case.

The upper part of the table refers to results obtained by the Agency, while the lower part gives data obtained from the same sera by Dr W. Henle (Children's Hospital of Philadelphia, USA). Although the titres found in Dr Henle's laboratory are regularly lower than those found at the Agency, there is excellent correlation as can be seen by the ranking of the cases. When each Burkitt's lymphoma case is compared with its five matched controls, eight out of ten cases rank either 1 or 6 in both Dr Henle's and the Agency laboratories. The probability of this happening by chance is 0.0034 for the Agency data and 0.047 for Dr Henle's data.

Fig. 8 Epstein-Barr virus reactivities before tumour development compared in ten Burkitt's lymphoma (BL) patients and ten controls

VCA—viral capsid antigen; EA—early antigen; ENBA—Epstein-Barr nuclear antigen; CF—complement fixing antigen

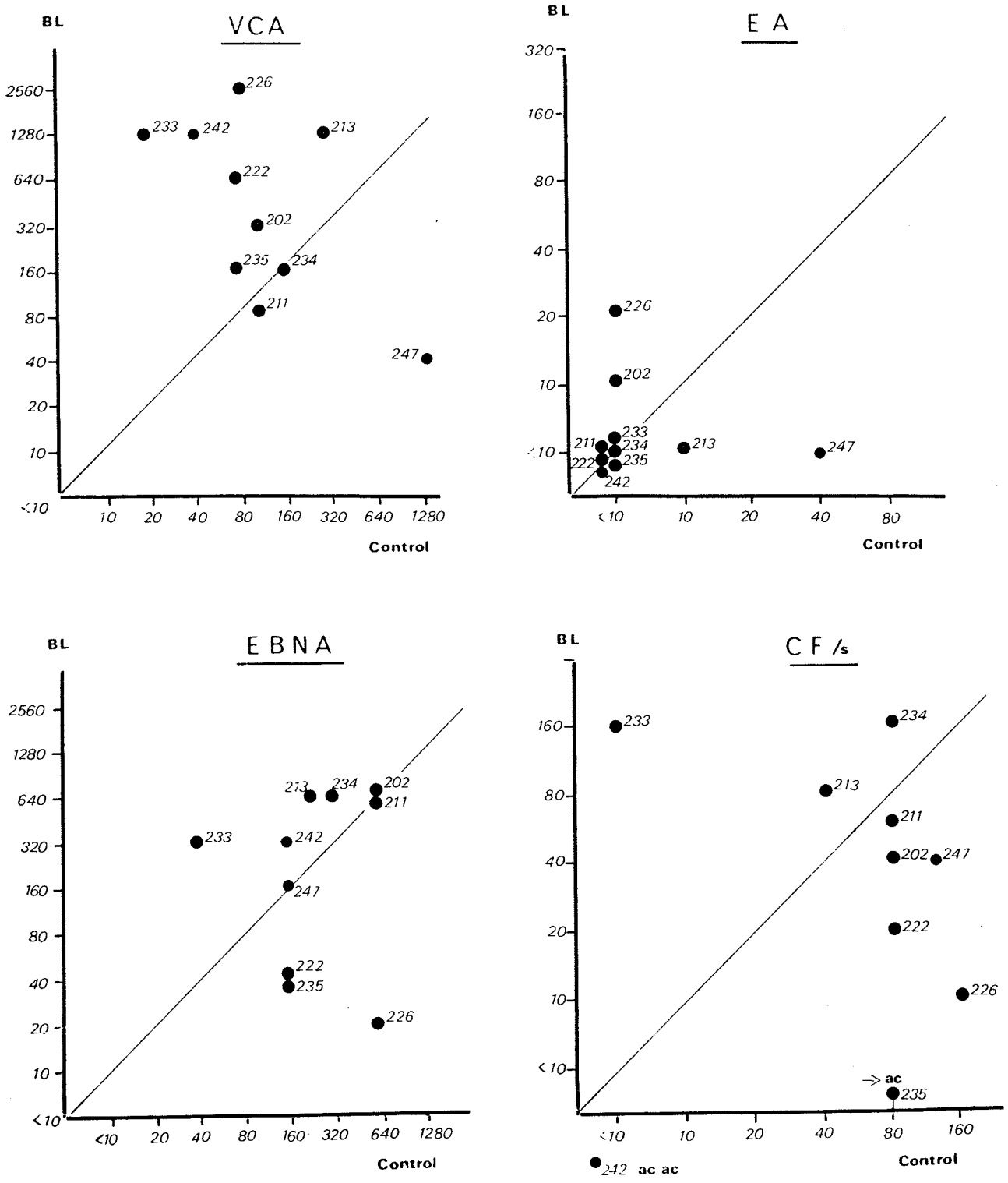


Fig. 9 Viral capsid antigen (VCA) titres in pre-Burkitt's lymphoma (BL) sera (*) and in those from neighbours (o)

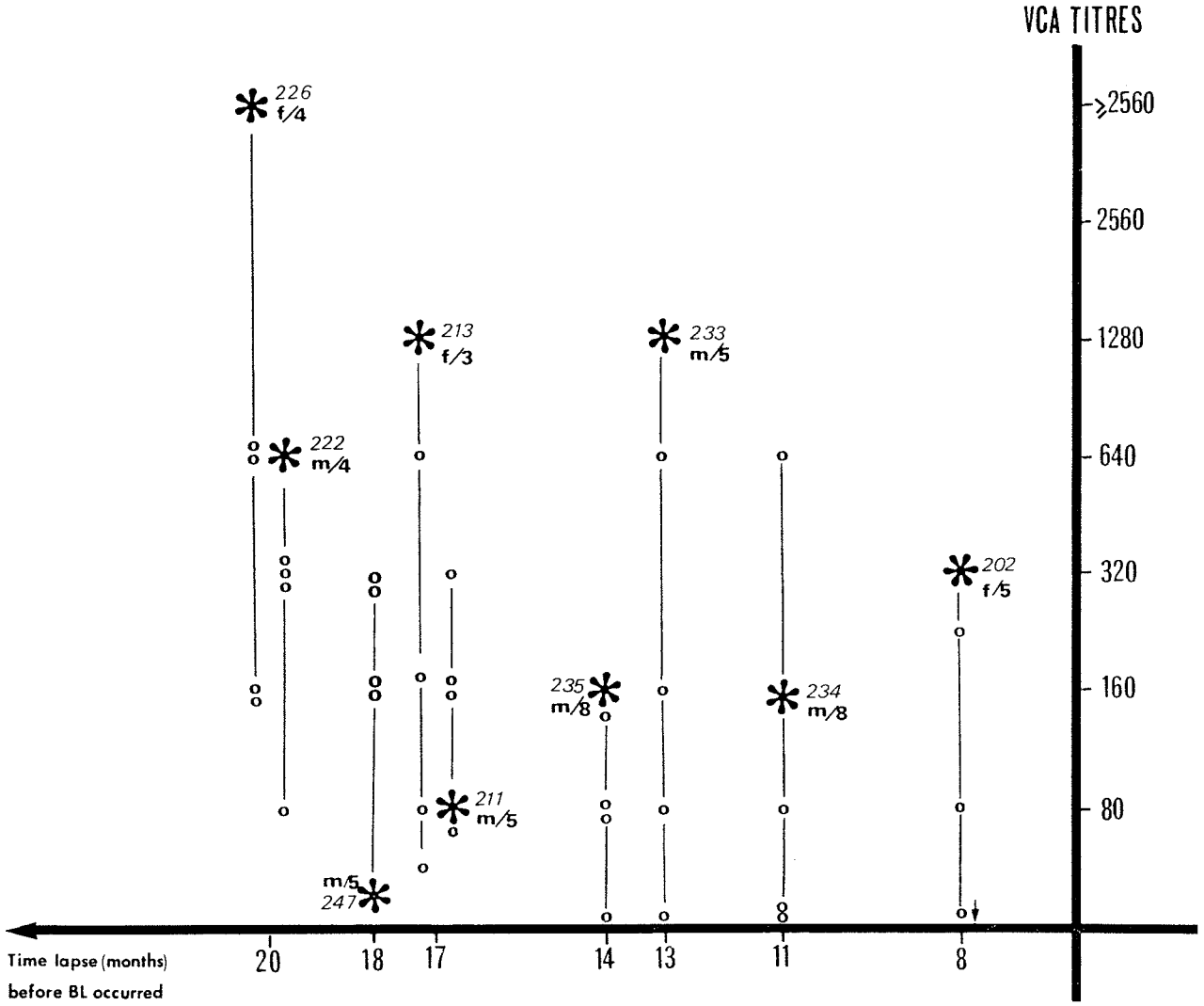


Figure 11 indicates that five cases out of ten are in the highest 15% of the titres for viral capsid antigen when matched by age to the general population. Figure 12 gives the serological profiles for Epstein–Barr virus in pre- and post-disease sera from Burkitt's lymphoma patients as compared with those from controls. This figure shows (that there is no major serological difference between pre- and post-Burkitt's lymphoma sera as compared to matched controls, except for the presence of antibodies directed against early antigens: although a sero-conversion from negative to positive was observed in a few cases, in the rest of the cases an increase in early antigen titres was seen between pre- and post-lymphoma sera.

Frozen biopsy specimens were available from seven of the pre-bled Burkitt's lymphoma cases, and these were sent to Dr zur Hausen for detection of Epstein–Barr virus genome by DNA hybridization. Five gave clear positive results, showing the presence of DNA from the viral genome into the tumour cells. Two of the tumours, however, from Burkitt's lymphoma cases Nos 234 and 247, gave results below the sensitivity of the assay. These results may explain the low antibody titres seen for these same two tumours (see Table 10).

Fig. 10 Viral capsid antigen (VCA) titres in pre-Burkitt's lymphoma (BL) sera (*) compared to the geometric mean titre of the corresponding age groups of the general population (———)

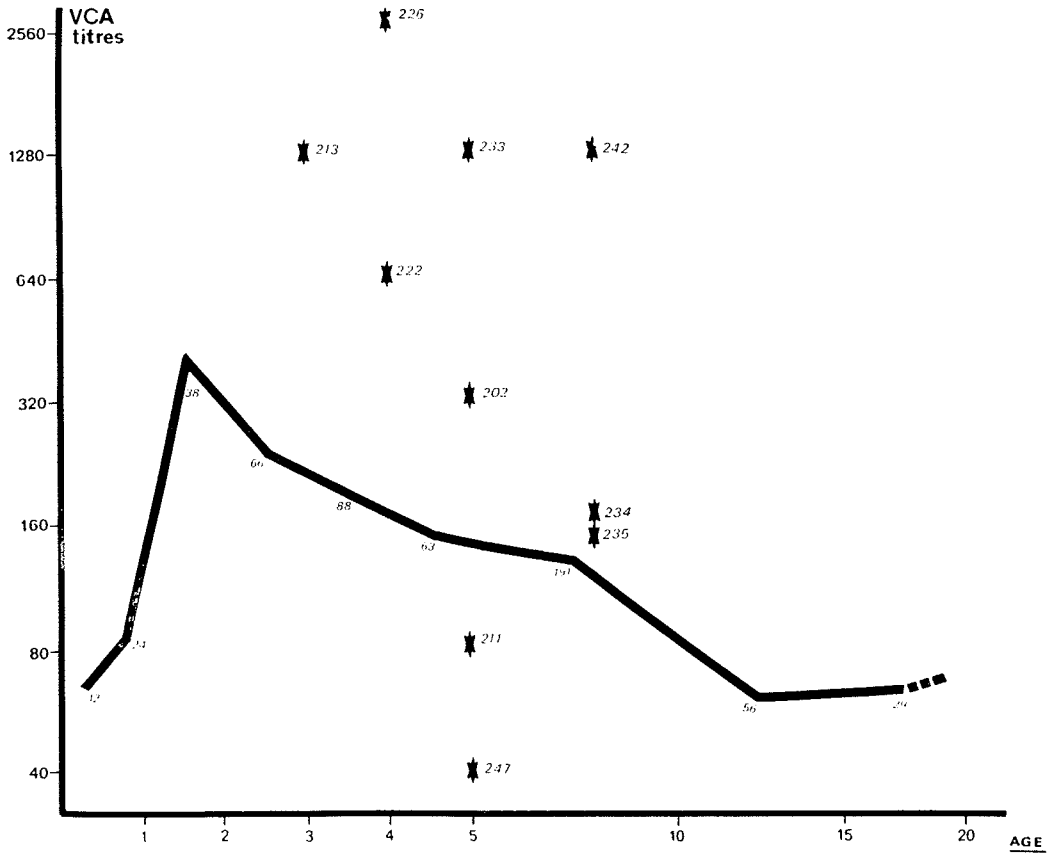


Fig. 11 Percentages of the general population in the 3-4, 4-5 and 5-9 year age groups with a viral capsid antigen titre at or above the level shown by the horizontal black lines, as compared with Burkitt's lymphoma cases (squares)

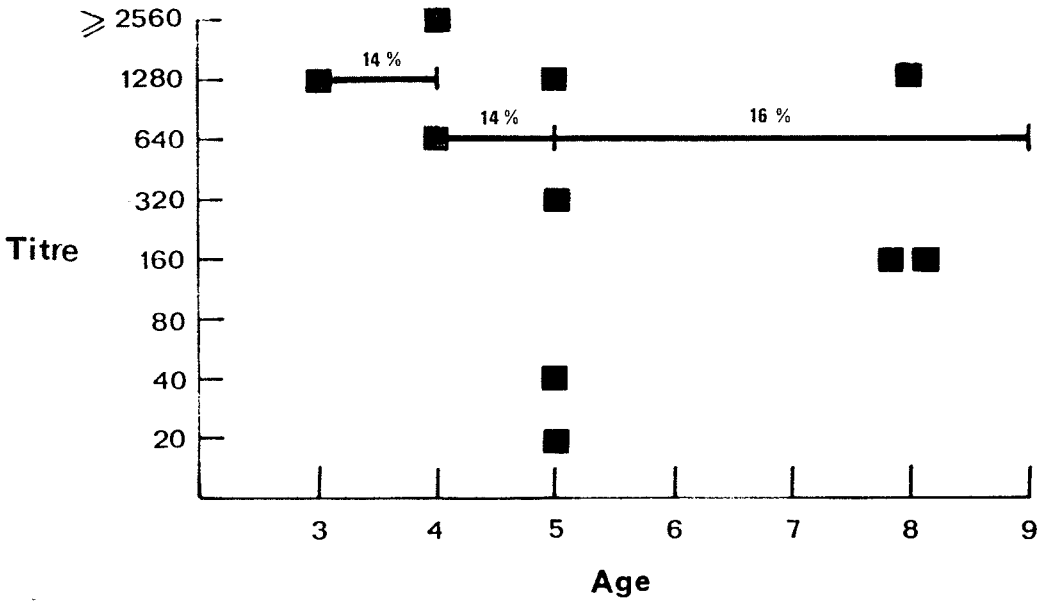


Fig. 12 Epstein-Barr virus profiles of pre- and post-disease sera from Burkitt's lymphoma (BL) cases (●) as compared to controls (△)
VCA — viral capsid antigen; EBNA — Epstein-Barr nuclear antigen; EA — early antigen

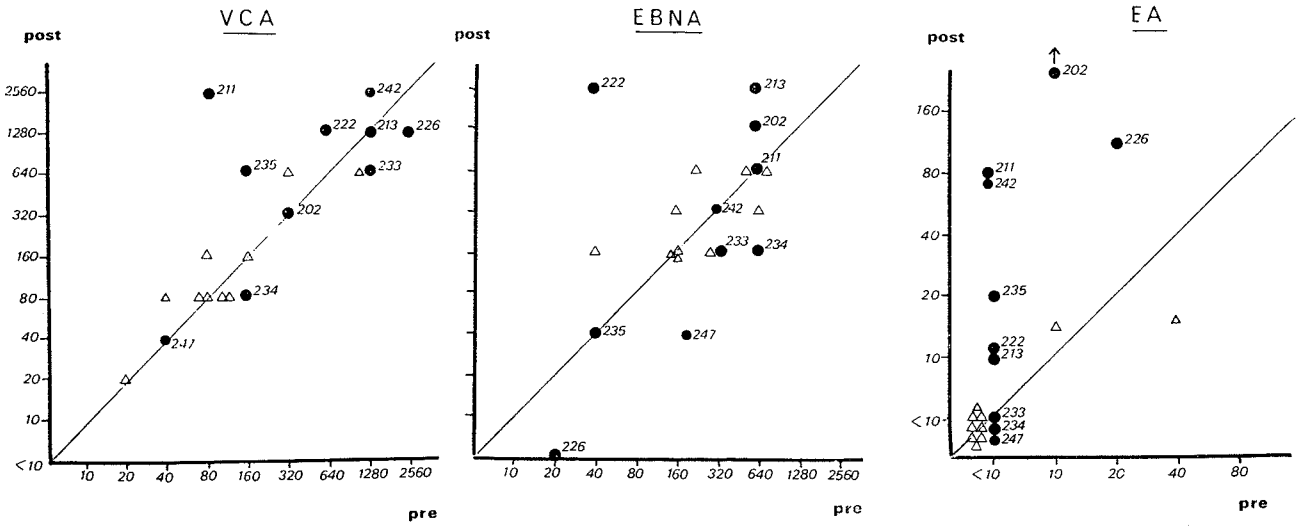


Table 10. Pre-Burkitt's lymphoma (BL) Epstein–Barr viral capsid antigen titres for 10 cases and for their five matched controls. Results from IARC and from Dr W. Henle (Philadelphia, USA)

BL case no.	Pre-BL titre	Controls				Rank of case		Proportion of general population with a higher titre
IARC RESULTS								
202	320	80/160	320	160/320	80	10	1 ½	0.27
211	20	80/160	160	80	320	160	6	0.93
213	1 280	320	40/80	640	80	160	1	0.09
222	640	80	320	80	320	320	1	0.10
226	⩾ 2 560	80	640	640	160	160	1	0.01
233	1 280	20	160	80	640	40	1	0.08
234	160	160	40	640	80	40	2 ½	0.38
235	160	80	40	160	80	80	1 ½	0.38
242	1 280	40	80	320	320/640	80	1	0.08
247	40	1 280	320	160	320	160	6	0.80
							P< 0.0034	P = 0.047
DR HENLE'S RESULTS								
202	40	40	160	40	40	5	(3)	
211	10	40	40	80	80	40	6	
213	160	80	40	160	40	40	1 ½	
222	640	80	80	40	80	160	1	
226	⩾ 2 560	40	80	80	40	80	1	
233	320	10	40	20	80	10	1	
234	40	80/160	20	80	40	10	3 ½	
235	80	40	10	80	40	40	1 ½	
242	320	20	20/40	40	80	40	1	
247	20	80	40	80	160	320	6	
							P = 0.047	

Three basic hypotheses were originally proposed:

(i) Pre-Burkitt's lymphoma titres would be lower than those in the sera of the general population.
(ii) Pre-Burkitt's lymphoma titres would be higher than those in the sera of the general population.
(iii) Pre-Burkitt's lymphoma titres would not differ from those of the sera of the general population.

The results show that eight out of ten cases appear to be extreme, six having higher viral capsid antigen titres than expected and two having lower ones. It is most interesting to see that one of the odd cases represents an Epstein–Barr virus-free lymphoma. An analysis of all the data is in progress.

These preliminary results suggest the following:

1. Antibodies directed against Epstein–Barr virus seven to thirty-one months prior to onset do not protect against the development of Burkitt's lymphoma.
2. On the contrary, high viral capsid antibody titres seem to be a factor which favours the development of the lymphoma.
3. The rise in early antigen antibody titres after lymphoma development probably reflects the reactivation of a heavy, chronic Epstein–Barr viral infection.

These results are consistent with the second hypothesis, that a heavy, chronic Epstein–Barr viral infection is a factor which favours the development of Burkitt's lymphoma. However, since most of the sera were taken seven to 20 months prior to development of the tumour, these results cannot yet rule out the first hypothesis. Furthermore, the above results are consistent with the hypothesis discussed below in section 2.2 that perinatal Epstein–Barr viral infection might be a crucial risk factor for development of Burkitt's lymphoma.

The testing of pre-Burkitt's lymphoma sera for antibodies against viruses other than Epstein–Barr virus, such as measles virus or herpesvirus, herpes simplex virus or cytomegalovirus, should also be carried out, since it will be important to know if the high viral capsid reactivity observed in the pre-Burkitt's lymphoma sera represents a specific immune response against Epstein–Barr virus or merely a general increase in response against a variety of infections. However, we do not want to deplete our stock of this highly valuable sera, and so such testing will probably not be made until the end of the study.

(c) *Re-bleeding in sample groups in the West Nile District ('in depth study')*

Sixteen sample groups, each comprising approximately 75 zero to nine-year-old children plus their families, are being re-bled every 6–10 months in order to study changes with time in Epstein–Barr virus antibody titres and malaria infection in the cohort under study in the West Nile District of Uganda. In January 1976, two further groups from the highlands of Okoro County, situated in the south-east corner of the District, were included. This plateau, which reaches a height of more than 5 000 feet, is practically free of Burkitt's lymphoma. It therefore seemed of interest to compare Epstein–Barr virus antibody titres in this area to those prevailing in the lowlands of the West Nile District where Burkitt's lymphoma is relatively frequent. The sera from the West Nile plateau will be tested for

Epstein–Barr virus antibodies and compared with those in sera from high and low areas in North Mara District, Tanzania, where an equally wide differential in Burkitt's lymphoma incidence exists.

2.2 *Neonatal Epstein–Barr virus infection and risk for Burkitt's lymphoma*

As shown in the preceding Annual Report [1], the age incidence of Epstein–Barr viral infection varies greatly in different geographical areas and among ethnic groups which are at different risks for Epstein–Barr virus-associated diseases. It has been shown that primary Epstein–Barr viral infection occurs in 99% of Ugandan children before the age of three, whereas such primary infection does not occur in Indians or in Singapore Chinese before 10 or 15 years of age. It was therefore decided to investigate whether early Epstein–Barr viral infection could represent a risk factor for the development of Burkitt's lymphoma. A hypothesis was made that peri- or neonatal primary Epstein–Barr viral infection, possibly in the presence of maternal antibodies, takes place in tropical areas where Burkitt's lymphoma is endemic and represents a risk factor for development of the tumour. The relationship which exists between neonatal infection by measles virus and the development, years after, of sub-acute sclerosing panencephalitis represents an example of this hypothesis.

The study is designed to determine if peri- or neonatal infection by Epstein–Barr virus takes place in Uganda. Techniques for the detection of infectious and transforming Epstein–Barr virus in the saliva of newborns and for the detection of immunoglobulin M (IgM)/Epstein–Barr virus antibodies in sera from newborns have been developed in the Agency laboratories. In a three-month pilot study carried out in Arua by Dr E. H. Williams to assess the feasibility of collecting monthly blood and saliva samples from babies, it was found that venous blood can be obtained from children after the age of four to six months. Implementation of the project will take place in the autumn of 1976.

2.3 *Co-factors in the etiology of Burkitt's lymphoma*

(*a*) *Malaria*

Since it is obvious that the omnipresent Epstein–Barr virus cannot be the only cause of the highly restricted Burkitt's lymphoma, a search for co-factors is built into all the ongoing field studies of this tumour. Malaria is still the most probable co-factor, since high incidences of Burkitt's lymphoma are restricted to areas in which malaria is highly endemic. In order to determine more accurately the level of malaria endemicity compatible with high Burkitt's lymphoma incidence, malaria surveys, described in the preceding Annual Report [2], were carried out in high and low areas of the West Nile District, Uganda and of the North Mara District, Tanzania. The results of these surveys are summarized in Table 11, in terms of malaria parasitaemia and presence of malaria antibodies. It can be seen that the prevalence of malaria in children is high (45.6–68.4%) in areas of low altitude in both districts and much lower (8.87–23.8%) on the Burkitt's lymphoma-free plateaux. Repeated malaria surveys in the North Mara District produced the surprising result that there is a much higher prevalence of malaria in the dry than in the wet season; for a better

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 67.
[2] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, pp. 62–63.

understanding of this finding, the malaria surveys are being repeated in North Mara in May-June 1976.

Table 11. Incidence of Burkitt's lymphoma and prevalence of malaria infection in East Africa at different altitudes

Area	Annual incidence of Burkitt's lymphoma	Malaria in 0–9-year-old children		Level of malaria endemicity
		Parasitaemia	Immunofluorescent antibodies	
West Nile District, Uganda (2 000–4 000 ft)	2.8/ 1 000 pop.	All seasons 68 %	100 %	hyperendemic
North Mara lowlands, Tanzania (2 000–5 000 ft)	2.8/ 1 000 pop.	Dry season 46 % Wet season 29 %	100 %	hyperendemic
High plateau, North Mara, Tanzania (5 000–7 000 ft)	Nil	Dry season 24 % Wet season 4 %	35 %	hypoendemic

(*b*) *Malaria suppression trial in Mara region, Tanzania*
Shirati Hospital, Tanzania

Principal investigator: Dr G. Brubaker

In order to establish whether or not hyperendemic malaria is essential for maintaining a high incidence of Burkitt's lymphoma in a population, an intervention trial is being planned in the Mara region. Feasibility studies continued during the period under review, mainly to ascertain that the incidence of Burkitt's lymphoma in the area is high enough to permit a meaningful interpretation of the results. Active case detection was introduced in South Mara from September 1975, and, while only one case was reported there during the first eight months of 1975, an additional six cases were detected during the last four months. In North Mara, five new cases were detected in 1975, making a total of 12 for the year in the whole region; this is near to the minimum number needed for the trial.

Continued scrutiny of newly established cooperative villages *(ujamaas)* confirmed that a drug distribution scheme would be feasible in view of the community organization that has been established in the entire region. At the beginning of 1976, the Tanzanian Government gave its formal approval to the research project, and preparatory work continues in the field.

(*c*) *Other co-factors in the etiology of Burkitt's lymphoma*

(i) *Environmental factors*

It should be kept in mind that the geographical association between malaria and Burkitt's lymphoma might well be due to a concealed variable, i.e., to other factors which coincide with malaria, either because they are transmitted by the same vectors or simply because they prevail in the same environment. One such factor is malnutrition, since

protein and other deficiencies are common in young children in the tropics and might conceivably increase their susceptibility to Burkitt's lymphoma. Observations made in the West Nile District project seem to rule out this possibility, however, since the high incidence of Burkitt's lymphoma in 1975 was preceded by several years of regular rainfall; there was thus an abundant supply of food in the area and no period of unusual nutritional deficiency.

(ii) *Genetic factors*

Burkitt's lymphoma seems to occur with equal frequency in different tribes and populations living in areas with hyperendemic malaria; it is thus generally thought that genetic susceptibility plays a minor role in determining the distribution of the tumour.

Although environmental factors could have been implicated, the possible role of genetic factors in the etiology of Burkitt's lymphoma might be indicated by the finding in the Mara region of multiple cases of Burkitt's lymphoma and other cancers within families. Since 1965, four such families have been found in a population of about one million:

1st family: two brothers died of Burkitt's lymphoma;

2nd family: three brothers (two full brothers, one half-brother) died of Burkitt's lymphoma;

3rd family: one boy died of Burkitt's lymphoma, and his sister developed chronic leukaemia one year later;

4th family: the father of a boy who died of Burkitt's lymphoma developed nasopharyngeal carcinoma in the same year.

Studies using the Caucasian sera panel have shown no relationship between Burkitt's lymphoma and any particular HLA pattern.

3. EPIDEMIOLOGY OF EPSTEIN–BARR VIRAL INFECTION

The aim of this programme was to investigate the epidemiological characteristics of Epstein–Barr viral infection in populations at different risk for diseases associated with Epstein–Barr virus in order to formulate or test the hypothesis of a causal relationship between viral infection and development of the tumour. As described previously [1], the age-specific incidence of infection differed widely in areas of high risk for Burkitt's lymphoma as compared to ethnic groups at high risk for nasopharyngeal carcinoma. On the basis of such results it can be hypothesized that there is a relationship between eventual peri- or neonatal Epstein–Barr viral infection and risk for developing Burkitt's lymphoma years later (see section 2.2).

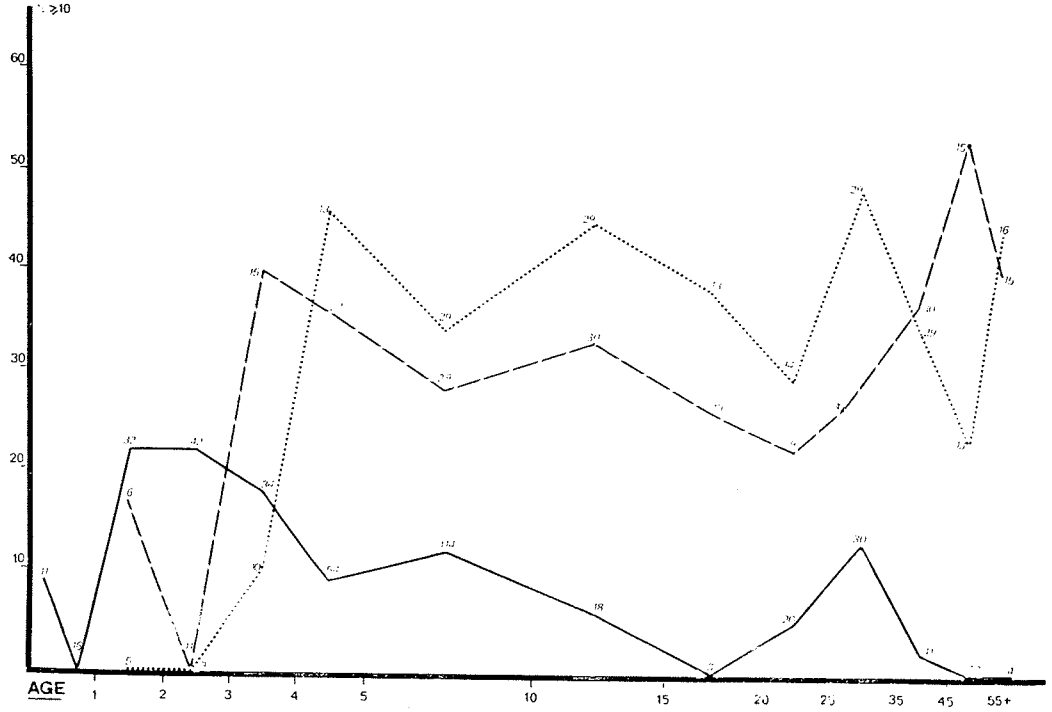
The most important results obtained during the review period concern the early antigen profiles of sera from Chinese, Indian, Ugandan and Caucasian populations. Figure 13 shows a peak occurrence of antibody against early antigen in Ugandan children of between one and four years of age, probably reflecting very early primary infection by the virus. By contrast, a step rise in the proportion of children with antibodies directed against early antigen occurs in Chinese children between the ages of three and five years and subsequently continues as a plateau. The high proportion (35–40%) of individuals with antibodies

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, pp. 66–68.

against early antigens among all ages of the general population of Chinese and Indians in Singapore might indicate a high rate of reactivation in these groups.

1 294 sera were sent in early 1975 to Dr Feorino (National Disease Center, Atlanta, Ga, USA), covering representative samples of the general populations of Uganda, Hong Kong, Singapore and Nancy, to be tested for antibodies against herpes simplex virus type one and against cytomegalovirus. When all of the results are available, they will be analysed statistically to compare the age-specific prevalence of infection by the three viruses, Epstein–Barr, herpes simplex and cytomegalovirus.

Fig. 13 Early antigen antibodies in sera from both sexes of three ethnic groups: ——— Africans: Uganda; -------- Chinese: Singapore; – – – – Indians: Singapore



4. ETIOLOGICAL STUDIES ON NASOPHARYNGEAL CARCINOMA

4.1 *Epidemiological studies* (Dr A. Geser and Miss B. Charnay)

Data from the nasopharyngeal carcinoma case-control study in Hong Kong have been analysed statistically, and preliminary results indicate that the consumption of salted fish was higher in nasopharyngeal carcinoma patients than in controls, and that salted fish was given to children during weaning more frequently in families of nasopharyngeal carcinoma patients than in control families. Many other variables were examined, such as demographic and socio-economic conditions, dietary and smoking habits and previous illnesses in the ear-nose-throat area: no significant differences between nasopharyngeal carcinoma patients and controls were observed.

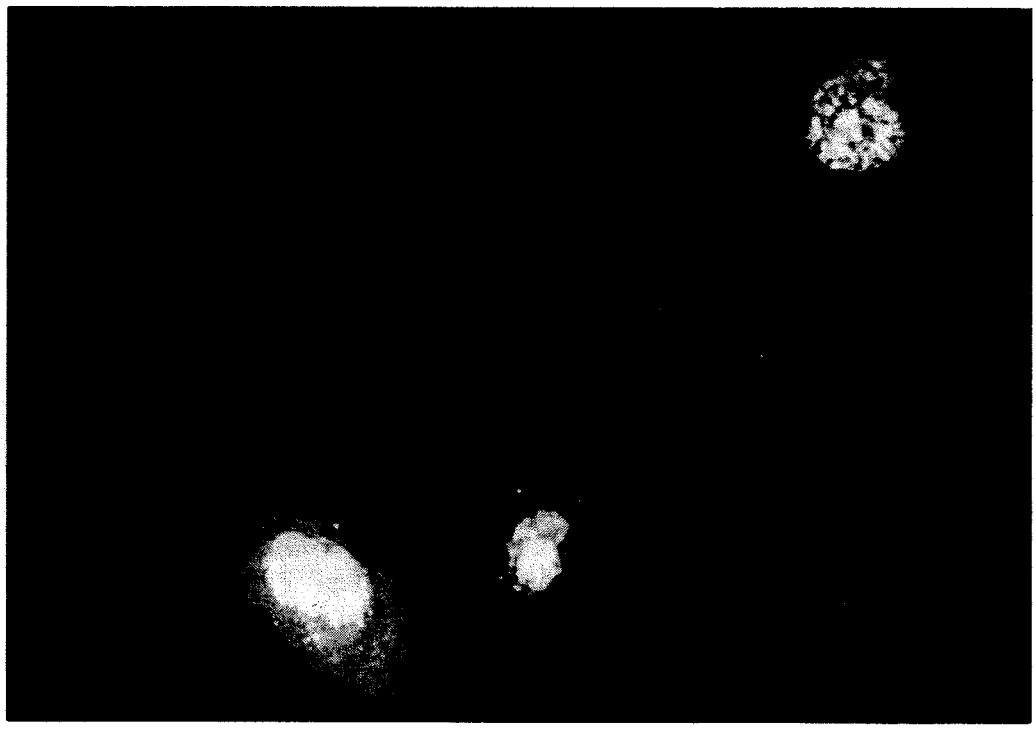
4.2 *Relationship between Epstein–Barr virus and nasopharyngeal carcinoma: markers for viral infections* (Mrs C. Desgranges-Blanc and Miss M. C. Favre) *Hershey Medical School, Hershey, Pa., USA*

Principal investigator: Dr R. Glaser

It was reported previously [1, 2] that nasopharyngeal epithelial carcinoma cells contain Epstein–Barr virus DNA, as shown by nucleic acid hybridization in separated cell populations; however, *in vivo* viral expression is limited to the synthesis of Epstein–Barr virus-specific nuclear antigen [3].

In order to ascertain whether nasopharyngeal carcinoma epithelial cells contain Epstein–Barr virus receptors and can be induced for further expression of the viral genome, nasopharyngeal carcinoma biopsies were cultured and treated either with 60 μg per ml IUdR or with P3HR1 virus (10^6 early antigen inducing units per ml). Three days later some cultures showed synthesis of early antigen in about 10% of the cells (Fig. 14). These results indicate that epithelial tumour cells from nasopharyngeal carcinomas possess Epstein–Barr virus receptors, and that dual repression of the viral genome is possible in these cells [4].

Fig. 14 Immunofluorescence photomicrograph of an epithelial cell explant (tumour 75-1531) treated with IUdR for three days. Note presence of Epstein-Barr virus early antigen-positive cells



[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, pp. 81–82.

[2] Desgranges, C., Wolf, H., de-Thé, G., Shanmugaratnam, K., Cammoun, N., Ellouz, R., Klein, G., Lennert, K. & zur Hausen, H. (1975) *Int. J. Cancer*, **16**, 7–15.

[3] de-Thé, G., Ablashi, D. V., Favre, M. C., Mourali, N. & Ellouz, R. (1975) In: Clemmesen, J. & Yohn, D. S., eds, *Comparative Leukemia Research*, Tokyo, University of Tokyo Press, pp. 101–103.

[4] Glaser, R., de-Thé, G., Lenoir, G. & Ho, J. H. C. (1976) *Proc. nat. Acad. Sci. (Wash.)*, **23**, 468–469.

The fact that the viral genome present in epithelial tumour cells can be induced to produce early and late viral antigen allows the isolation of Epstein–Barr viruses from the tumour cells themselves. In view of the very restricted areas and ethnic groups in which nasopharyngeal carcinoma occurs, it is of prime importance to try to obtain a wild, nasopharyngeal carcinoma-derived virus for use instead of the strain presently available, which is probably derived from B lymphocytes of patients with this tumour. It is possible that the two viruses are different, and one of the objectives of the Agency is to study the different biotypes of Epstein–Barr virus isolated from tumour cells from different geographical areas and from normal individuals in various parts of the world (see section 4.4). A search has also been made for the presence of Epstein–Barr virus receptors on the surface of epithelial cells from normal nasopharyngeal mucosa, with, however, up to now, negative results.

4.3 *Characterization of Epstein–Barr virus antigens; development of field-adaptable serological tests* (Dr G. Lenoir)

The Zoological Society of London, Nuffield Institute of Comparative Medicine, London

Principal investigator: Dr A. Voller

The Epstein–Barr virus nuclear antigen described in the last Annual Report [1] is presently being purified by techniques described elsewhere [2] and by the use of preparative acrylamide electrophoresis and columns. As soon as purification of this antigen reaches a satisfactory degree, a specific hetero-antiserum will be prepared for the development of a radioimmune assay. Such a highly specific test would be useful not only in titrating large numbers of sera in the field for specific antibodies, but also for detecting antigens in tumours and therefore allowing the screening of large numbers of tumours for the specific viral markers associated with them.

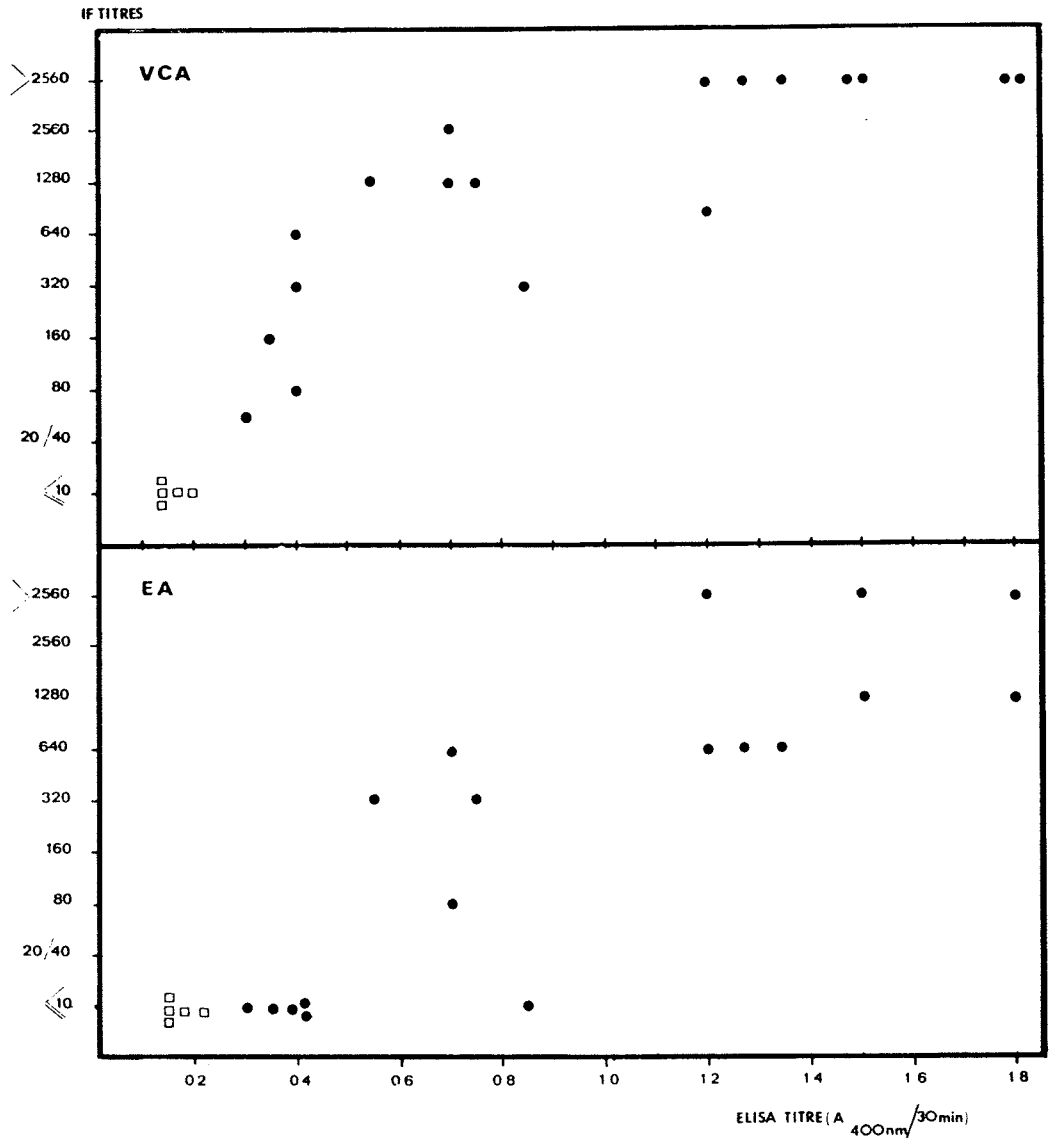
The characterization of Epstein–Barr virus early antigens is also of importance, since the antibodies against such antigens reflect active Epstein–Barr virus infection in the host and are of prognostic value in cases both of Burkitt's lymphoma and of nasopharyngeal carcinoma. The development of tests based on complement fixation allows biochemical analysis of these antigens [3]. This technique and gel filtration analysis indicate that early antigen activity is supported by a 150 000 daltons molecule.

In collaboration with Dr Voller, a new serological test using alkaline phosphatase-labelled antihuman immunoglobulin has been applied as an indirect test to the detection of Epstein–Barr virus antigens (either with crude antigen from the Pfizer Company, under contract with the National Cancer Institute Virus Cancer Program, or with the semi-purified virus or Epstein–Barr virus antigens prepared by Dr Lenoir). Results of a simple colorimetric test (which is readily applicable to field conditions) show that antibodies against Epstein–Barr virus capsid antigen and against early antigen can easily be detected (Fig. 15).

---

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 77.
[2] Lenoir, G., Berthelon, M. C., Favre, M. C. & de-Thé, G. (1976) *J. Virol.*, **17**, 672–674.
[3] Lenoir, G., Berthelon, M. C., Favre, M. C. & de-Thé, G. (1975) *Biomed.*, **23**, 461–464.

Fig. 15 Relation of micro-elisa titres (obtained with P3-HR1 extract as source of antigen) to titres of viral capsid antigen (VCA) and early antigen (EA) in Epstein-Barr virus (EBV)-negative sera (□) and in EBV-positive sera (●)



4.4 In vitro *and* in vivo *virological studies* [coordinated by IARC (Dr G. Lenoir and Mrs C. Desgranges-Blanc), but carried out mostly by national institutes]

Institute for Scientific Research on Cancer, National Centre for Scientific Research, Villejuif, France

Principal investigators: Dr P. Sheldrick, Dr P. Dubouch and Dr J. C. Salomon

Rush-Presbyterian-St. Luke's Medical Centre, Chicago, Ill., USA

Principal investigators: Dr F. Deinhardt and Dr L. Falk

The *in vitro* transforming activity of Epstein–Barr virus is being investigated in order to establish whether different biotypes of Epstein–Barr virus occur, either in association with diseases (Burkitt's lymphoma and nasopharyngeal carcinoma) or in different geo-

graphical areas. Although no differences in serotype have been found up to now, viruses isolated from the saliva of patients with either infectious mononucleosis or nasopharyngeal carcinoma in Hong Kong or Tunisia differ in their cell-virus relationships with human B lymphocytes from cord blood: production of late viral antigens occurs only in cultures transformed by Epstein–Barr virus associated with nasopharyngeal carcinoma and not in those transformed by the Epstein–Barr virus associated with infectious mononucleosis.

Dr Sheldrick is investigating the infection of DNA with herpes virus, in order to obtain molecular markers for the biological differences described above.

The *in vivo* oncogenic potential of the Epstein–Barr virus is being investigated by Dr Dubouch in an experimental model system. These studies, using *Callithrix jacchus* marmosets from The Netherlands Cancer Research Institute in Amsterdam, are being carried out in collaboration with Drs Deinhardt and Falk in Chicago. The purpose of these studies is to induce tumours (lymphomas and, whenever possible, carcinomas) which resemble those found in humans, and to investigate the role of co-factors. Up to now, parallel studies in cottontop marmosets and the more common *Callithrix jacchus* marmosets have shown that although typical lymphomas are obtained with B95-8 virus, inoculation with M81 virus (produced by transforming *Callithrix jacchus* lymphocytes with a nasopharyngeal carcinoma-derived Epstein–Barr virus strain) results in lympho-proliferative diseases; this calls to mind the infiltration observed in infectious mononucleosis. A further series of experiments on *Callithrix jacchus* marmosets resulted in the development of what appears to be reticulum-cell sarcomas invading the haematopoietic tissues.

Dr Salomon is inoculating nude mice with nasopharyngeal carcinoma biopsies and with cell hybrids developed by Dr Glaser in order to investigate the *in vivo* inducibility of Epstein–Barr virus early antigens in epithelial tumour cells.

4.5 *Genetic markers in nasopharyngeal carcinoma* (See also report of the Singapore Research Centre, p. 120)

WHO Immunology Research and Training Centre, Singapore
Principal investigator: Dr M. J. Simons

Blood Transfusion Centre, Beynost, Lyon, France
Principal investigator: Dr H. Bétuel

Salah-Azaiz Institute, Tunis
Principal investigators: Professor N. Mourali and Dr T. Souissi

Faculty of Medicine, University of Tunis, Tunis
Principal investigator: Professor R. Sohier

The discovery by Dr Simons of a specific HLA haplotype A2-BSin2 associated with a high risk of nasopharyngeal carcinoma among Cantonese Chinese had led to the hypothesis that there is a nasopharyngeal carcinoma disease susceptibility gene. Its localization requires further characterization of the HLA profile associated with risk of this carcinoma and a search for genes with a stronger association than that already established for the HLA haplotype A2-BSin2.

Since this gene should occur in all nasopharyngeal carcinoma patients, Chinese and non-Chinese, the investigation of genetic markers in this tumour must be carried out in

parallel in high, intermediate and low risk areas, i.e., in south-east Asia, in North Africa and in Europe or the United States.

Using a panel of 195 sera, 58 nasopharyngeal carcinoma patients from Singapore have been typed as part of locus D studies, and the typing of 43 samples received from Hong Kong is in progress. Typing of cord-blood lymphocytes from 32 normal Cantonese, Hokkein and Teochew Chinese and from nine Malays has begun.

Typing for the immune-response-associated gene of the major histocompatibility complex is another step in the characterization of the HLA haplotype associated with nasopharyngeal carcinoma risk. In a first batch of 72 sera from pregnant women in Singapore, four of 21 unselected cord-blood samples were found to contain this gene; these four sera will be used later as typing reagents.

It has been shown that the Sin2 antigen associated with a high nasopharyngeal carcinoma risk in Chinese is absent in the Tunisian population [1], and no association with any known HLA antigen has yet been found. The aim of the 1976 programme is to study the HLA pattern of the general population, using, whenever possible, panels of Tunisian sera instead of those from European or American populations. Thus, sera of multiparous women are being collected in order to establish the HLA profile of the general population.

4.6 *Relationship of immune status to the development of nasopharyngeal carcinoma*

Queen Elizabeth Hospital, Hong Kong (Coordinator: Dr J. P. Lamelin, consultant to IARC)

Principal investigator: Dr J. H. C. Ho

Department of Biochemistry, University of Hong Kong, Hong Kong

Principal investigator: Dr M. H. Ng

WHO Immunology Research and Training Centre, Singapore

Principal investigator: Dr M. J. Simons

Department of Microbiology, University of Singapore, Singapore

Principal investigator: Dr S. H. Chan

Salah-Azaiz Institute, Tunis

Principal investigators: Dr N. Mourali, Dr M. Cammoun and Dr R. E. Ellouz

University of Lyon, Lyon, France

Principal investigator: Dr J. P. Revillard

Laboratory Section for Clinical Studies, National Cancer Institute, Bethesda, Md., USA

Principal investigator: Dr P. Levine

Immunological investigation within the nasopharyngeal carcinoma programme can serve to answer the following questions:

(i) Do nasopharyngeal carcinoma patients exhibit an Epstein–Barr virus-specific, cell-mediated immune response relevant to the development of this tumour?

(ii) Does the presence of a nasopharyngeal carcinoma influence the general level of cell-mediated immunity against non-Epstein–Barr virus-specific (i.e., environmental) 'antigens'?

---

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 74.

(iii) Are the genetic factors associated with nasopharyngeal carcinoma, which are possibly related to the major histocompatibility complex, mediated through immunopathological processes?

(*a*) In vivo *studies* (Dr J. H. C. Ho)

Using membrane extracts provided by Litton Bionetics, Kensington, Md., USA (under contract to the National Cancer Institute Viral Cancer Program), and prepared from the HKLY-28 cell line originating from a Hong Kong Chinese nasopharyngeal carcinoma biopsy [1], and using various 'environmental' antigens, the skin reactivity of nasopharyngeal carcinoma patients at different stages of the disease was determined before and at different intervals after radiotherapy. As seen in Table 12 the percentage of untreated patients with skin testing positive to HKLY-28 membrane extracts increased between stages 1 and 4 of the disease. After radiotherapy there was a progressive decrease with time in the percentage of positive skin reactions to HKLY-28 extract, although the level of reactivity to 'environmental antigens' remained stable. A lower skin reactivity to HKLY-28 after radiotherapy was associated with good clinical status of the patient between stages 1 and 5. A negative HKLY-28 skin test, before and after radiotherapy, was similarly associated with good clinical status in patients between stages 1 and 3. The findings of these cross-sectional and longitudinal studies are consistent with the hypothesis that skin reactivity to HKLY-28 cell extract may be related to tumour burden between stages 1 and 4, since at stage 5 fo tumour development cell-mediated immunity appears to be depressed.

Table 12. Skin reactivity to HKLY-28, *Tricophyton* and *Monilia* in nasopharyngeal carcinoma patients before and after radiotherapy

Stage	Test antigen	Before		2 months after		6 months after		12 months after	
I	HKLY-28	2/7	(28.6 %)	0/5	(0 %)	0/3	(0 %)	0/1	(0 %)
II	HKLY-28	9/16	(56.3 %)	2/9	(22.2 %)	1/9	(11.1 %)	2/8	(25.0 %)
III	HKLY-28	24/37	(64.9 %)	13/29	(44.8 %)	4/17	(23.5 %)	3/10	(35.0 %)
IV	HKLY-28	6/9	(66.7 %)	4/8	(50.0 %)	4/6	(66.7 %)	0/3	(0 %)
	HKLY-28	41/69	(87.5 %)	19/51	(37.3 %)	9/35	(25.7 %)	5/22	(22.7 %)
I-IV	*Trichophyton*	51/56	(91.1 %)	35/43	(81.4 %)	26/35	(74.3 %)	17/22	(77.3 %)
	Monilia	49/56	(87.5 %)	33/35	(94.3 %)	28/35	(80.0 %)	19/22	(86.4 %)

(*b*) In vitro *studies*

(i) *Hong Kong*

Dr Ng has developed an original technique for solubilizing membrane extracts from nasopharyngeal carcinoma biopsies and lymphoblastoid cell lines. Further to the pre-

[1] de-Thé, G., Ho, H. C., Kwan, H. C., Desgranges, C. & Favre, M. C. (1970) *Int. J. Cancer*, **6**, 189–206.

liminary results presented in the last Annual Report [1], these studies were extended to involve different *in vitro* cell-mediated immunity tests, such as macrophage migration inhibition assays (which are more sensitive than lymphocyte proliferation assays) and inter feron release tests; however, these failed to discriminate between control lymphocytes and those from nasopharyngeal carcinomas. Dr Ng has also characterized the cell extracts biochemically; he identified 22 polypeptides in a Raji cell antigenic preparation, and two of these, of membrane origin, were labelled with radioactive iodine. Better characterization and purification of the antigens used in such *in vitro* cell-mediated immunity assays is essential for further advances in this field; and the efforts being made in Agency laboratories with respect to Epstein–Barr virus nuclear antigen and early antigen are obviously of great importance in the coordination and standardization of *in vitro* cell-mediated immunity studies in collaborating laboratories.

(ii) *Singapore*

Dr Simons and Dr Chan have continued the use of counterimmunoelectrophoresis and radioelectrocomplexing tests for measuring antibody avidity. The interest of such studies rests on the hypothesis that antibody avidity might be controlled by the nasopharyngeal disease susceptibility gene.

Dr Chan, as an Agency Research Fellow, spent part of the review period in the laboratory of Dr P. Levine at the National Cancer Institute (USA). His studies there involved the evaluation and utilization of a new cell-mediated immunity assay, the lymphocyte adherence inhibition assay, on cancer patients. On his return to Singapore, he will continue his programme on cell-mediated immunity.

(iii) *Collaborating laboratories*

In collaboration with the WHO Immunology Unit in Geneva, Dr G. Gabbiani (Department of Pathology, University of Geneva) and Dr J. P. Revillard (Department of Immunology, Claude Bernard University, Lyon, France) have carried out studies on serum factors associated with cell-mediated immunity. The increased frequency of circulating immune complexes, cold lymphocytotoxins and smooth-muscle antibodies in experimental situations ascribable to acute viral infections or to tumour development have made their studies of special interest in nasopharyngeal carcinoma.

(iv) *Circulating immune complexes*

Studies have shown that nasopharyngeal carcinoma sera contain a high level of circulating immune complexes. However, due to growing concern regarding the specificity of such immune complexes in leading laboratories throughout the world, the WHO Immunology Unit has initiated a comparative study of the various techniques involved. The Agency has consequently halted their studies in that area but have provided the WHO with sera from nasopharyngeal carcinoma patients. As soon as the situation is clarified the Agency will continue its study of whether the sera from patients with nasopharyngeal carcinoma and Burkitt's lymphoma contain Epstein–Barr virus-specific immune complexes.

---

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 80.

(v) *Cold lymphocytotoxins* (Dr J. P. Revillard)

Results of these studies (Table 13) have shown that nasopharyngeal carcinoma patients have a higher frequency and a higher geometrical mean titre of cold lymphocytotoxic antibodies than controls in all geographical areas. Table 14 shows the relationship between cold lymphocytotoxin titre and that of antibodies against Epstein–Barr virus: there is some degree of correlation with antibodies against viral capsid antigens and against the Epstein–Barr virus-specific nuclear antigens but not with antibodies directed against early antigens.

Table 13. Percentage of sera with cold lymphocytotoxic activity and geometrical mean titre (GMT) of positive sera in nasopharyngeal carcinoma (NPC) and control groups from different geographical areas

	% of positive sera			GMT		
	NPC	Controls	P	NPC	Controls	P
Caucasians	62 (8/13) [a]	17 (10/60)	< 0.001	1.7	1.1	< 0.05
Tunisians	64 (27/42)	55 (17/31) [b] 29 (16/56) [c]	N.S. < 0.05	2.4	1.4 [d] 2.1 [d]	< 0.01 N.S.
Chinese	84 (36/43)	28 (8/29)	< 0.0005	3.2	1.2	< 0.01

[a] actual numbers in parentheses
[b] unrelated individuals
[c] family controls
[d] The GMTs of related and unrelated controls differ significantly ($P < 0.01$).
N.S. Not significant

Table 14. Relationship between cold lymphocytotoxin titres and anti-Epstein–Barr virus titres [anti-viral capsid antigen (VCA), anti-Epstein–Barr nuclear antigen (EBNA) and anti-early antigen (EA)]

	anti-VCA		anti-EBNA		anti-EA	
	r[a]	P	r	P	r	P
Chinese No. of NPC cases	0.31 43	< 0.05	Not done		Not done	
Tunisians No. of NPC cases	0.33 38	< 0.05	0.53 27	< 0.01	— 0.085 26	N.S.
Caucasians No. of NPC cases	0.50 13	N.S.	Not done		0.411 13	N.S.

[a] Correlation coefficient with cold lymphocytotoxin titre
N.S. Not significant

(vi) *Smooth-muscle antibodies* (Dr G. Gabbiani)

Sera from 39 Tunisian nasopharyngeal carcinoma patients, 72 relatives of such patients and 17 healthy volunteers from Tunisia were examined for smooth-muscle antibodies. These were found more often in the nasopharyngeal carcinoma group (seven out of 29) than in the familial and unrelated control groups; this difference was statistically significant.

Similar antibodies were described in patients with active hepatitis, with leukaemia and with lymphomas. No correlation was found in the nasopharyngeal carcinoma patients between smooth-muscle antibody and Epstein–Barr virus antibody reactivities, nor with the titre of cold lymphocytotoxin, when evaluated in the same sera.

5. INTERNATIONAL REFERENCE PROGRAMME FOR ONCOGENIC HERPES VIRUSES

Professor R. Sohier, Honorary Professor at the University of Lyon, presently at the University of Tunis, and Dr G. Pardoe, University of Birmingham, UK, served as part-time consultants. The programme comprises two areas of activity.

The first involves the collaborating laboratories coordinated by the eight Herpes Advisory Teams. Some (those investigating simian viruses) have been very active in determining criteria for reference material in the oncogenic herpesvirus field and, whenever possible, in preparing antisera, reference viruses and cell lines.

The second area of activity is the preparation and distribution of two newsletters by the Agency. These newsletters include technical sheets and function as an information exchange service. Furthermore, whenever possible, biological specimens obtained from field studies have been sent to laboratories requesting them. Between July 1975 and June 1976, specimens (blood, sera, biopsies) were received from Uganda (52), Tunis (15), Hong Kong (26), Singapore (2) and the Gustave Roussy Institute, Paris (15). A total of 58 biological specimens were sent to collaborating laboratories and requesting institutes in Australia, Argentina, Cameroon, Canada, the Federal Republic of Germany, France, Greece, India, Israel, Japan, Singapore, Sweden, Switzerland, Czechoslovakia, the UK, the USA and other countries.

The activities of this programme will be reviewed during the third International Symposium on Oncogenesis and Herpesviruses to be held in July 1977 at the Science Center, Harvard University, Boston, Mass., USA, organized jointly by the National Cancer Institute Virus Cancer Program (USA) and the Agency.

The Unit participated actively in the course on immunovirology of cancer organized by the Agency this year (see p. 109).

Preparations are well ahead for the symposium on Etiology and Control of Nasopharyngeal Carcinoma to be held in Kyoto in April 1977, under the joint auspices of the National Cancer Institute Virus Cancer Program (USA), the Japan Society for Advance of Science and the Agency.

6. RELATIONSHIP BETWEEN INFECTIOUS MONONUCLEOSIS AND HODGKIN'S DISEASE (Dr N. Muñoz)

Difficulties in obtaining information from Sweden have left this project at the same level as reported last year [1]. It is hoped rapidly to complete the study.

---

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, pp. 85–86.

7. SEARCH FOR VIRAL MARKERS IN CERVICAL CARCINOMA (Dr N. Muñoz)

Uganda Cancer Institute, Kampala
Principal investigator: Dr C. Olweny

Tadj Pahlavi Cancer Institute, Teheran
Principal investigator: Dr A. Mojtabai

University of Pernambuco, Recife, Brazil
Principal investigators: Dr M. Lôbo Jardim and Dr A. R. L. de Carvalho

The collection of tissue specimens from cervical carcinomas and non-tumoral cervices has continued in Kampala (a high incidence area for cervical cancer) and Teheran (a low incidence area) and has been extended to Recife, Brazil (another high incidence area). These specimens are being examined for genome fragments of the three viruses which are known to infect the uterine cervix: herpes simplex virus type 2, cytomegalovirus and papillomavirus. Serum samples have also been collected from the same patients and from controls, and these are to be examined for specific antibodies to the same viruses. Our experience during this period has shown that it is extremely difficult to collect the large specimens (minimum weight, 10 g) needed for the hybridization experiments, since nearly all large tumours are treated by radiotherapy in most hospitals throughout the world.

Details of material received so far from the three areas are summarized in Table 15. Five cervical cancer specimens and five controls from Uganda and one cervical cancer specimen from Teheran have been examined for the presence of cytomegalovirus DNA by Dr E. Huang (University of North Carolina, Chapel Hill, N.C., USA) using DNA-DNA reassociation kinetics. Genome fragments were found in similar amounts in three cervical cancers and in three controls. Dr H. zur Hausen (Institute for Clinical Virology, Erlangen, Federal Republic of Germany) is examining samples of the collected specimens for papilloma DNA.

Table 15. Tissue specimens and sera received at the Agency from patients with cervical cancer and control patients

Area	Tissue specimens		Serum samples	
	Cases	Controls	Cases	Controls
Kampala	11	6	12	5
Teheran	5	4	4	5
Recife	10[a]	1	10[a]	1
Total	26	11	26	11

[a] 6 cervical cancers, 3 cancers of the penis and 1 cancer of the vulva

4. UNIT OF CHEMICAL CARCINOGENESIS

Dr L. TOMATIS (Chief)

1. INTRODUCTION

The Unit of Chemical Carcinogenesis has continued the identification of environmental carcinogenic chemicals and the evaluation of their carcinogenic risk to man. Up to June 1976, all available epidemiological, experimental, production and occurrence data had been screened for a total of 272 chemicals. This survey resulted in the preparation of further monographs in the series *Evaluation of Carcinogenic Risk of Chemicals to Man*, eleven volumes of which have so far been published. Unequivocal or strong circumstantial evidence of carcinogenicity to man was found for 20 of the 272 chemicals considered; most of these chemicals were of industrial origin, and, while the general population is or could be exposed to some of them, exposure to all of them has been highest during their manufacture and/or use in a particular occupation. Five of the 20 chemicals carcinogenic to man were medical drugs.

The Unit has also made a survey of the facilities which exist throughout the world for carrying out long-term testing of carcinogens and of those chemicals currently being tested for carcinogenicity. In 1975, 89 institutes in 19 countries were involved in the long-term carcinogenicity testing of a total of 828 chemicals, of which 341 had not been tested before.

The Unit has concentrated a large part of its activity on the development and improvement of rapid screening tests for preselecting environmental chemicals for long-term testing and on the development of criteria to allow a better assessment of the significance to man of results obtained in experimental carcinogenesis. These two aspects are closely linked, since the emphasis which is at present laid on much-needed short-term tests cannot be justified until the validity of the results obtained in long-term bioassays have been solidly established. In fact, the efficiency of short-term tests is often assessed by whether or not the results can be matched to those obtained in long-term bioassays. Particular emphasis is being given to developing a system to reduce and finally eliminate false negative results.

In all of these programmes, collaboration with national programmes has been expanded. In June 1975, a workshop on rapid screening tests was held in Brussels; this was organized jointly by the Agency and the Commission of the European Communities and was partly supported by the Belgian Government. The proceedings of this workshop have now been published [1]. In October 1975, the Agency, in conjunction with the German Cancer Research

[1] Montesano, R., Bartsch, H. & Tomatis, L., eds (1976) *Screening Tests in Chemical Carcinogenesis*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* No. 12).

Centre, Heidelberg, Federal Republic of Germany, and the Ministry of the Interior of the Federal Republic of Germany, convened a working group on air pollution and cancer at the School of Medicine, Hanover, Federal Republic of Germany. The proceedings of this meeting are in press [1].

The second volume of the *Manual on the Pathology of Tumours in Laboratory Animals* has been published [2]; volumes covering mice and hamsters will appear soon. The Unit is also involved in the preparation of a booklet, *The Safety of Handling Carcinogens in the Laboratory.*

In collaboration with the Unit of Biological Carcinogenesis and the Unit of Epidemiology and Biostatistics, the Unit is participating in an investigation on the possible role of prenatal events in the occurrence of cancer in children. The Unit is also contributing to the implementation of a cancer registry in the Lombardy region of Italy, with particular emphasis on occupational risks.

2. COORDINATION OF CARCINOGENICITY DATA

2.1 *Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*

(Dr C. Agthe and Mr J. D. Wilbourn)

The last Annual Report [3] listed the substances which were included in Volumes 8 and 9 of these monographs. Since then, three further working groups have met. Volume 10, published in May 1976, is the result of a meeting on some naturally occurring substances (Table 16). A questionnaire was distributed with Volume 10 to all recipients of the monographs, of which there are approximately 800. These were sent by surface rather than air mail; thus, up to June 1976 only 182 replies had been received, almost exclusively from European countries. Of these, 122 indicated that they have consulted the monographs frequently and 35 that they do so occasionally; none reported that they have never consulted them. Recipients in the following countries indicated that, to their knowledge, regulatory measures had been taken on the basis of the evaluations contained in the monographs: Austria, Belgium, Canada, Czechoslovakia, Denmark, Egypt, Federal Republic of Germany, France, German Democratic Republic, Hungary, Iran, Italy, Luxembourg, The Netherlands, Norway, Poland, Portugal, Romania, Spain, Sweden, Switzerland, UK, USA and USSR. Analysis of the complete data from these questionnaires will be carried out at a later date.

In December 1975, a working group met to re-evaluate asbestos, cadmium and nickel; in February 1976, a further working group evaluated some epoxides, miscellaneous compounds of industrial importance and some volatile anaesthetics. Volume 11, which was published in July 1976, covers the substances considered by both of these working groups, with the exception of asbestos (see Table 16). Publication of the monograph on asbestos has been postponed, and a further meeting has been convened for December 1976.

---

[1] Mohr, U., Schmähl, D. & Tomatis, L., eds (1976) *Air Pollution and Cancer in Man*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 16**) (in press).

[3] Turusov, V., ed. (1976) *Pathology of Tumours in Laboratory Animals*, Vol. I, *Tumours of the Rat*. Part 2, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 6**).

[2] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 90.

Two working groups will convene in June and October 1976 to consider some carbamates and thiocarbamates and some miscellaneous drugs, respectively. It would be impossible to deal with all drugs for which evidence of carcinogenicity exists in experimental animals and/or man, and therefore no antineoplastic drugs or sulphonamides will be considered; these will be evaluated at a future meeting.

A total of 272 compounds have been considered in Volumes 1–11. In addition to the 19 chemicals reported in the last Annual Report, one further compound, chloramphenicol, has been associated with cancer induction in man. In experimental animals, 137 chemicals have been found definitely to be carcinogenic. For 47 compounds the data were considered inadequate to make an evaluation, and for 22 the available data did not reveal a carcinogenic effect (Table 17).

Table 16. Substances included in *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Volumes 10 and 11

	Volume		Volume
Actinomycins	10	Glycidaldehyde	11
Adriamycin	10	Glycidyl oleate	11
Aflatoxins	10	Glycidyl stearate	11
Azaserine	10	Griseofulvin	10
Benzyl chloride	11	Hydroxysenkirkine	10
β-Butyrolactone	11	Isatidine	10
γ-Butyrolactone	11	Jacobine	10
Cadmium and cadmium compounds	11	Lasiocarpine	10
Cantharidin	10	Luteoskyrin	10
Chloramphenicol	10	Mitomycin C	10
Cholesterol	10	Monocrotaline	10
Coumarin	10	Native carrageenans	10
Cycasin	10	Nickel and nickel compounds	11
Cyclochlorotine	10	Ochratoxin A	10
Daunomycin	10	Parasorbic acid	10
Diepoxybutane	11	Patulin	10
Diglycidyl resorcinol ether	11	Penicillic acid	10
Dinitrosopentamethylenetetramine	11	Propylene oxide	11
1,4-Dioxane	11	Reserpine	10
Epichlorohydrin	11	Retrorsine	10
1-Epoxyethyl-3,4-epoxycyclohexane	11	Riddelliine	10
3,4-Epoxy-6-methylcyclohexylmethyl-3,4-epoxy-6-methylcyclohexane carboxylate	11	Safrole, isosafrole and dihydrosafrole	10
		Seneciphylline	10
		Senkirkine	10
cis-9,10-Epoxystearic acid	11	Sterigmatocystin	10
Ethylene oxide	11	Styrene oxide	11
Ethylene sulphide	11	Tannic acid and tannins	10
Fusarenon X	11	Trichloroethylene	11
General considerations on volatile anaesthetics	11	Triethylene glycol diglycidyl ether	11
		4-Vinylcyclohexene	11

Table 17. Analysis of the evaluations made by working groups on substances included in the *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Volumes 1–11

Number of chemicals evaluated	272
Number of chemicals carcinogenic to man	20
Number of chemicals definitely carcinogenic in experimental animals only	137
Number of chemicals for which data were inadequate for evaluation or indicated a possible carcinogenic effect	93
Number of chemicals for which the available data did not reveal a carcinogenic effect	22

The following is a list of experts who contributed to the preparation of monographs published in Volumes 8–11 (those who were involved in monographs comprising Volumes 1–7 are listed in the 1974 Annual Report [1]):

Dr A. Annoni, International Labour Office, Geneva, Switzerland
Dr B. K. Armstrong, University Department of Medicine, Perth Medical Centre, Shenton Park, Western Australia
Dr E. Arrhenius, Department of Environmental Toxicology, University of Stockholm, Wallenberg Laboratory, Stockholm
Dr E. A. Bababunmi, University of Ibadan, Ibadan, Nigeria
Mr H. Baxter, Laboratory of the Government Chemist, London
Dr J. Bignon, Pneumo-Phtisiology Clinic, Laennec Hospital, Paris
Dr R. K. Boutwell, McArdle Laboratory for Cancer Research, Madison, Wis., USA
Professor E. Boyland, London School of Hygiene and Tropical Medicine, London
Dr I. Chernozemsky, National Center of Oncology, Medical Academy, Sofia
Professor A. M. Clark, The Flinders University of South Australia, Bedford Park, South Australia
Dr J. J. Clary, E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del., USA
Dr T. H. Corbett, US Veterans Administration Hospital, Ann Arbor, Mich., USA
Dr C. C. J. Culvenor, Commonwealth Scientific and Industrial Research Organization, Parkville, Victoria, Australia
Professor F. Dickens, Findon Valley, Worthing, Sussex, UK
Dr D. B. Douglas, Department of Employment, Employment Medical Advisory Service, London
Dr U. H. Ehling, Society for Radiation and Environmental Research Ltd., Munich, Institute of Biology, Neuherberg, Federal Republic of Germany
Dr G. Eisenbrand, German Cancer Research Centre, Heidelberg, Federal Republic of Germany
Dr P. S. Elias, Department of Health and Social Security, London
Dr H. L. Falk, National Institute of Environmental Health Sciences, Research Triangle Park, N.C., USA
Dr L. Fishbein, National Center for Toxicological Research, Jefferson, Ark., USA
Dr R. C. Garner, Cancer Research Unit, University of York, Heslington, Yorks, UK
Dr D. E. Hathway, Imperial Chemical Industries Ltd, Alderley Park, Cheshire, UK
Dr L. Kinlen, Department of the Regius Professor of Medicine, University of Oxford, Radcliffe Infirmary, Oxford, UK
Dr P. Kleihues, Max-Planck Institute for Brain Research, Cologne, Federal Republic of Germany
Dr E. G. Knox, Health Services Research Centre, The Medical School, Edgbaston, Birmingham, UK

[1] International Agency for Research on Cancer (1974) *Annual Report, 1974*, Lyon, pp. 70–73.

Dr R. Kroes, National Institute of Public Health, Bilthoven, The Netherlands
Dr P. Krogh, National Food Institute, Division of Pesticides and Contaminants, Søborg, Denmark
Dr R. A. Lemen, National Institute of Occupational Safety and Health, Cincinnati, Ohio, USA
Professor N. Loprieno, CNR Laboratory of Mutagenesis and Differentiation, Pisa, Italy
Dr H. V. Malling, National Institute of Environmental Health Sciences, Research Triangle Park, N.C., USA
Dr G. Mohn, Central Laboratory for Mutagenicity Testing of the German Research Association, Freiburg, Federal Republic of Germany
Dr P. J. O'Connor, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, UK
Dr S. Odashima, Department of Chemical Pathology, National Institute of Hygienic Sciences, Tokyo
Professor F. Oesch, Johannes Gutenberg-University of Mainz, Pharmacology Institute, Mainz, Federal Republic of Germany
Dr E. Pedersen, The Cancer Registry of Norway, Oslo
Dr F. Peers, Tropical Products Institute, London
Dr M. Piscator, The Karolinska Institute, Department of Environmental Hygiene, Stockholm
Professor R. Preussmann, German Cancer Research Centre, Heidelberg, Federal Republic of Germany
Dr L. Rinzema, Dow Chemical Europe S.A., Horgen, Switzerland
Dr G. Rudali, Curie Foundation—Radium Institute, Paris
Dr U. Saffiotti, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md., USA
Dr M. Saito, The Institute of Medical Science, University of Tokyo, Tokyo
Dr R. Saracci, CNR Laboratory for Clinical Physiology, University of Pisa, Pisa, Italy [1]
Professor H.-W. Schlipköter, Medical Institute for Atmospheric Hygiene and Silicosis Research, Düsseldorf, Federal Republic of Germany
Professor D. Schmähl, German Cancer Research Centre, Heidelberg, Federal Republic of Germany
Professor T. Schramm, Central Institute for Cancer Research, Academy of Sciences, Berlin, German Democratic Republic
Professor I. J. Selikoff, Mount Sinai School of Medicine, New York, N.Y., USA
Dr R. J. Shamberger, The Clinic Center, Cleveland Clinic, Cleveland, Ohio, USA
Dr P. Shubik, The Eppley Institute for Research in Cancer, Omaha, Nebr., USA
Dr F. W. Sunderman, Jr, Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington, Conn., USA
Dr B. Teichmann, Central Institute for Cancer Research, Academy of Sciences, Berlin, Democratic Republic of Germany
Dr L. Teppo, Finnish Cancer Registry, Helsinki
Dr B. Terracini, Institute of Anatomy and Pathological Histology, University of Turin, Turin, Italy
Professor R. Truhaut, Toxicological Research Centre, Faculty of Pharmaceutical and Biological Sciences of René Descartes University, Paris
Dr V. S. Turusov, Cancer Research Center, USSR Academy of Medical Sciences, Moscow
Dr B. L. Van Duuren, New York University Medical Center, Institute of Environmental Medicine, New York, N.Y., USA
Dr J. C. Wagner, Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, Glamorgan, UK

[1] Present address: International Agency for Research on Cancer, Lyon, France.

Dr J. K. Wagoner, National Institute of Occupational Safety and Health, Cincinnati, Ohio, USA
Dr J. S. Wassom, Environmental Mutagen Information Center, Oak Ridge National Laboratory, Oak Ridge, Tenn., USA
Dr E. Weisburger, Carcinogen Metabolism Toxicology Branch, National Cancer Institute, Bethesda, Md., USA
Dr P. Westerholm, National Board of Occupational Safety and Health, Medical Department, Stockholm
Dr G. N. Wogan, Massachusetts Institute of Technology, Department of Nutrition and Food Science, Cambridge, Mass., USA
Professor F. Zajdela, Radium Institute, Faculty of Sciences, Orsay, France
Dr F. K. Zimmermann, Darmstadt Technical University, Biology Department, Darmstadt, Federal Republic of Germany

Representatives from the National Cancer Institute, Bethesda, Md., USA

Dr J. A. Cooper, Deputy Associate Director, Carcinogenesis Program, Division of Cancer Cause and Treatment
Dr M. Litwack, Division of Cancer Cause and Prevention
Dr S. Siegel, Carcinogen Bioassay and Program Resources Branch

Representatives from the Stanford Research Institute, Menlo Park, Calif., USA

Dr O. H. Johnson, Chemical Environmental Program
Dr K. E. McCaleb, Chemical Environmental Program
Dr R. H. Reinfried, Stanford Research Institute, Zurich, Switzerland
Mr D. E. Schendel, Chemical Information Services

2.2 *Survey of chemicals being tested for carcinogenicity*

(Dr C. Agthe, Dr H. Bartsch and Mrs M. J. Ghess)

Two surveys were undertaken in 1973 and 1974, and the results were made available to collaborating laboratories and to any other interested parties in the form of Information Bulletins 1–3 and 4–5, respectively. Reporting of compounds being tested for carcinogenicity in these two surveys was not complete since a considerable proportion of the testing performed in the USA was of a confidential nature. Since implementation of the Freedom of Information Act, however, the Agency has been able to obtain this information; thus, the survey initiated in 1975 has resulted in nearly complete reporting of substances under test in non-commercial laboratories or under a contract with the National Cancer Institute of the USA.

The data collected from the autumn 1975 survey were summarized in Bulletin 6, which was issued in March 1976. This bulletin gives information received from 89 institutes in 19 countries and includes a total of 828 chemicals. For the first time, this bulletin also includes an appendix giving information on chemicals for which epidemiological investigations are in progress. This information was made available by the Clearing House for On-going Research in Cancer Epidemiology, organized jointly by the Unit of Epidemiology and Biostatistics and the German Cancer Research Centre, Heidelberg, Federal Republic of Germany.

Of the 828 compounds reported in Bulletin 6 as being under test approximately 75% were known to be manufactured and used or to occur naturally. Therefore, about 25%

of the tests must be regarded as being of no practical value at present. Use and occurrence of substances under test were established by reference to the *Merck Index* [1] and to the *Directory of Western European Chemical Producers* [2] (Table 18).

Table 18. Uses of chemicals under test

Use of chemicals	No. of chemicals
Dyes	20
Flavour and fragrance chemicals	28
Pesticides	63
Pharmaceutical preparations and veterinary drugs	92
Plastics	8
Rubber processing chemicals	17
Solvents	17
Naturally occurring chemicals not in any other class of use	40
Chemicals produced for purposes other than those mentioned above	194

Of the 828 chemicals, 317 had already been tested for carcinogenicity [3]; thus, 511 compounds were being tested for the first time.

Of the compounds under test 73 have been evaluated by Agency working groups. Of these, 57 were evaluated as being carcinogenic in experimental animals and/or man and did thus not necessarily need further testing; however, for six substances the evaluation indicated only some carcinogenic effect, and for six no evaluation could be made, suggesting that further testing was needed.

There are, however, 47 further substances for which Agency working groups have been unable to make evaluations. This indicates that more use could be made of the monographs in determining priorities for testing and demonstrates the close relationship between the two programmes. Since information gathered from these surveys may become valuable in selecting chemicals for which monographs should be prepared, it is hoped that investigators collaborating in this programme will publish the results of their studies rapidly after completion.

2.3 *Carcinogenicity testing*

(*a*) *DDT* [4]

Results of metabolic and mutagenicity studies with various DDT metabolites are given in section 3.3. Concurrent investigations on the carcinogenicity of some of these metabolites in rodents are under way.

---

[1] Stecher, P. G., ed (1968) *The Merck Index*, 8th ed., Rahway, NJ, Merck & Co.

[2] Chemical Information Services, Ltd (1975) *Directory of Western European Chemical Producers, 1975/76*, Oceanside, NY.

[3] Carcinogenesis Program National Cancer Institute (1974) *Survey of Compounds which have been Tested for Carcinogenic Activity*, Washington DC, US Government Printing Office (*Public Health Service Publication* No. 149).

[4] 1, 1, 1-Trichloro-2, 2-di(4-chlorophenyl)ethane.

(i) *Department of Experimental Oncology, University of Genoa, Italy*
Principal investigators: Professor L. Santi and Dr L. Rossi

Long-term testing of DDT by administration to rats at a level of 500 mg/kg of diet for lifespan has been terminated. The results indicate that exposure to DDT results in an increased incidence of liver tumours late in life.

(ii) *Department of Occupational Health, Hadassah Medical School, Jerusalem* (RA/69/005)
Principal investigator: Professor M. Wassermann

The evaluation of DDT and polychlorinated biphenyl levels in human normal and tumoral tissues is continuing. Results of the first investigation show that there is an increased concentration of organochlorine compounds in tumoral tissue of the mammary gland as compared to adjacent normal tissue. These analyses of the levels present in extractable lipids also showed different levels of various metabolites of DDT and of the chlorinated biphenyl compounds [1]. An evaluation of levels of organochlorine insecticides and polychlorinated biphenyls was also carried out on maternal adipose tissue, maternal blood, foetal blood, maternal uterine muscle, placenta and amniotic fluid.

(*b*) *Maleic hydrazide* (Dr V. Ponomarkov)

Long-term testing of maleic hydrazide by subcutaneous or oral administration has been initiated in C57 black mice.

National Institute of Public Health, Utrecht, The Netherlands
Principal investigators: Dr G. van Esch and Dr R. Kroes

Long-term testing of maleic hydrazide in rats is in progress. Preliminary results indicate a slightly decreased kidney function in treated animals.

(*c*) *Styrene* (Dr V. Ponomarkov)

The first study, in which O_{20} mice received weekly oral doses of 1350 mg/kg styrene, has been terminated. The high dose administered considerably shortened the lifespan of the treated animals; most of the untreated control animals are still alive so a final evaluation of the data has been postponed. In two further studies, BD IV rats and C57 black mice are being treated orally with weekly doses of 500 and 300 mg/kg, respectively.

(*d*) *Styrene oxide* (Dr V. Ponomarkov)

Styrene oxide is being given orally to O_{20} mice in weekly doses of 600 mg/kg.

(*e*) *Vinylidene chloride* (Dr V. Ponomarkov)

Vinylidene chloride is being given to BD IV rats and to C57 mice orally in weekly doses of 150 and 70 mg/kg, respectively.

(*f*) *2-Chlorobutadiene* (Dr V. Ponomarkov)

2-Chlorobutadiene is being administered to BD IV rats weekly in doses of 50 mg/kg. A preliminary survey in animals which died within the first month after the beginning of

---

[1] Wassermann, M., Nogueira, D. P., Tomatis, L., Mirra, A. P., Shibata, H., Arie, G., Cucos, S. & Wassermann, D. (1976) *Bull. Environm. Contam.*, **15**, 478–484.

the experiment indicates a relatively high frequency of liver damage and, in particular, of multiple focal necrosis and haemorrhage.

(*g*) *Phenobarbital* (Dr V. Ponomarkov)

Lifetime exposure of CF1 mice to phenobarbital resulted in a high incidence of liver-cell tumours in both male and female animals. The final results of this study have been published [1].

Department of Experimental Oncology, University of Genoa, Italy
Principal investigators: Professor L. Santi and Dr L. Rossi

The final results of studies involving the administration to rats of phenobarbital continuously for lifespan show that the incidence of liver tumours in animals dying at a late age was increased.

(*h*) *Combined exposure to multiple carcinogens*

(i) *Research Institute of Oncology, Leningrad, USSR*
Principal investigator: Dr N. P. Napalkov

The combined effect of prenatal exposure to nitrosoethylurea and postnatal X-irradiation or treatment with the same carcinogen was studied in F_1 and F_2 BALB/c mice. It was found that transplacental pretreatment with 20 mg/kg nitrosoethylurea enhanced the carcinogenic effect of postnatal X-irradiation, resulting chiefly in a higher incidence of lung adenocarcinomas. This effect was particularly strong in F_1 females and was still evident in experiments in which the prenatal dose of nitrosoethylurea was only 2 mg/kg. The carcinogenic action of nitrosoethylurea could be seen in F_2 progeny of animals treated during pregnancy, and this effect is considered to be a result of exposure to the carcinogen before conception. Pretreatment with nitrosoethylurea before conception did not alter the postnatal sensitivity of F_2 mice to the same carcinogen (in comparison with the higher response of F_1 mice), and tumour response of the ovaries in F_2 females subjected to postnatal X-irradiation was reduced.

(ii) *School of Medicine, Hanover, Federal Republic of Germany*
Principal investigator: Professor U. Mohr

Courtauld Institute of Biochemistry, London
Principal investigator: Professor P. N. Magee [2]

Treatment with actinomycin D did not significantly modify the incidence of kidney tumours induced by a single dose of nitrosodimethylamine, but a shorter latent period was observed [3].

(*i*) *Magenta*

School of Medicine, Hanover, Federal Republic of Germany
Principal investigator: Professor U. Mohr

Long-term carcinogenicity tests on the effects of commercial magenta and pararosaniline have been initiated in two animal species. Syrian golden hamsters and Sprague-Dawley

---

[1] Ponomarkov, V., Tomatis, L. & Turusov, V. (1976) *Cancer Lett.*, **1**, 165–172.
[2] Present address: Temple University School of Medicine, Philadelphia, Pa., USA.
[3] Hilfrich, L., Haas, H., Montesano, R., Mohr, U. & Magee, P. N. (1975) *Brit. J. Cancer*, **32**, 578–587.

rats are treated twice weekly with oral doses of the test compounds. Treatment will continue for lifespan.

(*j*) N-*Phenyl-2-naphthylamine*

School of Medicine, Hanover, Federal Republic of Germany
Principal investigator: Professor U. Mohr

Long-term carcinogenicity tests have been initiated in Syrian golden hamsters and in Sprague-Dawley rats. The test compound is administered by gavage twice weekly, and treatment will continue for lifespan.

(*k*) *2-Chloroethylene oxide and 2-chloroacetaldehyde*

National Institute of Health and Medical Research, Orsay, France
Principal investigator: Dr F. Zajdela

Preliminary results of studies involving repeated subcutaneous administration of 2-chloroethylene oxide indicate that this results in the occurrence of local tumours. A final evaluation of the data cannot, however, be made until the end of the experiment. Repeated subcutaneous administration of 2-chloroacetaldehyde proved to be impracticable, due to the strong necrotic activity of this compound. A two-stage experiment has been initiated in which the treatment is limited to skin painting.

(*l*) *Methyl-2-benzimidazole-carbamate (carbendazim) and butylcarbamoylmethyl-2-benzimidazole carbamate (benlate)*

National Institute of Public Health, Budapest
Principal investigator: Dr M. Börzsönyi

The carcinogenic effect of carbendazim and benlate, given in conjunction with sodium nitrite, was investigated by administration to pregnant Swiss mice. Carbendazim and sodium nitrite produced tumours, particularly lymphomas, in the progeny[1]. Benlate, administered with sodium nitrite, was also carcinogenic. Further studies on other carbamates are in progress.

2.4 Manual on the Pathology of Tumours in Laboratory Animals

Tumours of the Rat, Volume I, Part 2, is now in press, and volumes on *Tumours of the Mouse* and *Hamster* will be published in 1977.

3. EVALUATION OF THE SIGNIFICANCE FOR MAN OF DATA ON EXPERIMENTAL CHEMICAL CARCINOGENESIS AND OF THE DEVELOPMENT OF RAPID SCREENING TESTS

3.1 *Metabolism of carcinogens and DNA repair studies* (Dr R. Montesano, Dr H. Bartsch, Dr G. Margison, Dr L. Zardi and Miss H. Brésil)

Studies in this programme concern the metabolism of nitrosamines and the persistence of alkylated purine bases in DNA induced by these carcinogens, the metabolism of vinyl chloride and DDT and the effect of multiple doses of carcinogens on DNA repair processes.

---

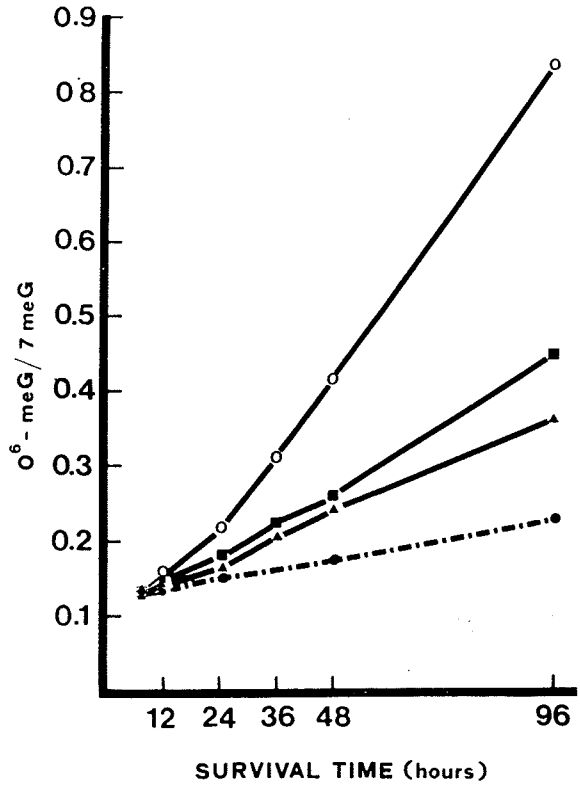
[1] Börzsönyi, M., Pintér, A., Surján, A. & Farkas, I. (1976) *Int. J. Cancer*, **17**, 742–747.

(*a*) *The role of DNA repair processes in the carcinogenicity of nitrosamines*

Recent studies in rats [1] have shown that the persistence of certain alkylated bases in DNA, namely O^6-alkylguanine, may be a determining factor in the organ-specific carcinogenicity of *N*-nitroso compounds.

Using a single dose of nitrosodimethylamine (NDMA), which produces a 30% incidence of liver tumours in hamsters [2], the relative stabilities of the alkylated purines were investigated in various organs of this species [3]. Liver DNA was alkylated to the greatest extent; lung and kidney DNA were alkylated to 8% and 3% of the liver value, respectively. The O^6-methylguanine:7-methylguanine ratios were initially the same in all three organs and in the liver DNA of rats given similar doses; O^6-methylguanine was found to be the most persistent alkylated purine in all three hamster tissues (Fig. 16). There was also evidence of excision of 7-methylguanine, the highest activity being present in the liver. The minor products, 3-methyladenine, 1-methyladenine, 3-methylguanine and 7-methyladenine, were detected in most hamster tissues, and their individual rates of loss from liver DNA were determined. Ring labelling of the normal purines in DNA was highest in the liver, and 80% of that value was seen in lung tissue; on the other hand, incorporation in the kidney was only 3% of the liver value.

Fig. 16 Relative amounts of 0^6 and 7-methylguanine (0^6-meG: 7-meG) in hamster tissue DNA at various times after intraperitoneal injection of ^{14}C-nitrosodimethylamine (25 mg/kg, 3.166 mCi/mmol) in liver (o), lung (■) and kidney (▲). The broken line represents the increase in the 0^6-meG: 7-meG ratio during incubation *in vitro* of DNA isolated from liver 7 h after injection of ^{14}C-nitrosodimethylamine.



[1] Goth, R. & Rajewsky, M. F. (1974) *Z. Krebsforsch.*, **82**, 37–64.
[2] Tomatis, L. & Cefis, F. (1967) *Tumori*, **53**, 447–451.
[3] Margison, G., Margison, J. & Montesano, R. (1976) *Biochem. J.*, **157**, 627–634.

These results are compatible with the hypothesis that the presence of O^6-alkylguanine in DNA is an important factor in the induction of tumours by alkylating agents: the DNA in hamster liver, the target organ, maintained the highest content of this product throughout the experimental period. In rats, however, single doses of NDMA did not induce liver tumours; this was attributed in part to the capacity of rat liver to excise O^6-methylguanine from its DNA enzymatically.

In another series of experiments [1] the effect of continuous treatment with alkylating agents on the activity of DNA 'excision' repair enzymes in rat liver was examined. In rats, the principal target organ is determined mainly by the dose schedule: a single exposure to a very large dose, or a limited number of exposures to fairly large doses, produce kidney tumours in the survivors; more prolonged exposure to low individual doses but to similar total doses results in a high incidence of liver tumours.

In attempts to explain the organ specificity of NDMA given in different dose schedules, the possibility that chronic administration of NDMA might produce liver tumours by inhibiting the O^6-methylguanine excision system was explored. Such administration would allow O^6-methylguanine to be present in DNA for a more extensive period and would thus increase the chances that a miscoding event would take place during DNA replication.

Rats were exposed chronically to unlabelled NDMA (25 ppm in the drinking-water) and were then given a single dose of *N*-nitroso-*N*-(^{3}H)methylurea (10 mg/kg). This compound, and not NDMA, was chosen, since it does not require metabolism for its reaction with nucleic acids; thus, excision repair capacity could be determined in control and NDMA-pretreated rats under conditions in which the initial extent and time of maximum DNA alkylation were identical.

Levels of 7- and O^6-methylguanine were determined in DNA from the livers of normal and NDMA-treated animals at various times after administration of *N*-nitroso-*N*-(^{3}H)-methylurea. Animals treated with NDMA for 8 ½ weeks had a slightly higher level of alkylation than did control rats, but the rates of loss of the various alkylation products were the same in control and in NDMA-pretreated animals (Fig. 17). The O^6-methylguanine: 7-methylguanine and 3-methyladenine:7-methylguanine ratios are better indicators of excision activity since they compensate for any differences between groups of animals in the absolute amounts of alkylation products; these were almost identical in control and NDMA-pretreated animals.

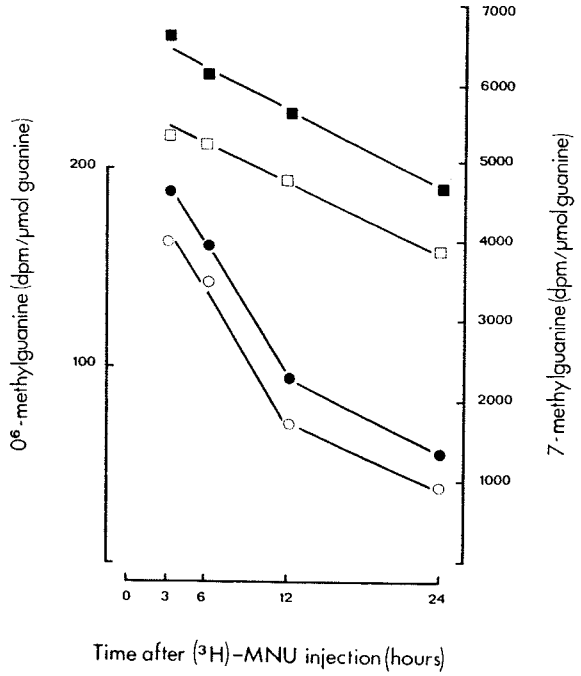
These results indicate that the high incidence of liver tumours during chronic administration of NDMA cannot be explained by a lower rate of excision of O^6-methylguanine (or 3-methyladenine) from the liver DNA of NDMA-pretreated animals.

(*b*) *Effects of split doses of nitrosomethylurea on unscheduled DNA repair synthesis in cultured mammalian cells*

In these experiments [2] the effect of either single or split doses of nitrosomethylurea on DNA 'excision' repair enzymes was examined in three cultured cell lines. These experiments were designed partly to examine whether splitting of doses of a carcinogen could decrease the capacity for excision repair of DNA damage induced by the carcinogen, as compared to the capacity after a single exposure to the carcinogen. The extent of excision repair was determined by unscheduled DNA-synthesis, as measured by ^{3}H-thymidine in

[1] Margison, G. P., Brésil, H., Margison, J. M. & Montesano, R. (1976) *Cancer Lett.* (in press).
[2] Zardi, L., Barbin, A., Saint-Vincent, L., Montesano, R. & Margison, G., submitted for publication.

Fig. 17 Levels of methylated purines in liver DNA at various times after administration of ^{3}H-nitrosomethylurea (MNU) (10 mg/kg). Open symbols, control rats; closed symbols, rats pretreated with nitrosodimethylamine; square symbols, 7-methylguanine (7-meG); round symbols, 0^6-methylguanine (0^6-meG)



the presence of hydroxyurea, which inhibits semi-conservative DNA synthesis by more than 95%. Rat liver epithelial cells (IAR-20), human liver mesenchymal cells (IAR-28) and mouse C3H 10T½ cells were used. The two former cell lines were initiated and maintained in the Agency laboratory. The mouse cell line was kindly provided by Professor C. Heidelberger, Madison, Wisc., USA. Unscheduled DNA repair synthesis was quantitated by determining the total radioactivity in acid extracts of the washed cells by scintillation counting and was expressed as cpm per cover-slip. Autoradiography was also used.

Nitrosomethylurea was found to induce DNA excision repair in confluent monolayer cultures of all the cell lines examined. ^{3}H-TdR incorporation in each cell line increased up to a concentration of approximately 200–400 μg nitrosomethylurea/ml, indicating that the enzymes involved in DNA repair synthesis may have become saturated. The different levels of maximal ^{3}H-TdR incorporation, which were two to three times the control level in rat and human cells and six times the control in 10T½ cells, may reflect either different extents of DNA repair or different sizes of the nucleotide pools.

(*c*) *Metabolism of vinyl chloride*

Results of previous experiments have indicated that:

(1) the mutagenic effect of vinyl chloride depends on the formation of alkylated agents by microsomal mixed-function oxidases [1];

(2) of the putative or identified vinyl chloride metabolites examined, chloroethylene oxide showed the strongest mutagenic effect on bacteria, yeasts and mammalian cells[2–4]; and

[1] Bartsch, H., Malaveille, C., Montesano, R. & Tomatis, L. (1975) *Int. J. Cancer*, **15**, 429–437.
[2] Malaveille, C., Bartsch, H., Barbin, A., Camus, A. M. & Montesano, R. (1975) *Biochem. biophys. Res. Commun.*, **63**, 363–370.
[3] Loprieno, N., Barale, R., Barnoncelli, S., Bartsch, H., Bronzetti, G., Cammellini, A., Corsi, C., Frezza, D., Nieri, R., Leporini, C., Rosellini, D. & Rossi, A. M. (1976) *Cancer Res.* (in press).
[4] Huberman, E., Bartsch, H. & Sachs, L. (1975) *Int. J. Cancer*, **16**, 639–644.

(3) chloroethylene oxide is a strong alkylating agent (half-life, 1.6 min in aqueous solution at pH 7.4 and 37°C), and the adduct formed with 4-(4-nitrobenzyl)pyridine is identical to that obtained from a volatile vinyl chloride metabolite in a mixture generated from vinyl chloride and oxygen in the presence of fortified liver microsomes[1–3].

Some of the binding products of chloroethylene oxide (2-chloroacetaldehyde) with adenosine were isolated by Sephadex chromatography and compared with those formed in the presence of vinyl chloride, mouse liver microsomes and adenosine [1]. 3-β-Ribofuranosylimidazo[2,1-*i*]purine was identified and characterized as a common reaction product by Dr J. M. L'Hoste (Curie Foundation, Orsay, France) on the basis of analysis by nuclear magnetic resonance.

In a collaborative study, Dr H. F. Stich (University of British Columbia, Vancouver, Canada) examined unscheduled DNA repair synthesis by incorporation of ^{3}H-TdR into cultured human fibroblasts following exposure to vinyl chloride metabolites. Highest activity was observed with chloroethylene oxide; no ^{3}H-TdR incorporation was detected after treatment with 2-chloroacetaldehyde.

(*d*) *Metabolism of DDT* (Miss G. Planche, in collaboration with Dr A. Croisy and Dr P. Jacquignon, Institute of Chemistry of Natural Substances, Gif-sur-Yvette, France)

Conversion of some of the identified mammalian metabolites of DDT following incubation with mouse liver microsomal fractions *in vitro* has been studied [4]. DDNU (I) [2,2-bis-(*p*-chlorophenyl)ethylene] is converted into 2,2-bis(*p*-chlorophenyl)-ethane-diol-1,2 (III). The presumed precursor, 2,2-bis(*p*-chlorophenyl)-oxirane (II), the authentic compound, 2,2-bis(*p*-chlorophenyl)-ethane-diol-1,2 (III) and 2,2-bis(*p*-chlorophenyl)-acetaldehyde (IV) were all synthesized. In the presence of mouse liver microsomal fraction, DDNU (I) had no mutagenic effect in the *Salmonella*/microsome mutagenicity test with strains TA100 and TA98; nor was any mutagenic activity noted with the epoxide (II), the glycol (III) or the aldehyde (IV). A very high concentration of the epoxide (II) had weak alkylating activity, as detected with 4-(4-nitrobenzyl)pyridine in ethylene glycol. The data show that although DDNU may be epoxidated *in vivo*, the resulting compound (II) is a very weak electrophile as compared to structural analogues such as styrene oxide [5].

3.2 *Chemical carcinogenesis* in vitro (Dr R. Montesano, Dr T. Kuroki, Miss C. Drevon and Mrs L. Saint-Vincent)

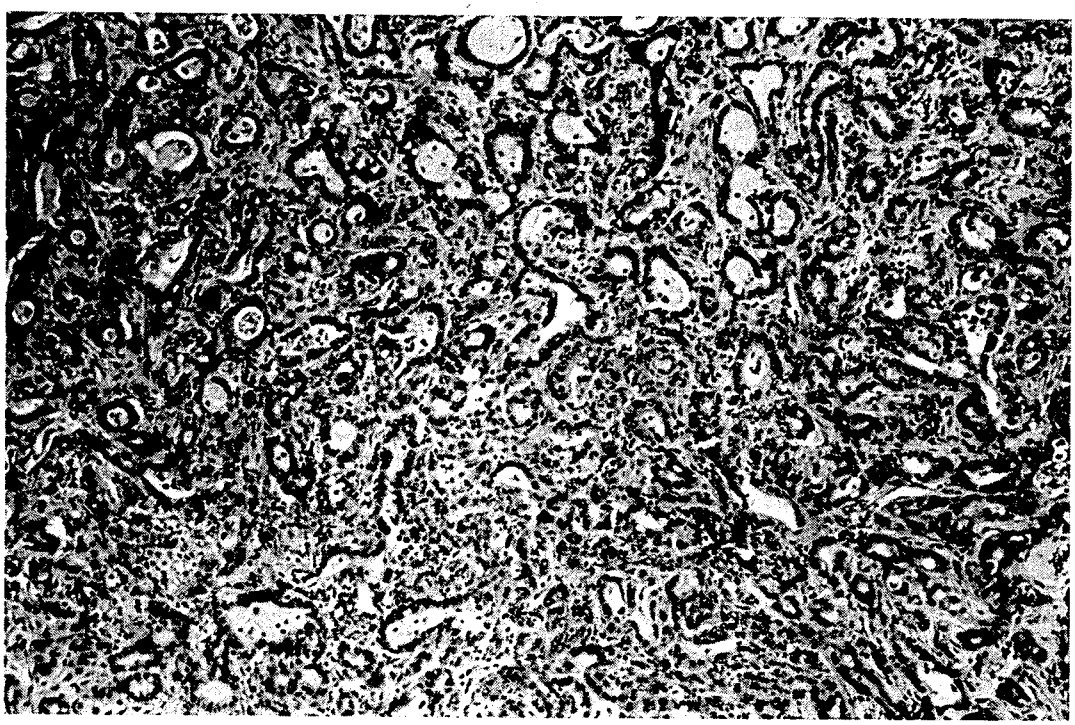
(*a*) *Rat liver epithelial-like cells*

Previous studies on the transformation of epithelial-like cells from the livers of BD rats 10 days or 8–10 weeks of age, have shown [6, 7] that these cells can be transformed *in*

[1] Barbin, A., Brésil, H., Croisy, A., Jacquignon, P., Malaveille, C., Montesano, R. & Bartsch, H. (1975) *Biochem. biophys. Res. Commun.*, **67**, 596–603.
[2] Bartsch, H. & Montesano, R. (1975) *Mutation Res.*, **32**, 93–114.
[3] Bartsch, H., Malaveille, C., Barbin, A., Brésil, H., Tomatis, L. & Montesano, R. (1976) *Environm. Hlth Persp.* (in press).
[4] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 100.
[5] Planche, G., Malaveille, C. & Bartsch, H., submitted for publication.
[6] Montesano, R., Saint-Vincent, L., Drevon, C. & Tomatis, L. (1975) *Int. J. Cancer*, **16**, 550–558.
[7] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, pp. 96–97.

vitro to produce carcinomas (Fig. 18) after back-transplantation into syngeneic rats. In most of the cultures examined, spontaneous transformation occurred in cells maintained in culture for approximately 50 weeks. However, treatment with certain carcinogens (*N*-itrosodimethylamine and *N*-nitroso-*N*-methyl-*N'*-nitroguanidine) resulted in transformation of the cells at an earlier stage in culture [1].

Fig. 18 Carcinomas produced by back-transplantation into syngeneic rats of cells transformed *in vitro* by chemical carcinogens



During the last year, we have concentrated on:

(1) establishing reliable criteria for distinguishing transformed from non-transformed epithelial cells *in vitro*, which is essential for the development of a quantitative assay;

(2) establishing the competence of these cells to metabolize certain carcinogens; and

(3) characterizing the epithelial nature of these cells.

In collaboration with Dr K. Sanford (National Cancer Institute, Bethesda, Md., USA) and Dr B. Weinstein (University of Columbia, New York, N.Y., USA), 12 cell lines, seven of which were tumorigenic and five not, were examined for their capacity to grow in soft agar, for their fibrinolytic activity [2] and for morphological changes in culture. Preliminary examination of the results shows a good correlation between growth in soft agar, morphological changes and tumorigenicity of the cells, whereas no such correlation was observed for fibrinolytic activity.

---

[1] Kuroki, T., Drevon, C., Saint-Vincent, L., Tomatis, L. & Montesano, R. (1976) *Colloque Internat. CNRS* (in press).

[2] Unkeles, J. C., Tobia, A., Ossowski, L., Quigley, J. P., Rifkin, D. B. & Reich, E. (1973) *J. exptl. Med.*, **137**, 85–111.

The occurrence of microsomal mixed-function oxygenases was investigated in IAR-20 cells of a non-transformed epithelial cell line which had been maintained in culture for 40 weeks. The cells were incubated with 100 μg/ml ^{14}C-nitrosodimethylamine for 4, 12, 24 hours or six days, and the total nucleic acids (DNA and RNA) or only DNA were determined in six-day samples by chromatography on Dowex-50. Acid hydrolysis for the presence of *N*-7-methylguanine was used to determine the capacity of these cells to metabolize nitrosodimethylamine into a methylating agent. The results (Table 19) show that some *N*-7-methylguanine was detected after 24 hours' or six days' incubation, but not after 4 or 12 hours' (The results obtained from the six-day sample are not expressed as dpm/μmol of guanine, since the DNA was isolated by the use of carrier DNA).

Table 19. Alkylation of total nucleic acids or of DNA only (6 days' sample) by ^{14}C-nitrosodimethylamine in IAR-20 cells

Exposure time	*N*-7-methylguanine (dpm/μmol guanine)
4 h	negative
12 h	negative
24 h	1451
6 days	positive

(*b*) *Transformation with C3H 10T½ mouse cells*

Quantitative chemical transformation is being carried out with 10T½ cells derived from embryos of C3H mice, kindly provided by Professor C. Heidelberger (University of Wisconsin, Madison, Wisc., USA). The transformations were strongly inhibited by additions of severals protease inhibitors, such as anti-pain, chemostatin, elastinal, leupeptin and pepstatin. This result suggests that the activation of proteases may be involved in the development of transformed cells.

A microsome-mediated transformation system is being established for efficient assay of chemical which require metabolic activation; this study is an extension of that involving microsome-mediated mutagenesis in V79 cells (see below). Measurements of the transforming abilities of vinyl chloride and related compounds in 10T½ cells are in progress.

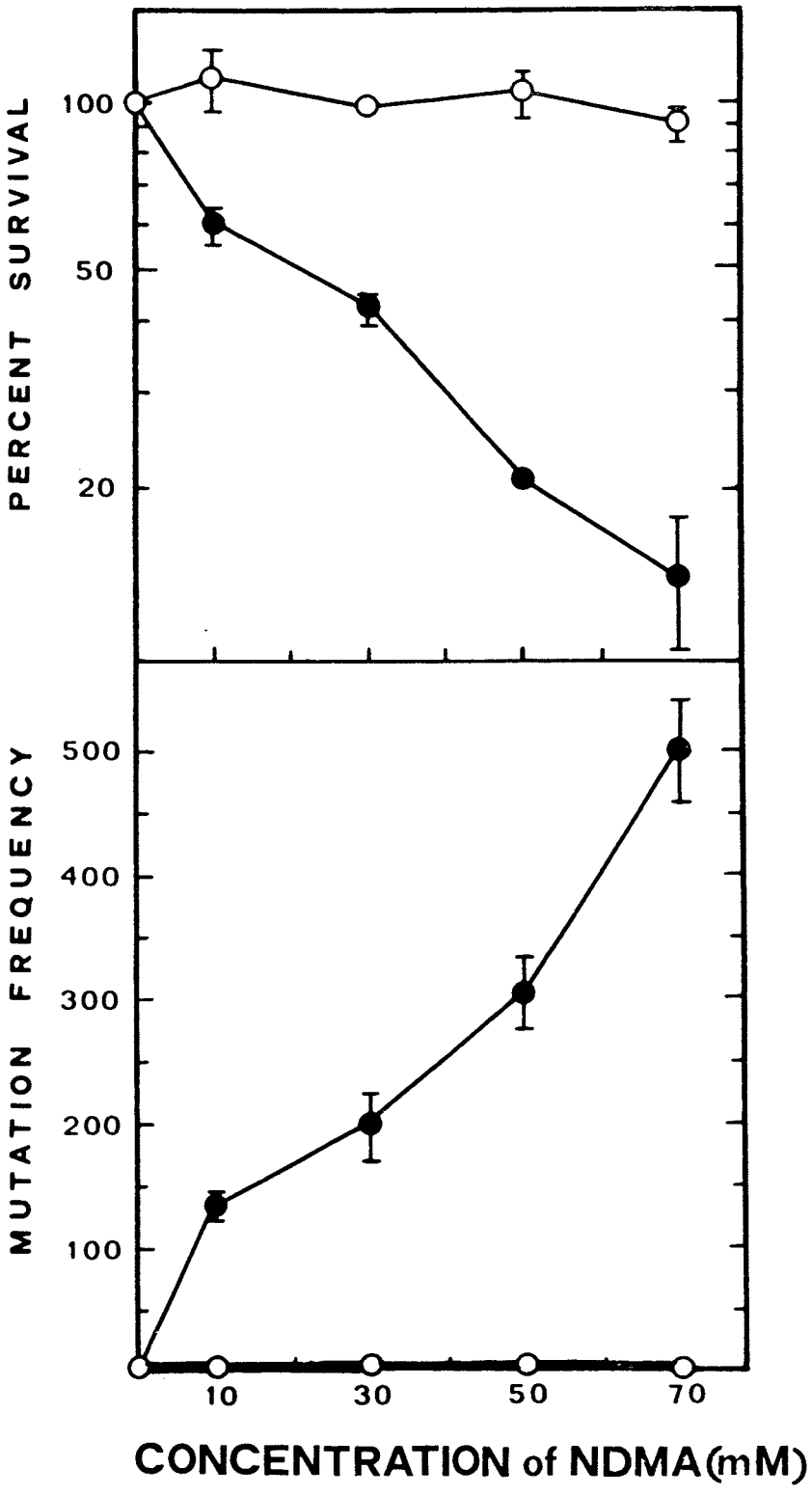
(*c*) *Chemical mutagenesis in a Chinese hamster cell line*

Microsome-mediated mammalian cell mutagenesis has been established in the V79 Chinese hamster cell line, kindly provided by Dr E. Huberman (The Weizmann Institute of Science, Rehovoth, Israel). Cells growing in a monolayer were incubated for one hour in a reaction mixture containing the liver microsome fraction (S-15) from phenobarbitone-pretreated rats, a NADPH-generating system and the chemicals to be tested. The cells were then washed and incubated in fresh culture medium for two to three hours and plated for mutagenesis and toxicity tests. 8-Azaguanine-resistance, which is known to be associated with genotype deficiency of hypoxanthine-guanine phosphoribosyltransferase, was used to determine mutagenesis. In our system, the microsome fraction itself was not toxic to the cells.

As shown in Figure 19, nitrosodimethylamine could induce mutagenesis and toxicity in a dose-related fashion in the presence of the microsome fraction; no induction was observed in the absence of the microsomes. Preliminary results of a series of investigations

using volatile chemicals, such as vinyl chloride monomer, indicate that this mammalian cell mutagenesis assay may be applicable to a wide range of chemicals which require metabolic activation.

Fig. 19 Toxicity (top) and mutagenicity (bottom) of nitrosodimethylamine (NDMA) for Chinese hamster V79 cells in the presence (o) or absence (●) of a microsomal activation system. Mutagenic effect is expressed as the number of 8-azaguanine-resistant mutants per 10^5 survivors



First Institute of Pathology, Medical University, Budapest (RA/70/003)
Principal investigator: Professor K. Lapis

Three cell lines, derived from 5–10-week-old human embryonic lungs, were cultured and after various times of growth *in vitro* were transplanted into normal and immunosuppressed adult mice and into newborn mice. Injected cells grew in immunosuppressed adult mice and in some cases killed the hosts [1]. Additional studies have been carried out with mouse embryo lung fibroblasts.

3.3 *Detection of chemical carcinogens as mutagens* (Dr H. Bartsch and Mr C. Malaveille)

(*a*) *Efficiency and reproducibility of the* S. typhimurium/*microsome mutagenicity test* (Miss A. M. Camus and Miss G. Planche)

Because of the relationship between carcinogenesis and mutagenesis, relatively quick and inexpensive mutagenicity tests have received much attention as a means of tracing carcinogens and/or mutagens in the complex environment of man and for pre-screening chemicals to be submitted to a more sophisticated set of bioassays. Tissue-mediated plate incorporation assays for detecting mutagenicity of chemical carcinogens may produce false negative results; these may be due to mutagen specificity, to high reactivity and/or volatility of the chemical or its metabolites or to lack of appropriate cofactors for activation [2, 3].

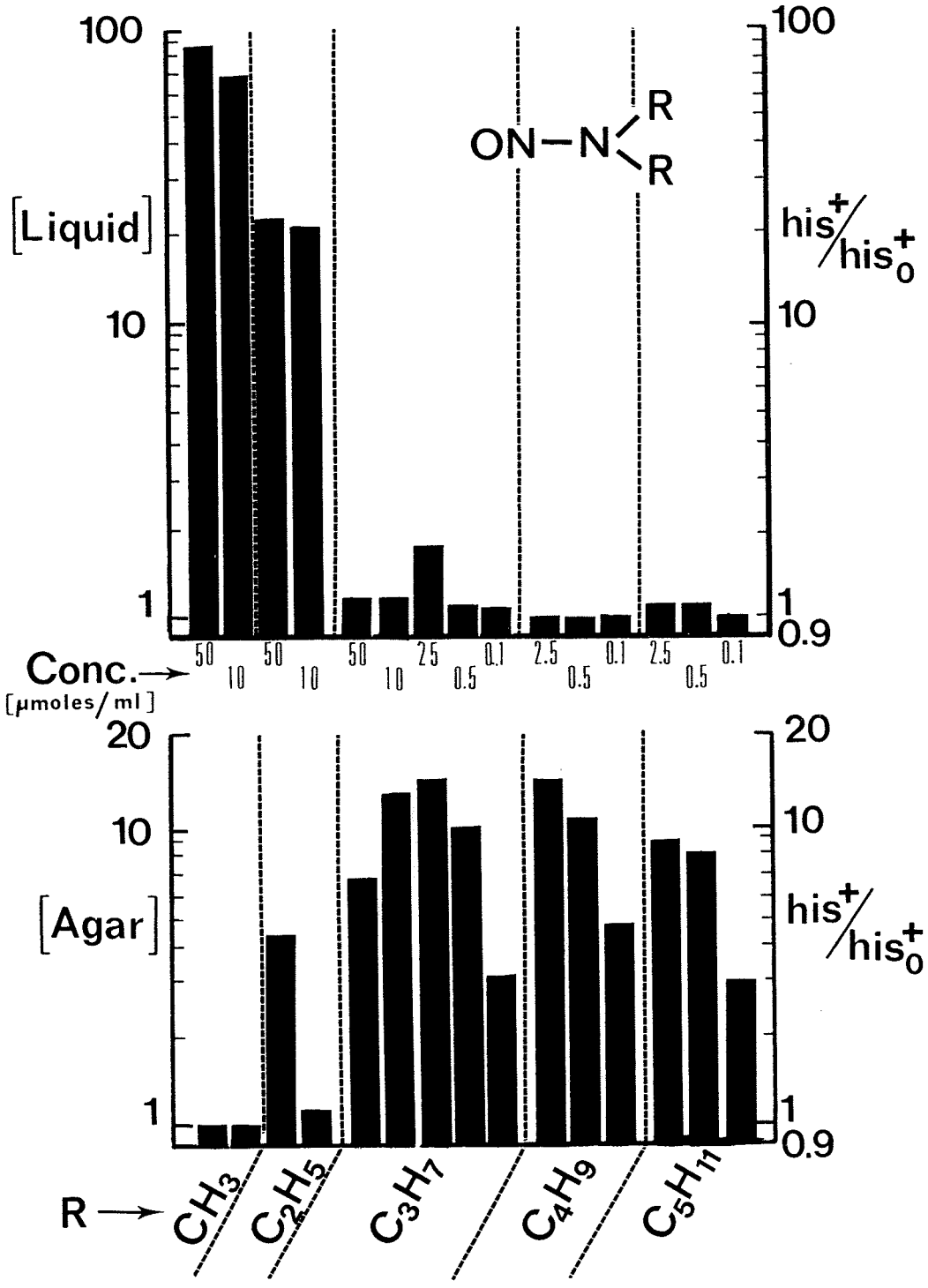
A quantitative comparison of the mutagenic effects of a series of aliphatic *N*-nitrosamines was made in a liquid incubation system and in a semi-solid agar assay (Fig. 20). When the mutagenic effects of nitrosodimethyl-, nitrosodiethyl-, nitrosodi-*n*-propyl-, nitrosodi-*n*-butyl- and nitrosodi-*n*-pentylamine were compared in *S. typhimurium* strain TA1530, in the presence of rat-liver fraction, nitrosodimethyl- and nitrosodiethylamine failed to give a response in the soft agar system, while nitrosodi-*n*-pentyl- and nitrosodi-*n*-butylamine were only marginally mutagenic in the liquid suspension system. The data emphasize the possibility that false negative results may be obtained with certain compounds if the two systems are not used in parallel or combined. The relative mutagenic strengths observed in the two systems (his^+/his_0^+) were not identical [4].

In another study [5], it was demonstrated that the plate incorporation assay in soft agar is more effective in detecting chemicals whose metabolic conversion into mutagens occurs at a low rate. This was shown experimentally by incorporating liver microsomal enzymes into a soft agar layer and by measuring their viability after different intervals of pre-incubation at 37°C. The fact that the viability was prolonged for up to several hours could explain why nitrosodi-*n*-butyl- and nitrosodi-*n*-pentylamine were not detectable in liquid suspension after 30 minutes' incubation. Furthermore, with directly acting carcinogens such as *N*-acetoxy-2-acetylaminofluorene, it was shown that the yield of mutant colonies of TA100 or TA98 strains in a plate incorporation assay in soft agar was strongly dependent on the state of bacterial growth, e.g., addition of the carcinogenic compound during the phase of logarithmic growth enhanced the mutagenic effect several fold.

---

[1] Kopper, L., Ferencz, G., Szender, B., Lapis, K. & Surján, M., submitted for publication.
[2] McCann, J. & Ames, B. N. (1976) *Proc. nat. Acad. Sci. (Wash.)*, **73**, 950–954.
[3] Bartsch, H. (1976) *Mutation Res.*, **38**, 177–190.
[4] Bartsch, H., Camus, A. & Malaveille, C. (1976) *Mutation Res.* (in press).
[5] Malaveille, C., Planche, G. & Bartsch, H., submitted for publication.

Fig. 20 Comparative mutagenicities of a series of *N*-nitrosodialkylamines assayed in liquid suspension (top) or in soft agar (bottom). The routine incubation systems contained a liver fraction from phenobarbitone-pretreated rats and an *N*-nitrosodialkylamine, ON-N < R_2, with the substituent R indicated at the bottom. The concentrations are expressed as μmol/ml of incubation medium or of soft agar overlay. *N*-Nitrosodimethylamine and *N*-nitrosodiethylamine were dissolved in saline; *N*-nitroso derivatives of di-*n*-propylamine, di-*n*-butylamine and di-*n*-pentylamine were assayed in DMSO solutions. Incubations were carried out simultaneously in assays in liquid suspension and in soft agar using the same S-9 liver preparation. The mutagenic strengths (his^+/his_0^+) were calculated by dividing the number of his^+ revertant colonies obtained in the routine assays by that measured in an appropriate control (his_0^+).



(*b*) *Liver microsome-mediated formation of alkylating agents from halogenated olefinic hydrocarbons* (Mr A. Barbin and Miss G. Planche)

The finding that vinyl chloride is a human and animal carcinogen has prompted studies on related olefinic compounds. Using a tissue-mediated mutagenicity assay with *S. typhimurium* strain TA100, the mutagenicity of a series of compounds was investigated as a sensitive indicator of DNA interaction. Petri dishes containing bacteria and fortified post-mitochondrial supernatants were exposed to various gaseous mixtures of the test compound in air. Removal of the substrate at different time intervals *in vacuo* permits the plotting of dose- and time-dependent mutagenicity curves after the mutant colonies are allowed to grow with further incubation for up to 48 hours. The actual concentration of the test compound in the aqueous phase was determined by gas-liquid chromatography.

The following mutation rates (values in brackets) are taken from the linear region of dose- and time-dependent assays with liver fractions from phenobarbitone-pretreated mice and are expressed as the number of histidine revertants per μmol of substrate per hour of incubation per plate: vinyl acetate, vinylidene fluoride or trichloroethylene (0); vinyl chloride (5.6); vinylidene chloride (14.6); vinyl bromide (25.9); 2-chlorobutadiene (51.2); 1-chlorobutadiene (157.5); and 3,4-dichlorobutene-1 (490). 1,4-Dichlorobutene-2 and tetrachloroethylene were assayed by the routine plate incorporation assay because of their high boiling-points. The mutagenic effect of 1,4-dichlorobutene-2, expressed as the number of histidine revertants per μmol of compound per plate, was 840; tetrachloroethylene was non-mutagenic under these conditions [1].

The enzymic formation of alkylating agents from vinyl chloride, vinyl bromide and 2-chlorobutadiene was demonstrated [1] after assaying by previously published procedures using 4-(nitrobenzyl)pyridine in ethylene glycol [2].

(*c*) *Carcinogen metabolism by human tissue biopsy specimens* (Dr N. Sabadie, Miss A. M. Camus and Mrs G. Brun, in collaboration with Dr M. Boiocchi and Dr G. Della Porta, National Institute for the Study and Therapy of Tumours, Milan, Italy; Dr H. B. Richter-Reichhelm, Dr J. Hilfrich and Professor U. Mohr, School of Medicine, Hanover, Federal Republic of Germany; and Dr E. Matos and Dr E. de Lustig, The Angel H. Roffo Institute of Oncology, University of Buenos Aires, Buenos Aires, Argentina)

In order to establish better experimental criteria for evaluating the carcinogenic risk of chemicals to man, more experimentation with human tissues or fluids is necessary; therefore, a network for collecting human tissue biopsy samples has been set up in collaboration with the national research institutions listed above. The capacities of such samples to convert carcinogens into electrophilic metabolites mutagenic for *S. typhimurium* have been measured in the S-9 fractions of homogenates of these biopsy specimens and compared with those obtained from mouse or rat tissues.

---

[1] Bartsch, H., Malaveille, C., Barbin, A., Planche, G. & Montesano, R. (1976) *Proc. Amer. Ass. Cancer Res.*, **17**, 17.

[2] Barbin, A., Brésil, H., Croisy, A., Jacquignon, P., Malaveille, C., Montesano, R. & Bartsch, H. (1975) *Biochem. biophys. Res. Commun.*, **67**, 596–603.

Tables 20 and 21 summarize the relative capacities of human biopsy samples to convert halogenated hydrocarbons or *N*-nitrosamines into mutagenic intermediates. The results are expressed as a percentage of an appropriate animal control; samples from human individuals are represented by different letters. Samples A, B, C and D (Table 20) from human liver, the target organ in which vinyl chloride causes cancer, could convert this carcinogen into electrophilic and mutagenic intermediates. Similarly, human liver specimens were active in converting vinyl bromide, vinylidene chloride, 2-chloro-1,3-butadiene and 1,4-dichlorobutene-2 into mutagens, the activity being in general lower than that in mouse liver. A number of *N*-nitrosamines, some of which are listed in Table 21, are known to be carcinogenic to various animal species, and man is exposed to some of them [1, 2]. Although no epidemiological investigations or case reports have so far given evidence that they have actually induced cancer in man, the possible biological hazard to man of these compounds is evident from the fact that liver samples A, B, C and D from four humans efficiently converted *N*-nitrosomorpholine into mutagenic intermediates. The average enzymic activity was closest to that of rat liver, in which *N*-nitrosomorpholine is a potent carcinogen. Other heterocyclic nitrosamines, such as *N*-nitrosopyrrolidine and *N*-nitrosopiperidine, which are hepatocarcinogens in rats, were also activated by human liver fractions; and the activities of samples X, Y and Z in converting these two compounds were from 50 to 200% of that with rat liver fraction, which is given as 100 (Table 21). *N*-Nitroso-*N'*-methylpiperazine, which induces nasal cavity tumours in rats, was the least active mutagen in this series. Human liver samples X, Y and Z were three to thirty times more active than rat liver.

To examine the hypothesis that a variability in the metabolic activation and detoxification of carcinogens among the human population may explain why some individuals are more susceptible than others to certain carcinogens (Fig. 21), the enzymic capacities of a series of human liver biopsy specimens to convert vinyl chloride into electrophilic mutagens were measured, and these were compared to their capacities to hydroxylate benzo[*a*]pyrene at the 3-position [aryl hydrocarbon hydroxylase (AHH) activity] [3]. Activities are expressed as pmol 3-hydroxybenzo[*a*]pyrene formed per minute per g of wet tissue (ordinate) and as the number of histidine-revertant colonies of *S. typhimurium* TA1530 strain after 4 hours' exposure to 20% vinyl chloride in air per 38 mg wet tissue per plate [4] (abscissa). Different human liver biopsies are represented by different letters. Spontaneous mutations have been subtracted; values were taken from linear dose- and time-dependent response curves. Even with the limited number of samples, a positive correlation between the two enzymic activities can be deduced; an absolute correlation between the rate of metabolism of benzo[*a*]pyrene and the rate of formation of vinyl chloride mutagens in different individuals would suggest that the two carcinogens are metabolized by a single enzyme system or by enzyme systems which are under a similar regulatory control. The data support the hypothesis that microsomal mixed-function oxidase in human liver activates vinyl chloride into electrophilic and mutagenic reactants. The enzymic capacities of the different specimens to hydroxylate benzo[*a*]pyrene or to convert vinyl chloride into mutagens varied within a ten-fold range.

---

[1] Montesano, R. & Bartsch, H. (1976) *Mutation Res.*, **32**, 179–228.

[2] Magee, P. N., Montesano, R. & Preussmann, R. (1976) In: Searle, C. E., ed., *Chemical Carcinogens*, ACS Monogr. (in press).

[3] Nebert, D. W. & Gelboin, H. V. (1968) *J. biol. Chem.*, **243**, 6242–6249.

[4] Bartsch, H., Malaveille, C. & Montesano, R. (1975) *Int. J. Cancer*, **15**, 429–437.

Table 20. Relative mutagenicity mediated by human and animal tissues

Compound	Organ fraction	Activity %	
		human	animal
Vinyl chloride	Liver	A 170 [a] B 70 C 64 D 46	Mouse (100)
Vinyl bromide	Liver	Z 32 Y 39 X 22	Mouse (100)
Vinylidene chloride	Liver	Z 18 Y 15 X 10	Mouse (100)
2-Chloro-1,3-butadiene	Liver	K 22 Z 0 Y 0 X 0	Mouse (100)
	Lung	L, M N, O, P (0)	Mouse (0)
1,4-Dichlorobutene-2	Liver	Z 83 [b] Y 37 X 17	Mouse (100)

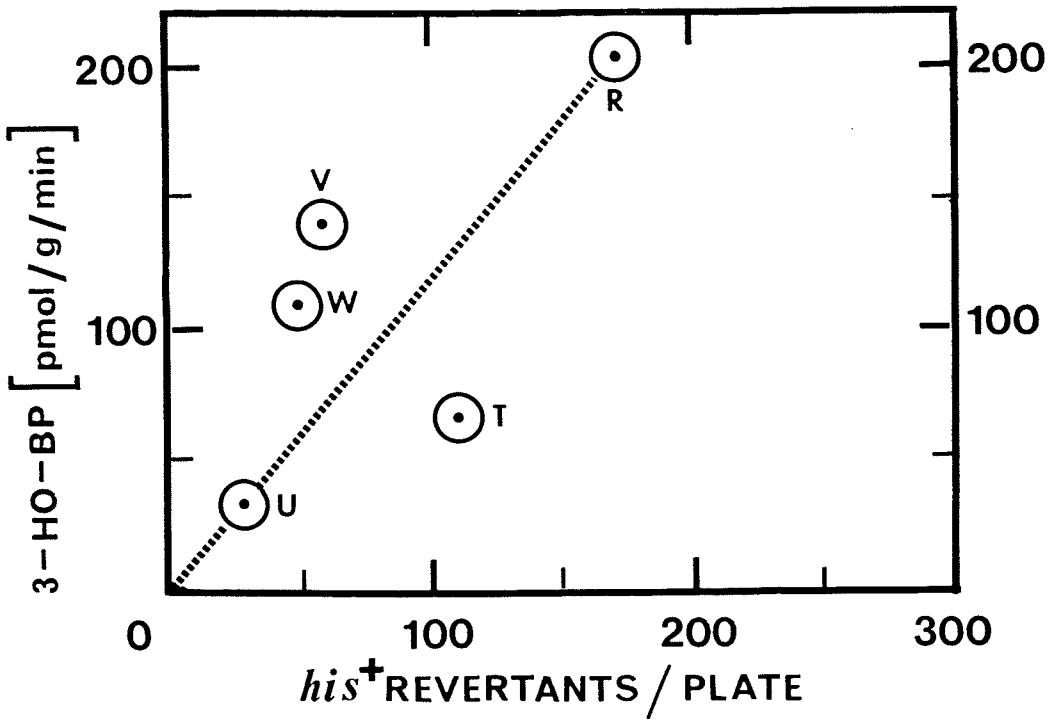
[a] Mutagenicity assay: Bartsch, H., Malaveille, J. & Montesano, R. (1975) *Int. J. Cancer*, **15**, 429–437.
[b] Plate incorporation assay: Ames, B. N., Durston, W. E., Yamasaki, E. & Lee, F. D. (1973) *Proc. nat. Acad. Sci. (Wash.)*, **70**, 2281–2285.

Table 21. Relative mutagenicity mediated by human and animal tissues

Compound	Organ fraction	Activity %	
		human	animal
N-Nitrosomorpholine	Liver	A 84 [a] B 47 C 37 D 28	Rat (100)
	Lung	E, F G, H (0)	Rat (0)
N-Nitrosopyrrolidine	Liver	Z 117 Y 91 X 54	Rat (100)
N-Nitrosopiperidine	Liver	Z 216 Y 189 X 86	Rat (100)
N-Nitroso-*N'*-methylpiperazine	Liver	Z 3 180 Y 1 800 X 370	Rat (100)

[a] Mutagenicity assay: Ames, B. N., Durston, W. E., Yamasaki, E. & Lee, F. D. (1973) *Proc. nat. Acad. Sci. (Wash.)*, **70**, 2281–2285.

Fig. 21 Enzymic capacities of human liver biopsy specimens (R, T, U, V, W) to convert vinyl chloride into electrophilic mutagens (expressed as the number of *his*$^+$-revertant colonies/plate *versus* their capacities to hydroxylate benzo[a]pyrene at the 3-position [aryl hydrocarbon hydroxylase (AHH) activity]



(*d*) *Biological activity of vinyl chloride metabolites* (Dr H. Bartsch, in collaboration with Professor N. Loprieno, CNR Laboratory of Mutagenesis and Differentiation, Pisa, Italy; Dr H. Stich, Cancer Research Centre, University of British Columbia, Vancouver, Canada; and Dr F. Zajdela, Radium Institute, Faculty of Sciences, Orsay, France)

2-Chloroethanol and 2-chloroacetaldehyde, two possible metabolites of vinyl chloride, and chloroethylene oxide, a metabolic intermediate characterized *in vitro*, were assayed for their genetic activity in the yeasts *S. pombe* and *S. cerevisiae*.

Chloroethylene oxide was the most effective in inducing forward mutations in *S. pombe* and gene conversions in *S. cerevisiae*, increasing the mutation and conversion frequencies to 340 and 350 times, respectively, as compared to those in controls. In both the presence and absence of mouse liver microsomes, 2-chloroacetaldehyde showed only feeble genetic activity, and 2-chloroethanol was completely inactive in both yeast strains. In contrast to vinyl chloride, 2-chloroacetaldehyde did not induce forward mutations in *S. pombe* in the host-mediated assay in mice. The results strongly support the hypothesis that chloroethylene oxide is one of the principal mutagenic agents formed from vinyl chloride in the presence of mouse liver enzymes [1].

Chloroethylene oxide, 2-chloroacetaldehyde and 2-chloroethanol were also assayed for their capacity to induce unscheduled DNA repair synthesis in cultured human fibroblasts [2]. Chloroethylene oxide was the most active compound in this system; 2-chloro-

[1] Loprieno, N., Barale, R., Baroncelli, S., Bartsch, H., Bronzetti, G., Cammellini, A., Coris, C., Frezza, D., Nieri, R., Leporini, C., Rosellini, D. & Rossi, A. M. (1976) *Cancer Res.* (in press).
[2] San, R. H. C. & Stich, H. F. (1975) *Int. J. Cancer*, **16**, 284–291.

acetaldehyde was toxic but caused no detectable unscheduled incorporation of ^{3}H-TdR. *In vitro* transformation experiments with chloroethylene oxide are in progress in 10T½ cells.

(*e*) *Metabolic activation of carcinogens* (Miss A. M. Camus and Mrs G. Brun, in collaboration with Dr P. L. Grover and Dr P. Sims, Chester Beatty Research Institute, Royal Cancer Hospital, London; Dr G. Kolar and Dr M. Wiessler, German Cancer Research Centre, Heidelberg, Federal Republic of Germany)

The biological activities of synthetic putative or identified metabolites of several carcinogens, such as polycyclic aromatic hydrocarbons, substituted 3,3-dimethyl-1-phenyltriazenes and *N*-nitrosodialkylamines, are being measured *in vitro* in order to elucidate the mechanisms of metabolic activation *in vivo*.

(*f*) *Other mutagenicity tests*

Laboratory of Biophysics and Radiobiology, Free University, Brussels
Principal investigators: Dr M. Radman and Professor M. Errera

The role of misincorporation of non-complimentary nucleotides into defined intact or mutagen-modified templates has been elaborated, using a prokaryotic system. The possible use of a eukaryotic system is being investigated. Extracts of mammalian cells, treated with chemical carcinogens or X-irradiated, will be examined for their capacities to promote the misincorporation of the oxyribonucleotides into polynucleotides, and templates will be made in order to purify and characterize these error-prone polymerases.

3.4 *Research training in mutagenicity testing*

Dr M. Shariaty, from the Department of Cancer Research, University of Teheran, received training on the *Salmonella*/microsome mutagenicity test system during a three-month period. Subsequently, bread, tea and wheat from Gonbad, an area of Iran with a high incidence of oesophageal cancer, were tested using this technique. The material was extracted with different aqueous and non-aqueous solvents (in collaboration with Mr E. Walker and Mr M. Castegnaro of the Unit of Environmental Carcinogens), and the residues were applied to the routine *Salmonella*/microsome mutagenicity test. Apart from a marginal mutagenic effect in tea, no significant activity was noted in the extracts.

A short training course on the *Salmonella*/microsome mutagenicity test system was held in January 1975, with 20 participants from four countries. The theoretical background and limitations of this particular test as related to other short-term assays were discussed. Practical demonstrations and pertinent references in the literature were provided.

3.5 *Workshop on Rapid Screening Tests in Chemical Carcinogenesis and related activities*

The proceedings of the workshop, which was convened jointly by the Agency and the Commission of the European Communities and held in Brussels from 9–12 June 1975, were published in April 1976 [1]. The proceedings include 34 papers on the metabolism of carcinogens, carcinogenesis *in vitro*, mutagenesis and DNA repair monitoring as a function

---

[1] Montesano, R., Bartsch, H. & Tomatis, L., eds (1976) *Screening Tests in Chemical Carcinogenesis*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* No. 12).

of chemically-induced genetic damage. The vast programme being carried out in Japan, The Netherlands and the USA to compare data on mutagenicity, long-term bioassays *in vivo* and transformation *in vitro* is also reviewed.

4. TRANSPLACENTAL CARCINOGENESIS

4.1 *Experimental studies*

A survey of studies carried out on the effects of prenatal administration of chemical carcinogens has indicated that at least 39 chemicals produced tumours in the progeny of mothers exposed during pregnancy [1].

In support of the results obtained with nitrosomethylurea, it was shown that administration of nitrosoethylurea to pregnant BD rats produced a high incidence of tumours in the F_1 generation and an increased cancer risk in subsequent generations [2].

School of Medicine, Hanover, Federal Republic of Germany
Principal investigator: Professor U. Mohr

A study of the effects on progeny of administration of 7,12-dimethylbenzanthracene to pregnant C57 black mice is in progress. The study is being extended over three subsequently untreated generations, and the experimental design includes cross-breeding of mice of the first generation with progeny of untreated controls.

Cancer Research Center, Academy of Medical Sciences of the USSR, Moscow
Principal investigator: Dr V. S. Turusov

A study of the persistence of cancer risk in progeny of animals exposed to a carcinogen during intra-uterine life has been initiated.

4.2 *Prenatal events in childhood cancer*

Under the leadership of Dr N. Muñoz (Interdisciplinary Programme and International Liaison, see page 116), a multidisciplinary programme involving the Unit of Epidemiology and Biostatistics (Dr N. Day) is investigating the possible role of prenatal events in the development of cancer in progeny. Particular attention is being given to the role of prenatal events in the occurrence of congenital cancer, i.e., that observed at birth or within 28 days after birth.

The Agency has continued its sponsorship of a cancer registry in the Lombardy region of Italy (Dr F. Berrino, National Institute for the Study and Therapy of Tumours, Milan, Italy), with emphasis on the identification of occupational carcinogens and of events possibly related to the occurrence of cancer in childhood.

---

[1] Tomatis, L. (1976) *Nat. Cancer Inst. Monogr.* (in press).
[2] Tomatis, L., Ponomarkov, V. & Turusov, V. (1976) *Int. J. Cancer* (in press).

5. UNIT OF RESEARCH TRAINING AND LIAISON

Dr W. DAVIS (Chief)

1. INTRODUCTION

The fellowships programme has continued this year with a slightly higher budget than for the last two years, and three courses were organized. Two new titles were added to the *IARC Scientific Publications* series.

2. THE FELLOWSHIPS SELECTION COMMITTEE

The meeting of the Fellowships Selection Committee was held in April 1976 to review applications for fellowships and to discuss general policy. Some modifications to the fellowships rules were made. The physical difficulties and financial implications of interviewing all the candidates were taken into consideration, but the general view was that the role of the interview was of paramount importance in assessing the candidates. Whilst interviewing by telephone might be admissible if it were carried out in the candidate's mother tongue, it could not be used generally as a substitute for the 'site visit'. The Committee agreed with the need to continue the practice of interviewing as many candidates as possible and set as a minimum requirement the interviewing of all those candidates whose application was of high quality, regardless of field of study, or which was in the priority fields of epidemiology and environmental carcinogenesis. In order to give the committee more time to complete its interviewing commitment, the closing date for the receipt of applications was brought forward to January 31st.

The members of the Committee were:

Professor M. Bagshaw, Department of Radiobiology, Stanford University, Stanford, Calif., USA *(Radiobiology)*; also Chairman of the Commission on Fellowships and Personnel Exchange of the UICC

Professor C. Heidelberger, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisc., USA *(Chemical Carcinogenesis)*

Professor N. P. Napalkov, Director, N. N. Petrov Research Institute of Oncology, Leningrad, USSR *(Chemical Carcinogenesis)*

Professor N. F. Stanley, Department of Microbiology, University of Western Australia, Perth, Australia *(Microbiology)*

Professor H. Sugano, Director, Cancer Institute (Japanese Foundation for Cancer Research), Tokyo *(Experimental Pathology)*

3. RESEARCH TRAINING FELLOWSHIPS

Of the 77 applications reviewed by the Committee, 13 were recommended to receive an award. As in the previous two years, priority was given to applicants in the fields of epidemiology and environmental carcinogenesis, and Table 22 shows the distribution by scientific discipline.

Table 22. Distribution of fellowships by scientific discipline, 1976

Research Training Fellowships		Travel Fellowships	
Biochemistry	2	Biochemistry	1
Cell biology	1	Immunology/Virology	1
Epidemiology	3	Molecular biology	1
Experimental carcinogenesis	1		
Genetics	1		
Immunology	1		
Molecular biology	1		
Virology	3		

4. TRAVEL FELLOWSHIPS

Thirty-nine applications for travel fellowships were received during 1976; however, only three fellowships could be awarded due to the limited availability of funds. In view of the very small percentage of successful candidates, it was felt by the Committee that continuation of the travel fellowships programme was no longer justifiable, and it has therefore been suspended until new funds make possible the awarding of fellowships to at least one out of every six applicants.

5. CORVISSIANO FELLOWSHIPS

The receipt of a legacy of one million French francs 'to be devoted to cancer research in France' from the estate of Mr P. Corvissiano has enabled the Agency to set up a new programme of fellowships which will enable scientists from national laboratories throughout the world to contribute their special skills to cancer research in France and internationally by coming to work in the Agency laboratories.

The first Corvissiano fellow, Dr T. Yokota, from Fukushima, Japan, arrived in Lyon in January 1976 and will stay for 12 months to work on cancer immunology.

6. SPECIALIZED COURSES

6.1 *Immunovirology of cancer*

Following the success of the first immunovirology course held in Lyon in 1974, a second course on this subject was held from 10–22 May 1976. The coordinator was again Professor N. F. Stanley, University of Western Australia. He was supported particularly by Professor A. S. Evans (Yale University School of Medicine, New Haven, Conn., USA), Professor C. A. Mims (Guy's Hospital Medical School, London), Professor N. A. Mitchison (University College, London), Dr J. P. Revillard (Edouard Herriot Hospital, Lyon), Dr D. V.

Ablashi (National Cancer Institute, USA) and Dr Natalie Teich (Imperial Cancer Research Fund Laboratories, London) and members of the Agency's staff. Several special lectures were given by other invited scientists.

Half of the sessions were held in the Agency and half in the Hôtel Beaulieu, Charbonnières-les-Bains, France. Thanks to the generous collaboration of Professor J. Traeger, it was again possible to hold certain practical demonstrations in the laboratories of the nephrology clinic of the Edouard Herriot Hospital, Lyon. The handbook of techniques prepared for the 1974 course was updated and improved, with contributions from Agency staff members and leading scientists throughout the world. The manual was edited by Drs J. P. Lamelin and G. Lenoir of the Unit of Biological Carcinogenesis; important contributions were also made by Dr J. P. Revillard and his colleagues at the Edouard Herriot Hospital, Lyon.

Twenty-six participants from 17 countries took part in the course.

6.2 *Epidemiology of cancer*

Under the direction of Professor P. Cole (Department of Epidemiology, Harvard University, Cambridge, Mass., USA), a course on cancer epidemiology will be held at the Agency from 30 August to 10 September 1976. Other lecturers include Professor E. D. Acheson (University of Southampton, UK) and Professor D. D. Reid (London School of Hygiene and Tropical Medicine, London).

Fig. 22 Some of the participants at the course on cancer epidemiology (Photo by courtesy of Becam, Lyon)

In collaboration with the National Cancer Division of Brazil (Director, Dr H. Torloni), the Agency is organizing a one-week course on cancer epidemiology to be held in Brasilia from 29 November to 4 December, 1976. The course coordinator will be Dr Pelayo Correa (Louisiana State University Medical Center, New Orleans, La., USA).

7. PUBLICATIONS

Two more titles have appeared in the *IARC Scientific Publications* series — *Oncogenesis and Herpesviruses II* and *Screening Tests in Chemical Carcinogenesis*. Five more are in press—*Environmental Pollution and Carcinogenic Risks* (joint publication with the French National Institute of Health and Medical Research); *Pathology of Tumours in Laboratory Animals*, Volume I, *The Rat*, Part 2; *Environmental* N-*Nitroso Compounds—Analysis and Formation ; Cancer Incidence in Five Continents*, Volume III; and *Air Pollution and Cancer in Man*. Finally, in the series *Pathology of Tumours in Laboratory Animals*, Volume II, *The Mouse*, and Volume III, *The Hamster*, are in preparation. The list of publications in the series is given in Table 23.

Up-to-date figures for the distribution of the scientific publications and monographs are given in Table 24.

Table 23. List of *IARC Scientific Publications*

No.	Title	Year of publication
1	*Liver Cancer*	1971
2	*Oncogenesis and Herpesviruses*	1972
3	N-*Nitroso Compounds — Analysis and Formation*	1972
4	*Transplacental Carcinogenesis*	1973
5	*Pathology of Tumours in Laboratory Animals*, Vol. I: *The Rat*, Part 1	1973
6	*Pathology of Tumours in Laboratory Animals*, Vol. I: *The Rat*, Part 2	1976 [a]
7	*Host Environment Interactions in the Etiology of Cancer in Man*	1973
8	*Biological Effects of Asbestos*	1973
9	N-*Nitroso Compounds in the Environment*	1974
10	*Chemical Carcinogenesis Essays*	1974
11	*Oncogenesis and Herpesviruses II*, Parts 1 and 2	1975
12	*Screening Tests in Chemical Carcinogenesis*	1976
13	*Environmental Pollution and Carcinogenic Risks*	1976 [a, c]
14	*Environmental* N-*Nitroso Compounds — Analysis and Formation*	1976 [a]
15	*Cancer Incidence in Five Continents*, Vol. III	1976 [a]
16	*Air Pollution and Cancer in Man*	1976 [a]
	Methods of Cancer Registration	1976 [b]
	Pathology of Tumours in Laboratory Animals, Vol. II: *The Mouse*	1977 [b]
	Pathology of Tumours in Laboratory Animals, Vol. III: *The Hamster*	1977 [b]

[a] In press
[b] In preparation
[c] Joint publication with the French National Institute of Health and Medical Research

Table 24. Distribution of *IARC Scientific Publications* and *Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*

	Official distribution	Sales
Scientific Publications		
No. 1	657	786
2	762	1 327
3	912	781
4	888	753
5	977	916
6 *	—	—
7	973	600
8	961	805
9	917	748
10	950	825
11 — Part 1	1 019	620
11 — Part 2	1 018	620
12	880	494
Monographs Series		
Vol. 1	2 597	1 931
2	1 735	1 659
3	1 777	1 608
4	1 589	1 414
5	1 744	1 325
6	1 576	1 310
7	1 851	1 189
8	1 752	956
9	1 780	924
10	1 657	797
11	1 690	504

* Not yet published.

8. SYMPOSIA

In collaboration with the French National Institute of Health and Medical Research, a very successful symposium on environmental pollution and carcinogenic risks was held at the Agency from 3–5 November 1975. The Agency was honoured by the presence of Madame Simone Veil, French Minister of Health, who presided at the opening ceremony. Madame Veil afterwards visited the Agency's laboratories and talked with staff members.

The symposium brought together representatives of all groups concerned with the cancer hazard posed by pollution of the environment: academic, governmental, industrial and trade union. The general feeling of the participants was of a successful fulfilment of the aim of the symposium: to enable representatives of industry and trade unions to understand better the possibilities and limitations of scientists and to enable the scientists to understand better what industrialists and trade union members, in turn, expect of them.

9. COORDINATING COMMITTEE FOR HUMAN TUMOUR INVESTIGATIONS

Plans for the Seventh Symposium on the Biological Characterization of Human Tumours, to be held in Budapest from 13–15 April 1977, are well under way. The programme will include meetings of study groups and discussions on the molecular basis of viral and chemical carcinogenesis, the chemotherapy of solid tumours and breast cancer.

6. INTERDISCIPLINARY PROGRAMME AND INTERNATIONAL LIAISON UNIT

Dr C. A. LINSELL (Chief)

1. INTRODUCTION

In addition to the maintenance of external liaison with the WHO units concerned with cancer, both at the Headquarters in Geneva and at regional offices, official liaison has been established with the World Bank and with the Food and Agriculture Organization of the United Nations (FAO). Interest in the closest liaison with the Agency has been expressed by the Food Policy and Nutrition Division of FAO, since the evaluation of carcinogenic risk of items in the diet of rural populations in developing countries is of major interest to that organization.

The Eastern Mediterranean Regional Office of WHO was visited by Dr Linsell and Dr Griciute for consultations on collaborative studies on bilharzia. The European Regional Office of WHO in Copenhagen was the site of the annual meeting of the WHO Interdisciplinary Team on Cancer. The Director and Dr Linsell attended the Conference on the Development of an Informational System for Coordination of Cancer Research, held in Moscow in December 1975, followed by a symposium at the International Institute for Applied Systems Analysis at Laxenburg in Austria, where the cancer component of the medical programme of the Institute was discussed.

Liaison and programme coordination with the International Union Against Cancer (UICC) continued; and an interdisciplinary programme on liver cancer has been established.

2. IMMUNOLOGY

2.1 *Standardization of α-fetoprotein* (Dr P. Sizaret)

The reference sample of α-fetoprotein (AFP) prepared at the Agency has been accepted by the WHO Expert Committee on Biological Standardization as the international standard, and the Agency has accepted the responsibility of providing it to national laboratories. AFP is a marker for prenatal diagnosis of neural tube defects and for liver-cell cancer. A collaborative study has confirmed that the Agency standard is suitable for assay of AFP in amniotic fluid or sera from mothers whose offspring are at risk for such congenital malformations.

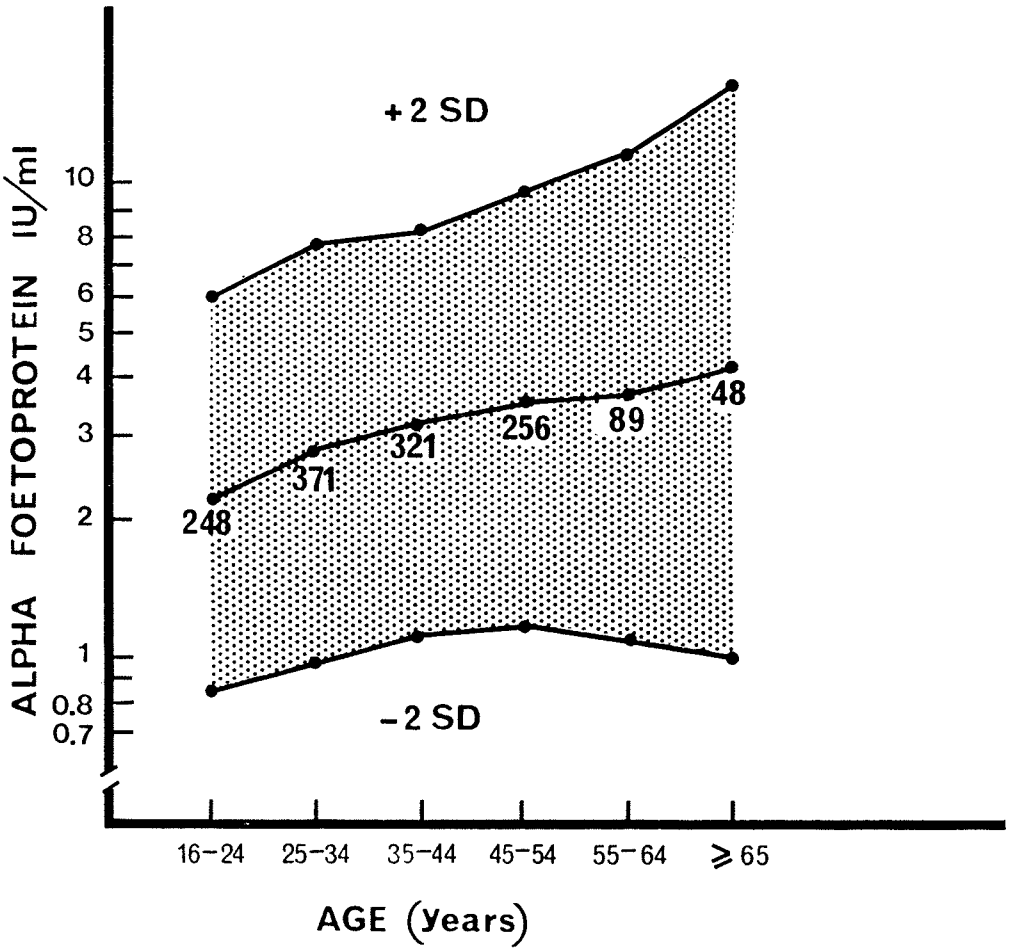
2.2 *Studies on Concanavalin-A α-fetoprotein variants* (Miss C. Dambuyant)

This programme has now been concluded: it has been shown that the various fractions of the sepharose 4B/Concanavalin-A complex are immunologically identical [1].

2.3 *α-Fetoprotein values in normal persons* (Dr A. Tuyns and Miss N. Martel)

Mean AFP levels in normal males over the age of 15 were established by numerous assays in control groups. It was found that such levels increase with age [2]. Standardization of the normal range in international units should facilitate evaluation of comparative studies on AFP (Fig. 23).

Fig. 23 Mean α-fetoprotein values in 1 333 males, by age group (± 2 S. D.)



[1] Dambuyant, C. (1974) Thesis, Claude Bernard University, Lyon, France.
[2] Sizaret, P., Martel, N., Tuyns, A., Jouvenceaux, A., Levin, A., Ong, Y. W., Rive, J. & Reynaud, S. (1976) *Digestion* (in press).

2.4 *β-1-Specific glycoprotein*

Following a report [1] in which the presence was described of the β-1 pregnancy specific glycoprotein (B1 SP1) in the serum of patients with trophoblastic tumours, the Agency organized a meeting in Lyon in November 1975. The participants were as follows:

Professor K. D. Bagshawe, Charing Cross Hospital, London (Chairman of the Committee on Trophoblastic Tumours of the European Organization for Research on Treatment of Cancer)
Dr P. W. Becker, Behringwerke AG, Marburg-Lahn, Federal Republic of Germany
Dr R. M. Lequin, Catholic University, Nijmegen, The Netherlands
Professor Y. S. Tatarinov, 2nd Moscow Medical Institute, Moscow

Two collaborative studies were established:

(1) A retrospective study to verify the specificity of the B1 SP1 in hydatidiform moles and choriocarcinoma. The sensitivity, also, of this marker will be compared with that of human chorionic gonadotrophin hormone. The reagents used for the radioimmunoassay have been elaborated by Drs Bohn and Becker of Behringwerke AG. Sera for this study have been obtained from France, The Netherlands, the UK and the USSR.

(2) A prospective study to assess the value of B1 SP1 identification in follow-ups of patients with hydatidiform moles and chorioepithelioma. The relative rarity of these tumours requires that the results of different series be pooled, and the Agency is coordinating the collection of sera from such patients with Professor K. D. Bagshawe (Charing Cross Hospital, London), Professors S. Saez and B. Lanèche (Léon Berard Centre, Lyon) and Professor N. Trapeznikov (Cancer Research Centre, Moscow).

If these studies show a suitable degree of specificity and sensitivity, the Agency will consider preparing a reference standard. With this in mind, the Agency has initiated the collection of sera from pregnant women by a number of colleagues in Lyon. This material could also be used for standardizing other proteins of placental origin, such as α-2 pregnancy-associated globulin, if its value in the follow-up of breast and other cancers is confirmed.

2.5 *Oncofetal antigens in rat liver cell lines* (Dr T. Yokota, in collaboration with the Unit of Chemical Carcinogenesis)

Dr T. Yokota, of the Fukushima Medical College of Japan, currently holds a Corvissiano fellowship awarded by the Agency. He is examining chemically transformed rat-liver cells for oncofetal antigens other than α-fetoprotein. Preliminary results indicate that IARC 6–1 (a non-AFP producing rat liver-cell line originally transformed by nitrosodimethylamine) produces an antigen which is associated with malignancy. The precise identity of this antigen is currently being determined.

2.6 *Cell-mediated immunity studies* (Dr A. G. Levin, IARC Research Centre, London)

(*a*) *Cell, tissue and serum bank*

A central collection of cells and sera from cancer patients and controls in Africa, India and the United Kingdom has been established at the Clinical Research Centre (CRC)

[1] Tatarinov, Y. S. (1974) *Int. J. Cancer*, **14**, 548–554.

of the Medical Research Council, London. Laboratory and office accommodation and storage space for the bank have generously been provided by the Centre. A technician and part-time secretary have been engaged. The coding and information retrieval system has been adapted for the computer system at the CRC, and there are currently 1 875 individual specimens of cells and sera in the collection, together with details of the clinical state of the patient. The serum specimens in the central collection in London have been divided into small aliquots to provide material for investigations by a number of collaborators in the CRC and other laboratories in London. Collection and preservation of cells, tissue and sera continues in Nairobi and Bombay; for the moment this is limited to specimens from the cancer patients in the follow-up programme to assess cell-mediated immunity after various treatment regimens.

(*b*) *Laboratory studies*

Aliquots of lymphocytes stored for over 3½ years have been tested at two laboratories (the CRC in London and the Medical Research Council Population and Genetics Unit in Edinburgh) for cell viability, rosette formation and response to stimulation with mitogens and lymphoblastoid cell lines. The tests, carried out by micro methods on aliquots of two to four million cells, showed the cells to be viable and stimulatable.

Sera and tumour tissue have been sent to Professor R. Baldwin (University of Nottingham, UK) for preparation of tumour-specific material for use in tests of cell-mediated immunity. Professor W. Bodmer (Department of Biochemistry, University of Oxford, UK) is testing sera for HLA antibodies and frozen lymphocytes for HLA antigens.

Analysis continues of baseline studies on the incidence of AFP, hepatitis B antigens and carcino-embryonic antigen (CEA) in East African patients and controls. Many of the African control samples used for previous studies of these markers have been obtained from blood banks, and it has now also been possible to obtain material from rural populations for comparison. Preliminary analysis suggests that there is little difference between the two populations as far as AFP, CEA and hepatitis B antigen and antibody are concerned. It is proposed to establish standard cell-mediated immunity testing of the material from Nairobi and Bombay in the laboratory of Dr Stella Knight in the Division of Surgical Sciences of the CRC, which specializes in research on cell-mediated immunity in preserved lymphocytes. Collaboration has also been established with Mr A. Kark, Head of the Oncology Unit of the Division of Surgical Sciences of the CRC, who will make available further material for comparative studies of cell-mediated immunity of cancer patients in London, Nairobi and Bombay.

3. PRENATAL EVENTS AND CHILDHOOD CANCER (Dr N. Muñoz, Dr L. Tomatis

and Dr N. E. Day, in collaboration with Professor K. H. Degenhardt, Institute for Human Genetics, Frankfurt/Main, Federal Republic of Germany; Dr C. Rumeau-Rouquette, National Centre for Scientific Research, Villejuif, France; Dr N. Wald, Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford, UK; Dr J. F. Bithell, Childhood Cancer Research Group, University of Oxford, Oxford, UK; Professor E. V. Kuenssberg, Royal College of General Practitioners, Edinburgh, UK; Professor L. Saxén, Department of Pathology, University of Helsinki, Helsinki;

Dr K. Koppe, Ziekenhuis Academy, Amsterdam, The Netherlands; Dr A. Czeizel, State Institute of Hygiene, Budapest; and Dr G. Fara, Department of Hygiene, Faculty of Medicin and Surgery, University of Milan, Milan, Italy)

The Agency is coordinating material from prospective studies designed to assess the role of prenatal events in the incidence of congenital malformations. Although these studies may individually yield sufficient numbers to study the role of prenatal events and congenital malformations, the relative rarity of cancer in children makes it necessary to pool the data for correlation with the occurrence of cancer. The studies record information on a number of potential dangers of pregnancy, specifically, evidence of viral diseases, diagnostic procedures, medical treatment and sociological factors. Identification of cases of cancer among offspring in these study groups is under way. Evaluation of the study was carried out at a workshop in Freudenstadt, Federal Republic of Germany, in September 1975, and Table 25 summarizes the data obtained to date.

The pregnancy records of mothers whose children have developed cancer are sent to the Agency for translation and circulation to the participating investigators. Serum samples were collected during pregnancy in 7 of the 14 cases of cancer detected so far. If a prenatal factor can be implicated, then appropriate tests to compare the cancer cases with controls will be elaborated.

Table 25. Collaborative, prospective study on childhood cancer (preliminary results)

Country	No. of pregnancies	Study period	No. of cancers		Serum collected
			Expected	Observed	
France	12 000	1963–68	11.0	8	4
Federal Republic of Germany	12 000	1964–73	9.0	5	3
UK					
Birmingham*	15 000	1964–65	15.0	–	–
Dundee*	15 000	1965–67	15.0	–	–
Oxford*	10 000	1972–76	1.4	–	–
Finland*	40 000	1969–72	15.0	–	–
Hungary	1 200	1962–63	1.0	1	–
Italy*	18 000	1974–	1.0	–	–
The Netherlands	1 000	1974–	0	–	–
TOTAL	144 200		68.4	14	7

* Study in progress

4. LIVER CANCER (Dr C. A. Linsell and Dr N. Muñoz)

An interdisciplinary programme has been established to evaluate field studies on the etiology of liver cancer. The association of liver cancer in some countries of Africa and Asia with aflatoxin levels has been demonstrated, and further epidemiological studies to monitor improved crop storage are being considered. The exposure levels which have been calculated in the Agency's studies in Kenya and Swaziland can be used to evaluate such projects for improved cereal storage [1]. The feasibility of using these sites is being explored.

[1] Peers, F. G., Gilman, G. A. & Linsell, C. A. (1976) *Int. J. Cancer*, **17**, 167–176.

Epidemiological evidence suggests that chronic infection with the hepatitis B virus (HBV) may also be a high risk factor for the development of liver cancer. This persistent infection can be detected serologically by the presence of the HB surface antigen. The possibility of follow-up studies on carriers and controls is also under review. It will be necessary to pool the information from the follow-up of population-based studies on HBV and from the extensive programmes for the detection of carriers among blood donors in a number of tropical countries.

The Agency is also considering studies, including intervention trials, to determine the combined roles of aflatoxin and HBV in the development of liver cancer. The major problem, as always in studying the etiology of a cancer in a developing country, is the establishment of an effective registration of cancer patients.

7. IARC RESEARCH CENTRE, NAIROBI

Professor A. WASUNNA (Head)

Professor Ambrose Wasunna, Professor and Chairman of the Department of Surgery of the Nairobi Medical School, has agreed to administer this research centre. In addition to offering assistance to IARC staff and projects in Eastern Africa, Professor Wasunna wishes to develop regional cancer research programmes. Liaison has been established with other research institutes and clinical departments in East Africa, and a programme involving clinical and epidemiological cooperation is being explored.

The cell, tissue and serum bank programme continues in Nairobi under Mrs Josephine Safari. Over 100 cancer patients at the Kenyatta National Hospital are under observation, and specimens of lymphocytes and serum are obtained at regular intervals. To assess the effects of varying treatment regimens and to preserve small aliquots of cells, fresh and preserved cells are checked by immunological studies in the Nairobi laboratory. Expansion of this programme under the supervision of the Department of Surgery and the Wellcome Trust Laboratories, using the micro techniques which have been developed both in Nairobi and London, is currently under discussion.

The Nairobi Research Centre continues to assist the studies in the Shirati area of Tanzania on families with multiple tumours.

Oesophageal cancer (Professor A. Wasunna)

A questionnaire for use in a case-control study has been established in Nairobi with the cooperation of the WHO Epidemiology Unit, and a preliminary trial has been established at the Kenyatta National Hospital. Areas of varying cancer incidence have been identified in Kenya, and it is planned to involve hospitals in these areas in a major case-control study. Professor Wasunna attended the IARC Workshop on Oesophageal Cancer among the Turkomans in East Iran in May 1976; this workshop was attended by experts from Africa and Europe as well as Iran, with the hope of developing collaborative studies with common protocols. The Nairobi Research Centre will cooperate in an assessment of the pathology of oesophageal cancer and possible precancerous lesions of the oesophagus.

8. IARC RESEARCH CENTRE, SINGAPORE

Professor K. SHANMUGARATNAM (Head)

1. THE SINGAPORE CANCER REGISTRY (RA/67/009)

Principal investigator: Professor K. Shanmugaratnam
Supporting staff: 3

Population-based cancer registration for the Republic of Singapore has been successfully maintained. A five-year analysis (1968–1972) of cases among the Chinese, Malay and Indian populations in Singapore is included in Volume III of *Cancer Incidence in Five Continents* [1].

The Registry continues to provide basic epidemiological data for on-going immunological and immunogenetic investigations on nasopharyngeal cancer and liver cancer, in collaboration with the Singapore General Hospital and the WHO Immunology Centre in Singapore. The Registry has also collaborated in studies on histologic-specific incidence rates for cancers of the lung, breast and gonads. A population-based histological study of lung cancer has been published [2].

2. IMMUNOGENETIC AND IMMUNOLOGICAL STUDIES ON NASOPHARYNGEAL CARCINOMA

Principal investigators: Dr M. J. Simons and Dr S. H. Chan
Supporting staff: 6

The association between the HLA phenotype A2-BSin2 and nasopharyngeal carcinoma in Chinese patients can now be regarded as established. There is preliminary evidence of an association between another HLA phenotype in association with the disease in the medium incidence population of Malays. Moves towards the establishment of a Ia typing programme are satisfactory, and the frequency of B-cell reacting sera suggests that typing reagents should be available within the next two to three months.

The leucocyte adherence assay has been successfully adapted to the detection of cell-mediated immunity to Epstein–Barr virus antigens. Counterimmunoelectrophoresis has

---

[1] Waterhouse, J. A. H., Muir, C. S, Correa, P. & Powell, J., eds (1976) *Cancer Incidence in Five Continents*, Vol. 3, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 15**) (in press).

[2] Law, C. H., Day, N. E. & Shanmugaratnam, K. (1976) *Int. J. Cancer*, **17**, 304–309.

been successfully employed in the detection of Epstein–Barr virus-positive lymphoblastoid cell line antigens and corresponding antibodies. Difficulties are being experienced in the establishment of radioelectrocomplexing to this system, but further approaches are being pursued.

It is expected that joint immunogenetic-immunological studies of nasopharyngeal carcinoma patients and their families can be undertaken within the next four to six months, as scheduled in the research programme.

2.1 *Immunogenetics*

Further progress has been made in analysing the association between HLA gene type and nasopharyngeal carcinoma. A study of 110 Singapore Chinese nasopharyngeal carcinoma patients and 91 negative (clinically suspected, but not histopathologically confirmed) controls provided confirmation of the association between an increased risk for nasopharyngeal carcinoma and the HLA genes, HLA-A2 and Singapore 2 (BSin2) [1]. The risk was found to be restricted to the co-occurrence of A2-BSin2, suggesting that the genotype which predisposed to the development of nasopharyngeal carcinoma was the A2-BSin2 haplotype. Studies of Chinese patients in Malaysia and Hong Kong have revealed a similar association [2], indicating that the increased risk for nasopharyngeal carcinoma associated with the A2-BSin2 phenotype is a feature common to Asian Chinese in at least three geographic locations.

On the basis of these results, we have proposed the existence of a nasopharyngeal carcinoma-disease susceptibility (DS) gene or genes in linkage disequilibrium with A2-BSin2, both of which carry a high risk for nasopharyngeal carcinoma and are found predominantly among southern Chinese. Two new findings bear out this hypothesis. Firstly, the linkage disequilibrium between A2 and BSin2 is twice as high in Cantonese as in Hokkien/Teochew controls ($\Delta = 0.053$ and 0.025, respectively). This difference parallels the two-fold higher incidence of nasopharyngeal carcinoma in Cantonese. Furthermore, among patients with this tumour, Cantonese show a greater linkage disequilibrium for the A2-BSin2 phenotype than do Hokkiens/Teochews ($\Delta = 0.126$ and 0.102, respectively). Secondly, evidence that the high risk HLA type is associated with disease susceptibility emerges from an examination of new cases among whom the relative risk conferred by A2-BSin2 is 3.4. In survivors of five years or longer, the relative risk associated with A2-BSin2 is 2.3, suggesting that the HLA type also confers a lower resistance to the course of the disease and is a marker of poor prognosis.

BSin2 has been detected only among mongoloid populations, and only Chinese have a disequilibrium between A2 and BSin2. It is therefore unlikely that these genes would be associated with nasopharyngeal carcinoma in other ethnic groups. If nasopharyngeal carcinoma is a single disease, and if there is a DS locus allele common to the majority, if not all, of nasopharyngeal carcinoma patients, other HLA genes can be expected to be associated with the disease in non-Chinese groups. We have hypothesized that HLA genes

---

[1] Simons, M. J., Wee, G. B., Goh, E. H., Chan, S. H., Shanmugaratnam, K., Day, N. E. & de-Thé, G. (1976) *J. nat: Cancer Inst.* (in press).

[2] Simons, M. J., Wee, G. B., Singh, D., Dharmalingam, S., Yong, N. K., Chau, J. C. W., Ho, J. H. C., Day, N. E. & de-Thé, G. (1976) In: Henderson, B. E., ed., *Proceedings of a Symposium on Epidemiology and Cancer Registries in the Pacific Basin, Hawaii, 1975* (in press).

in linkage disequilibrium in normal subjects of the medium-incidence ethnic group, such as the Malays, will be in even greater linkage disequilibrium among nasopharyngeal carcinoma patients of that ethnic group. Among Malay patients, locus A allele A9 and locus B BW15 occur frequently. There is no linkage disequilibrium between A9 and BW15 ($\triangle = 0.003$). By contrast, the delta value for A9 and B18 is 0.09. HLA studies of normal Malays, currently in progress, revealed a delta value for A9 and B18 of 0.019. This finding is consistent with the hypothesis and suggests that the association between HLA type and nasopharyngeal carcinoma among Malays involves a haplotype different from that in Chinese. To further characterize the HLA loci A and B associations in Chinese and Malays, studies involving the typing of approximately 100 Cantonese, 100 Hokkiens/Teochews and 100 Malays are in progress. To date, more than 60 subjects in each of these groups have been HLA-typed.

The stronger association between the locus B allele Singapore 2 (BSin2) and nasopharyngeal carcinoma suggests that the putative DS genes may be located along the chromosome toward the B locus side. The D locus controlling mixed-lymphocyte reactions is located in this region. In addition, recent developments indicate that a B lymphocyte alloantigenic polymorphism may also be controlled by genes in the region of the D locus. Our attempts to identify the nasopharyngeal carcinoma DS genes are therefore increasingly directed towards typing for alleles of these two loci.

Mixed-lymphocyte reaction typing among family members has been established using a microculture technique measuring both ^{3}H-thymidine and ^{75}SeMe uptake. However, the results are not easily interpretable on a single gene basis. Furthermore, there are practical difficulties in obtaining blood samples from A2-BSin2 homozygous individuals, some of whom are suspected to be homozygous for the D locus allele that is designated Singapore 2a. In an attempt to overcome the latter problem, lymphocytes from A2-BSin2 homozygous nasopharyngeal carcinoma patients and normal subjects have been established as lymphoblastoid cultures by Dr W. Liebold (Hanover, Federal Republic of Germany). The next stage involves evaluation of the suitability of these cells as D locus typing reagents.

In February, a major programme of gene typing of immune response-associated genes (Ia) was initiated. To date, 1 005 delivery sera have been collected and screened against total lymphocytes and separated B cells. Of the 360 sera tested, 28 (7.7%) showed only B cell reactivity. An additional 16 sera (4.5%) showed both anti-HLA and anti-B cell activities. It thus appears likely that B cell typing reagents will be identified by this approach. In order to expedite the identification of sera containing antibodies directed against B cell alloantigens associated with nasopharyngeal carcinoma, the sera are being screened with B cells from patients and will be screened against the lymphoblastoid lines. The screening programme is also expected to identify new sources of anti-Sin2 typing reagents.

2.2 *Immunology*

Working at the National Cancer Institute (USA), Dr S. H. Chan (under an IARC Research Training Fellowship) has adapted a leucocyte adherence inhibition assay for cell-mediated immunity testing. In Singapore, emphasis has been placed on the adaptation of counterimmunoelectrophoresis and radioelectrocomplexing to the detection of Epstein–Barr virus antigens and antibodies.

(a) Leucocyte adherence inhibition assay for detection of cell-mediated immunity

The response to purified protein derivative in the leucocyte adherence inhibition assay in individual normal subjects was compared to their past responses to skin tests performed within the last two years. Seven (30%) of 23 subjects gave a positive response to the two tests, and 11 (48%) were negative to both tests (Table 26) [1]. There were five (22%) individuals who gave a positive response in the leucocyte adherence inhibition test but had negative responses to the skin test. No individuals who were negative in the leucocyte adherence inhibition test showed positive skin tests. These findings were consistent with the interpretation that the former assay detects cell-mediated immunity and may be more sensitive than the *in vivo* Tine test.

Table 26. Correlation between leucocyte adherence inhibition (LAI) and skin testing with purified protein derivative (PPD)

		LAI response to PPD		
		+	—	
Skin test response (Tine test)	+	7	0	
	—	5	11	
Total				23

This assay has several advantages over others: only small amounts of antigen (10^{-4}-10^{-5} μg/well) are used; only small amounts of peripheral blood (2 ml) are required; the reaction time is short (2 hours); sterile conditions are not required. In addition, the antigen plates could be prepared in advance and stored at —70°C; and antigen and/or fixed plates are easily transported after completion of the assay for comparison between collaborators.

This assay has been applied at the National Cancer Institute (USA) to studies of response of breast cancer and lymphoma patients to viral and tumour-associated antigens[1, 2].

Studies have been initiated in Singapore to investigate the cell-mediated immunity responses of nasopharyngeal carcinoma patients to Epstein–Barr virus- and nasopharyngeal carcinoma-associated antigens using the leucocyte adherence inhibition assay.

(b) Counterimmunoelectrophoresis and radioelectrocomplexing for Epstein–Barr virus antigen and antibody

This programme has two objectives: firstly, to detect Epstein–Barr virus antibody, both in the free state and complexed to antigen as soluble antigen/antibody complexes; and, secondly, to determine the relative avidity of Epstein–Barr virus antibody. The former objective is based on the likelihood that Epstein–Barr virus antibody is present as an immune complex and that detection of this antibody requires identification of both free

[1] Chan, S. H., Wallen, W. C., Levine, P. H., Periman, P. & Perlin, E. (1976) *Int. J. Cancer* (in press).

[2] Chan, S. H., Wallen, W. C., Levine, P. H. & Soares, N. (1976) In: *Proceedings of the 3rd International Symposium on Detection and Prevention of Cancer New York, 1976* (in press).

and complex antibody. The second objective is based on the likelihood that the immunogenetic association with nasopharyngeal carcinoma is a manifestation of an immunodeficiency and that any immunodeficiency may be a qualitative rather than a quantitative deficiency.

EDTA shockates of lymphoblastoid cells provided by Dr M. H. Ng served as the source of antigens. Heteroimmune sera to these antigens, also provided by Dr Ng, were used as antibody. No antigen-antibody reactions were observed in immunodiffusion, even after staining; however, clear immunoprecipitates were obtained on counterimmunoelectrophoresis with these antigens. The antibody was detected in the sera of all four rabbits tested, higher titres being observed in two of them. These results indicate that the shockates contain an anodally migrating antigen or antigens, thereby meeting the prerequisite for adaptation to radioelectrocomplexing. To date, two ^{125}I-labelled preparations of antigens have been assessed, but the electrophoretic migration of both was poor. It is hoped that preparative electrophoresis of ^{125}I-labelled antigenic preparations may overcome this problem.

3. LUNG CANCER IN SINGAPORE CHINESE (Dr J. L. DaCosta and Dr Y. K. Ng) (see p. 41)

9. IARC RESEARCH CENTRE, TEHERAN

Dr B. ARAMESH (Head)
Dr J. KMET (IARC Team Leader)
Miss Paula COOK, British Medical Research Council (Consultant)
Professor T. HEWER, University of Bristol, UK (Consultant)
Mrs L. BANISADRE (Administrative Assistant)

Collaborating Institutes:

Institute of Public Health Research, University of Teheran (Director, Dr A. Nadim)
Tadj Pahlavi Cancer Institute, University of Teheran (Director, Dr A. Mojtabai)

1. CASPIAN CANCER REGISTRY

The work of the Caspian Cancer Registry has continued (RA/70/024), and data up to June 1974 have been received and analysed. Verification of the denominator data awaits the results of the 1976 census, after which an analysis of time trends is envisaged.

2. OESOPHAGEAL CANCER STUDIES

2.1 *Study of cases, controls and their households*

Interviewing of oesophageal cancer cases, controls and their households [1] finished at the end of Iranian year 1354 (21 March 1976). The target numbers and the numbers actually interviewed are given in Table 27. While nearly 80% of oesophageal cancer patients were traced to their homes, many were difficult to interview due to their illness; 53% of all cases were interviewed with two controls. For other forms of cancer the corresponding figures were 60% and 34%.

Preliminary analyses of the data indicate that it is highly unlikely that the study will incriminate sheep's milk or yoghurt or the chewing of *nass* as significant risk factors. Nor is there likely to be any epidemiological evidence of the possible carcinogenicity of bread in the high incidence areas, since this is a universal item of diet. The only factors which appear positively to be associated with the disease are a lower socio-economic status and a more restricted diet (Tables 28 & 29). Analysis of this extensive material continues.

[1] International Agency for Research on Cancer (1975) *Annual Report, 1975*, Lyon, p. 116.

Table 27. Numbers of patients with oesophageal cancer (OC) and with other cancers (Other) registered between March 1975–March 1976, by district, together with the proportion of such persons traced and followed to their homes and the proportion interviewed for whom the controls were also interviewed

	Gorgan & Gonbad		Babol [a]		Rasht		Ardebil		All regions [a]	
	OC	Other	OC	Other	OC	Other	OC	Other	OC	Other
Number of patients registered	310	141	180	158	134	239	78	67	702	605
% of patients followed up [b]	82.6	59.1	68.0	46.8	82.4	61.1	64.9	63.1	77.2	59.3
% of patients interviewed with two controls [c]	67.4	39.5	41.9	30.6	49.3	37.9	23.4	23.8	53.0	34.6

[a] Excludes the areas of Shahsavar and Nowshahr
[b] % of total number of patients registered
[c] % of total, minus cases excluded from study

Table 28. Owners of sheep (as an indication of socio-economic status[a]) among patients with oesophageal carcinoma and among controls [b]

Region	Males	Females	Both sexes
Gorgan and Gonbad	12 : 5	9 : 3	21 : 8
Babol	8 : 4	2 : 1	10 : 5
Rasht	1 : 0	0 : 0	1 : 0
Ardebil	6 : 1	1 : 1	7 : 2
All regions	27 : 10	12 : 5	39 : 15

[a] Ownership of sheep is not only an index of socio-economic status but also an index of the consumption of sheep's milk. Other socio-economic indications such as ownership of land show the same trend.
[b] In each pair of numbers, the first corresponds to the number of times the average value for the two controls was greater than the value for the case and the second, the number of times the value for the case was greater.
Overall $\chi^2 = 10.7$ $P = 0.001$

Table 29. Consumers of raw green vegetables (salad) among patients with oesophageal carcinoma and among controls [a, b]

Region	Males	Females	Both sexes	
Gorgan and Gonbad	22 : 9	10 : 7	32 : 16	$\chi^2 = 5.3$ $P = 0.03$
Babol	14 : 10	4 : 5	18 : 15	
Rasht	5 : 7	8 : 2	13 : 9	
Ardebil	5 : 7	1 : 3	6 : 10	
All regions	46 : 33	23 : 17	69 : 50	

[a] Many other dietary items show a similar trend. It is of particular interest that the low intake among cases is more marked in the high incidence (Turkoman) region.
[b] In each pair of numbers, the first corresponds to the number of times the average value for the two controls was greater than the value for the case and the second, the number of times the value for the case was greater.
Overall $\chi^2 = 3.0$

2.2 *Search for environmental carcinogens*

As noted in previous reports, the levels of volatile nitrosamines, polycyclic aromatic hydrocarbons and aflatoxins in diet are low and are substantially similar in high and low incidence areas. As no other item appears to be incriminated, attention has been focused on bread, the staple food of the high incidence area.

As bread itself is clearly not carcinogenic, its contaminants have been studied, in particular, fungi and their associated toxins and extraneous seeds. A possible role of mycotoxins is suggested by the mycotoxicosis observed in Kazakhstan, USSR, before the war, which was associated with over-wintered wheat. The toxin responsible was produced by a species of *Fusarium*, and in animal feeding experiments it produced non-malignant oesophageal lesions. Samples of wheat from the Turkoman area were sent to the Commonwealth Mycology Institute, London (Dr C. Booth) and to Rothamstead Experimental Station, Harpenden (Professor J. Lacey). These were cultured, and the resultant fungi were identified: *Alternaria triticine* was found in many of the samples, and *Aspergillus restrictus* was found as a heavy contaminant of wheat stored in traditional underground pits.

Wheat samples were examined at the official Seed Testing Station for England and Wales in Cambridge, UK, and seeds of over 50 adventitious species were identified, five of which occurred very frequently. The significance of these results remains to be assessed. Professor T. Hewer (University of Bristol, UK) is conducting a field botanical survey in the region to assess plant usage and wheat contamination.

Dr M. Shariaty (Tadj Pahlavi Cancer Institute, University of Teheran) spent three months at the Agency to become acquainted with techniques of mutagenicity testing; he is now examining bread, tea and sheep's milk. If indicated, animal feeding studies will be undertaken.

2.3 *Laboratory population studies*

The Iranian authorities have decided to undertake longitudinal health surveys in the area of the Caspian littoral where oesophageal cancer incidence is low (Gurab-Zamikh in the province of Gilan) and in another where the incidence is high (Gonbad). It is hoped the latter will be extended to include the more traditional Moraveh-Tappeh/Golidagh region largely inhabited by Turkomans.

The Institute of Public Health Research, which is organizing these surveys, will conduct a variety of studies, several of which are relevant to the oesophageal cancer problem.

(*a*) *Nutrition*

The finding of the quantitative food intake study [1] that the Turkoman diet is unusually limited in range is to be explored further. Biochemical and clinical studies of nutritional status will be conducted to determine whether there is physiological evidence of deficiencies of those nutrients for which the food consumption study suggested a very low intake (Dr H. Ghasemi, Dr S. Vaghefi and Professor D. S. MacLaren). Biochemical assays will be performed for the presence of vitamin A and the carotenoids, riboflavin, pyridoxine,

[1] International Agency for Research on Cancer (1974) *Annual Report, 1974*, Lyon, p. 104.

vitamin C, protein and serum amino acids, lipids, zinc, iron and selected trace elements. These studies will be complemented by further, more qualitative studies of dietary intake and food utilization on a year-round basis.

(*b*) *Genetic studies*

These include observations of family types, inbreeding patterns, twins occurrence, malformations, constitutional types, blood groupings, protein systems and cell enzymes (Dr D. Farhud).

(*c*) *Immunogenetic studies and relation between nutrition and immune response*

HL-A typing of population samples and oesophageal cancer patients is being undertaken (Dr F. Modabber and Dr N. Mohagheghpur), in addition to studies of T and B cell populations among oesophageal cancer patients.

(*d*) *Histopathological studies*

The morphology of the oesophagus adjacent to neoplasms in patients from the Gorgan area will be examined (Dr S. Sadeghi and Dr K. Dowlatshahi).

Annex 1

PARTICIPATING STATES AND REPRESENTATIVES AT THE FIFTEENTH SESSION OF THE IARC GOVERNING COUNCIL 29–30 APRIL 1976

Australia

Dr R. W. CUMMING *(Rapporteur)*
Assistant Director-General of Health
International Health Branch
Australian Department of Health
Canberra, A.C.T.

Mr J. RAVENSCROFT
Director, Australian Treasury Office
Australian Permanent Mission
Geneva, Switzerland

Belgium

Professor S. HALTER *(Chairman)*
Secretary-General
Ministry of Public Health and the Family
Brussels

Dr G. CLAUS
Director-General
Ministry of Public Health and the Family
Brussels

France

Professor E. J. AUJALEU
Honorary Director-General
National Institute of Health and Medical Research
Counsellor of State
Paris

Dr J. F. DUPLAN
Director of Research
National Institute of Health and Medical Research
Bordeaux

Federal Republic of Germany

Mr H. VOIGTLÄNDER
International Relations Section
Federal Ministry for Youth, Family Affairs, and Health
Bonn

Dr H. KAISER
Ministerial Council
Ministry of Finance
Bonn

Japan

Dr A. TANAKA
Ministry of Health and Welfare
Tokyo

Mr T. ONISHI
First Secretary
Permanent Delegation of Japan to the International Organizations in Geneva
Geneva, Switzerland

The Netherlands

Dr J. SPAANDER
Director-General
National Institute of Public Health
Bilthoven

Union of Soviet Socialist Republics

Professor N. BLOKHIN
Director, Cancer Research Centre
Academy of Medical Sciences
Moscow

Dr N. N. FETISOV
Deputy Chief, External Relations
USSR Ministry of Public Health
Moscow

Dr A. A. KLIMENKOV
Cancer Research Centre
Academy of Medical Sciences
Moscow

United Kingdom

Sir JOHN GRAY
Secretary, Medical Research Council
London

Dr Katherine LEVY
Medical Research Council
London

United States of America

Dr F. J. RAUSCHER
Director, National Cancer Institute
National Institutes of Health
Bethesda, Md.

Mr R. F. ANDREW
Director, Agency for Health and Drug Control
Department of State
Washington, D.C.

Dr M. D. LEAVITT *(Vice-Chairman)*
Director, Fogarty International Center
National Institutes of Health
Bethesda, Md.

World Health Organization

Dr T. A. LAMBO
Deputy Director-General

Mr W. W. FURTH
Assistant Director-General

Mr F. GUTTERIDGE
Director, Legal Division

Mr J. DONALD
Associate Chief, Finance and Accounts

Observer

Professor G. L. ADA
Outgoing Chairman of the IARC Scientific Council

Annex 2

MEMBERS OF THE SCIENTIFIC COUNCIL AT ITS TWELFTH SESSION, 8–9 JANUARY 1976

Professor G. L. ADA *(Chairman)*
Department of Microbiology
The John Curtin School of Medical Research
The Australian National University
Canberra

Professor Z. M. BACQ
Department of Physiopathology
Medical Faculty
State University
Liège, Belgium

Professor A. CAPUTO
Director, Regina Elena Institute for Cancer Research
Rome

Sir RICHARD DOLL
Regius Professor of Medicine, Oxford University
Radcliffe Infirmary
Oxford, UK

Dr J. F. DUPLAN
Director of Research
National Institute of Health and Medical Research
Bordeaux, France

Dr S. ECKHARDT
Director, National Institute of Oncology
Budapest

Dr T. HIRAYAMA
Chief, Epidemiology Division
National Cancer Center Research Institute
Tokyo

Professor C. MOFIDI
Professor of Human Ecology, University of Teheran
Secretary General, Central Council of Universities and Academic Institutions of Iran
Teheran

Professor K. MUNK
Director, Institute of Virology
German Cancer Research Centre
Heidelberg, Federal Republic of Germany

Professor N. N. TRAPEZNIKOV
(Vice-Chairman)
Deputy Director, Cancer Research Centre
Academy of Medical Sciences of the USSR
Moscow

Professor A. C. UPTON *(Rapporteur)*
Dean, School of Basic Health Sciences
State University of New York at Stony Brook
Stony Brook, N.Y., USA

Professor T. G. VAN RIJSSEL
Pathology Laboratory
Faculty of Medicine
National University of Leiden
Leiden, The Netherlands

Annex 3

RESEARCH AGREEMENTS IN OPERATION BETWEEN IARC AND VARIOUS INSTITUTIONS, JUNE 1975 – JUNE 1976

Support of IARC Research Centres

RA/68/002	University of Singapore (Contribution to the maintenance of an IARC Research Centre at the University of Singapore)
RA/75/020	University of Nairobi (Contribution to the maintenance of an IARC Research Centre at the University of Nairobi)

Reference centres/Serum banks

RA/67/019	Netherlands Cancer Institute, Amsterdam (Provision of tumour-bearing animals)
RA/73/029	Institute of Experimental Oncology, University of Genoa, Genoa, Italy (IARC Reference Centre for environmental carcinogenesis)
RA/73/033	Medical College, Hanover, Federal Republic of Germany (Creation of an IARC Reference Centre for environmental carcinogenesis)
RA/74/003	Institute for Documentation, Information and Statistics, German Cancer Research Centre, Heidelberg, Federal Republic of Germany (Clearing house for on-going research in cancer epidemiology)
RA/75/013	Cancer Research Centre, Academy of Medical Sciences of the USSR, Moscow (Creation of an IARC Reference Centre for environmental carcinogenesis)
RA/75/013a	Angel H. Roffo Oncological Institute, Buenos Aires (Creation of an IARC Reference Centre for environmental carcinogenesis)
RA/75/014	National Institute of Hygiene, Budapest (Creation of an IARC Reference Centre for environmental carcinogenesis)
RA/76/005	Department of Obstetrics and Gynaecology, Hôtel Dieu, Lyon, France (Supply of serum samples for the preparation of an international standard of pregnancy proteins)

RA/76/006 Minguettes Polyclinic, Vénissieux, France
(Supply of serum samples for the preparation of an international standard of pregnancy proteins)

RA/76/007 Tonkin Clinic, Villeurbanne, France
(Supply of serum samples for the preparation of an international standard of pregnancy proteins)

RA/76/009 Saint Augustin Clinic, Lyon, France
(Supply of serum samples for the preparation of an international standard of pregnancy proteins)

RA/76/010 Rillieux Polyclinic, Rillieux, France
(Supply of serum samples for the preparation of an international standard of pregnancy proteins)

Cancer registries/Incidence studies

RA/67/009 IARC Research Centre, University of Singapore
(Cancer registry at Singapore)

RA/70/024 Institute of Public Health Research, University of Teheran, Teheran
(Study on the incidence of cancer in the Caspian littoral of Iran)

RA/72/014 Department of Pathology, University of the West Indies, Kingston, Jamaica
(Partial support of the Jamaica Cancer Registry)

RA/73/016 International Association of Cancer Registries
(Provision of a secretariat and other supporting services)

RA/75/001 Department of Medicine, St Elizabeth Hospital, Copenhagen
(Study of cancer morbidity and causes of death among Copenhagen brewery workers)

RA/75/004 Birmingham Regional Cancer Registry, Birmingham, UK
(Production of Volume III of *Cancer Incidence in Five Continents*)

RA/75/016 Geneva Tumour Registry, Geneva, Switzerland
(Pilot study on determination of dietary and other habits in a sample of the population of the canton)

RA/75/017 Department of Medicine, St Elizabeth Hospital, Copenhagen
(Study of cancer morbidity and causes of death among Copenhagen brewery workers)

RA/75/019 Danish Cancer Registry, Copenhagen
(Study of cancer morbidity and causes of death among Copenhagen brewery workers)

RA/75/022 Medico-Social Research Board, Dublin
(Study of causes of death among Guinness brewery workers in Dublin)

RA/76/003 Royal College of General Practitioners, Birmingham, UK
(Record linkage from an outcome of pregnancy studies carried out in Birmingham and Dundee to the cancer registry records in Oxford)

RA/76/004 University of Helsinki, Helsinki
(Record linkage from a study of congenital malformation to cancer registry records in Finland)

RA/76/015 Geneva Tumour Registry, Geneva, Switzerland
(Participation in cost of meeting of Latin-tongued cancer registries)

RA/76/016 Medico-Social Research Board, Dublin
(Study of causes of death among Guinness brewery workers in Dublin)

Oesophageal cancer studies

RA/74/035 Biochemical Institute of Environmental Carcinogens, Hamburg, Federal Republic of Germany
(Analysis for polycyclic aromatic hydrocarbons in food samples from Iran)

RA/75/003 University of Teheran, Institute of Public Health Research, Teheran
(Joint Iran/IARC study of cases of cancer, controls and their households in the Caspian littoral of Iran)

RA/75/015 National Institute of Health and Medical Research, Division of Medico-Social Research, Le Vésinet, France
(Study of cases of oesophageal cancer and their controls in the Calvados region of France)

RA/76/011 Institute of Public Health Research, University of Teheran, Teheran
(Joint Iran/IARC botanical survey of areas of highest oesophageal cancer incidence in the Caspian littoral of Iran)

Studies on cancers linked with herpesviruses

RA/70/013 Hong Kong Anti-Cancer Society, Hong Kong
(Studies on the relationship between herpes-type infection and nasopharyngeal carcinoma)

RA/70/017 Department of Pathology, University of Singapore, Singapore
(Studies on the relationship between herpes-type infection and nasopharyngeal carcinoma)

RA/71/020 East African Virus Research Institute, Entebbe, Uganda
(Follow-up study on Burkitt's lymphoma and Epstein–Barr herpesvirus infection in the West Nile District of Uganda)

RA/72/030 Netherlands Cancer Institute, Amsterdam
(Collaborative studies on immunoserology of Burkitt's lymphoma and nasopharyngeal carcinoma)

RA/73/009 Shirati Hospital, Tarime, Tanzania
(Studies on the epidemiology of Burkitt's lymphoma in the North Mara District of Tanzania)

RA/73/013 Salah-Azaiz Institute, Tunis
(Experimental and epidemiological studies on nasopharyngeal carcinoma in Tunisia)

RA/73/017 Sainte Marie-Thérèse Clinic, Lyon, France
(Study of the role of the herpesvirus type Epstein–Barr in the establishment of permanent cell lines from cord blood specimens)

RA/73/038 University of Western Australia, Nedlands, Australia
(Studies on cell-mediated immunity to Epstein–Barr virus in patients with nasopharyngeal carcinoma)

RA/74/001 Association for the Development of Cancer Research, Primatology Laboratory, National Centre for Scientific Research, Villejuif, France
(Studies on the induction of lympho-epithelial tumours in the marmoset with Epstein–Barr virus)

RA/74/018 University of Hong Kong, Queen Mary Hospital Compound, Hong Kong
(Isolation and purification of Epstein–Barr virus specific antigens)

RA/75/002 Ross Institute, London School of Hygiene and Tropical Medicine, London
(Malaria antibody testing to be carried out by the Institute on sera from Burkitt's lymphoma studies in the West Nile District of Uganda and the Mara Region of Tanzania)

RA/75/005 Gustave Roussy Institute, Villejuif, France
(Study of the role of herpesvirus type Epstein–Barr in nasopharyngeal cancer)

RA/75/006 Lyon Blood Transfusion Centre, Plasma Desiccation Department, Histocompatibility Department, Beynost, Miribel, France
(HL-A typing of blood from families with nasopharyngeal carcinoma in Tunisia)

RA/75/007 University of Montreal, Department of Microbiology and Immunology, Montreal, Canada
(Use of antibody coupling to peroxydase for the detection of Epstein–Barr virus associated antigens)

RA/75/008 Edouard Herriot Hospital, Centre for Study and Research on Metabolic and Renal Diseases, Lyon, France
(Study on cellular immunity on the blood of nasopharyngeal carcinoma patients and their families from Tunis, Paris, Lyon and Marseille)

RA/75/009 Institute of Scientific Cancer Research, National Centre for Scientific Research, Villejuif, France
(Study of the biological activity of DNA of herpes simplex virus and Epstein–Barr virus on human lymphoblastoid lines)

RA/76/002 Zoological Society of London, London
(Development of micro-elisa test for Epstein–Barr virus serology)

Laboratory studies

RA/74/032 WHO International Reference Centre for Immunoglobulins, WHO Immunology Research and Training Centre, University of Lausanne, Lausanne, Switzerland
(Studies on the surface receptors of lymphoblastoid cell lines)

RA/74/047 Medical Research Council, London
(Contribution towards the cost of work to be undertaken by the Council's Clinical Population Cytogenetics Unit in respect of cell-mediated immunity utilizing preserved lymphocytes)

RA/75/012 University Hospital Centre, Saint-Louis-Lariboisière, Applied Microbiology Research Group, Paris
(Titration for measles infection of sera from 400 Ugandan children)

RA/75/018 François Baclesse Regional Centre, Caen, France
(Animal experiments on the possible carcinogenic effects of cider alcohols)

Liver cancer studies

RA/75/025 Department of Hygiene and Epidemiology, School of Medicine, University of Athens, Athens
(Contribution to the collection of sera from patients with liver disease and controls to assess α-fetoprotein levels)

RA/76/012 Geneva Tumour Registry, Geneva, Switzerland
(Study of liver disease, including primary liver cancer, in the canton of Geneva)

Studies on chemical carcinogens

RA/69/005 Department of Occupational Health, Hebrew University–Hadassah Medical School, Jerusalem
(Analysis of fat and other tissues for the presence of chlorinated hydrocarbons)

RA/70/002 Medical College, Hanover, Federal Republic of Germany
(Investigation of the effects of chemical carcinogens administered transplacentally on the fetal reproductive organs)

RA/70/003 Institute of Pathology, Medical University, Budapest
(Investigation of the effects of minute doses of chemical carcinogens on cells cultured *in vitro*)

RA/70/030 National Institute for the Study and Treatment of Tumours, Milan, Italy
(Investigation on DDT storage levels in various tissues of DDT-treated and control mice)

RA/72/011 Institute of Experimental Oncology, University of Genoa, Genoa, Italy
(Investigation on effects of long-term administration of DDT and phenobarbital to rats)

RA/72/031 Institute for Experimental Toxicology and Chemotherapy, Heidelberg, Federal Republic of Germany
(Study of elaboration of analytical methods for the identification and quantification of *N*-nitroso compounds in various environmental media)

RA/74/007 National Institute of Public Health, Bilthoven, The Netherlands
(Study on the potential carcinogenicity of maleic hydrazide)

RA/74/011 Ministry of Health, Institute of Experimental and Clinical Medicine, Tallinn, Estonian SSR
(Investigations on the combined carcinogenic action of asbestos dust and *N*-nitroso compounds in the hamster)

RA/74/034 P. Jacquignon Laboratory, Institute of Chemistry of Natural Substances, National Centre for Scientific Research, Gif-sur-Yvette, France
(Synthesis of reactive intermediates of some pesticides and organochlorine compounds)

RA/75/023 The Plant Pathology Department, Rothamsted Experimental Station, Harpenden, UK
(Study of microorganisms and toxins in Iranian wheat)

RA/75/024 Mycotoxin Research and Training Centre, Department of Biochemistry and Nutrition, University of Isfahan, Isfahan, Iran
(Study of mycotoxins in Iranian wheat)

RA/76/001 Free University of Brussels, Brussels
(Investigation of an *in vitro* biochemical assay for somatic mutagenesis by chemical mutagens/carcinogens)

RA/76/017 Cancer Research Centre, Academy of Medical Sciences of the USSR, Moscow
(Investigation on the effect of prenatal exposure to a chemical on successive untreated generations)

Studies on carcinogens other than chemicals

RA/72/034 Medical Research Council Pneumoconiosis Unit, Penarth, UK
(Research study programme on asbestos cancers)

RA/76/008 Joint European Medical Research Board, Liverpool, UK
(International epidemiological research programme to examine health effects, particularly as these concern cancer, of exposure to man-made mineral fibres in man)

Studies of various other cancer forms

RA/73/004 Department of Pathology, University of Iceland, Reykjavik
(Investigations on familiality of carcinoma of the breast)

RA/73/041 Radioimmunology Laboratory, University of Liège, Liège, Belgium
(Study on polypeptide hormones in different populations with different incidence figures for cancer of the breast)

RA/74/031 Uganda Cancer Institute, Kampala
(Collection of specimens from patients, controls and spouses to study the role of viruses on cervical cancer)

RA/74/043 Research Institute for Community Health, University of Kuopio, Kuopio, Finland
(Project on intestinal micro-ecology in Scandinavia)

RA/74/044 Department of Medicine, St Elizabeth Hospital, Copenhagen
(Project on intestinal micro-ecology in Scandinavia)

RA/74/045 Department of Pathology, University of Kuopio, Kuopio, Finland
(Project on large-bowel pathology in an autopsy series in Kuopio)

RA/75/021 Department of Internal Medicine, Copenhagen County Hospital, Copenhagen
(Analysis of data from a study of intestinal microecology and colon cancer in Scandinavia)

RA/75/026 Cancer Research Centre, Academy of Medical Sciences of the USSR, Moscow
(Editorial activities for the production of *Pathology of Tumours in Laboratory Animals)*

RA/76/014 Cancer Research Centre, Academy of Medical Sciences of the USSR, Moscow
(Editorial activities for the production of *Pathology of Tumours in Laboratory Animals)*

Annex 4

MEETINGS AND WORKSHOPS ORGANIZED BY IARC, 1975–76

Role of cancer registries in occupational cancer	Lyon, 22–26 September 1975
International association of cancer registries	Lyon, 27 September 1975
Study of risk of man-made mineral fibres	Lyon, 29–30 September 1975
Fourth meeting on the analysis and formation of *N*-nitroso compounds	Tallinn, Estonian SSR, 1–3 October 1975
Evaluation of carcinogenic risk of chemicals to man: naturally occurring substances	Lyon, 7–14 October 1975
Working group for investigation of carcinogenic burden by air pollution in man	Hanover, Federal Republic of Germany, 22–24 October 1975
IARC/INSERM symposium on environmental pollution and carcinogenic risks	Lyon, 3–5 November 1975
Collaborative study for serological detection of trophoblastic tumours	Lyon, 28 November 1975
Evaluation of carcinogenic risk of chemicals to man: asbestos, cadmium and nickel	Lyon, 9–11 December 1975
Malaria studies on Burkitt's lymphoma projects in Uganda and Tanzania	Lyon, 19 January 1976
Conference on mutagenicity testing	Lyon, 27–28 January 1976
Evaluation of carcinogenic risk of chemicals to man: epoxides, industrial chemicals and general considerations on volatile anaesthetics	Lyon, 3–9 February 1976
Meeting on latent carcinoma of the prostate	Lyon, 1–5 March 1976
Meeting on alcohol and cancer	Lyon, 8–10 March 1976
European sub-committee on methods of analysis of *N*-nitroso compounds	Lyon, 10–22 May 1976

Workshop on oesophageal cancer among Turkomans in north-east Iran	Teheran, 8–10 May 1976
Postgraduate course on immunovirology of cancer	Lyon, 10–22 May 1976
Evaluation of carcinogenic risk of chemicals to man: some carbamates, thiocarbamates and carbazides	Lyon, 9–15 June 1976

Annex 5

VISITORS TO IARC, JULY 1975 TO JUNE 1976

Dr D. V. Ablashi
National Cancer Institute, Frederick, Md., USA

Dr J. Albert
University Institute of Social and Preventive Medicine, Lausanne, Switzerland

Dr P. M. Alibazah
Deputy Rector, Academic Affairs, University of Indonesia, Djakarta

Dr. A. Amiruddin
Rector, Hasanuddin University, Hasanuddin, Indonesia

Mr E. S. Annaheim
Chief, Distribution and Sales, WHO, Geneva, Switzerland

Dr H. L. Arora
J. L. N. Medical College, Ajmer, India

Mr K. Asano
Director, National Hospital Division, Ministry of Health, Tokyo

Professor A. K. Bahn
Fox Chase Cancer Center, University of Pennsylvania, Philadelphia, Pa., USA

Dr Y. Becker
Laboratory for Molecular Virology, The Hebrew University, Hadassah Medical School, Jerusalem

Dr D. P. Beri
East African Virus Research Institute, Arua, Uganda

Dr J. Berlie
Cancer Section, Division of Medico-Social Research, Le Vésinet, France

Dr R. J. Biggar
National Cancer Institute, Bethesda, Md., USA

Mme F. Bonichon
Bergonié Foundation, Bordeaux, France

Dr N. Breslow
Department of Biostatistics, University of Washington, Seattle, Wash., USA

Dr G. Brubaker
Shirati Hospital, Musoma, Tanzania

Dr A. Brugarolas
Asturias General Hospital, Oviedo, Spain

Dr T. A. Brun
National Institute of Health and Medical Research, Paris

Dr M. Brunet
Cancer Section, Division of Medico-Social Research, Le Vésinet, France

Dr F. Cabanne
Salah Azaiz Institute, Tunis

Dr V. Casale
Regina Elena Institute, Rome

Professor L. Cayolla da Motta
Ministry of Social Affairs, Lisbon

Dr J. Charrier
Kodak-Pathé, Chalon-sur-Saône, France

Dr L. CHE-HSIN
Institute of Cancer Research, Academy of Medical Sciences, Peking

Dr J. CLEMMESEN
Danish Cancer Registry, Copenhagen

Dr M. CRESPI
Regina Elena Institute, Rome

Dr J. A. DEERING
Acting Director, National Institute on Alcohol Abuse and Alcoholism, Rockville, Md., USA

Dr E. DE LUSTIG
Angel H. Roffo Oncological Institute, Buenos Aires

Professor A. DESPOPOULOS
Ciba-Geigy Limited, Basel, Switzerland

Mr P. DIETHELM
Data Processing, WHO, Geneva, Switzerland

Dr H. DOLS
University of Utrecht, Utrecht, The Netherlands

Dr C. C. DRAPER
London School of Hygiene and Tropical Medicine, London

Mr J. F. DRILLEAU
National Institute of Agronomic Research, Rennes, France

Dr J. M. EASTON
National Cancer Institute, Bethesda, Md., USA

Dr G. B. ELLIOTT
Royal Jubilee Hospital, Victoria, B.C., Canada

Dr J. ESTEVE
Claude Bernard University, Villeurbanne, France

Dr J. FAIVRE
General Hospital, III Medical Department, Dijon, France

Dr J. FALCONER
Chief Medical Officer, Kodak Limited, London

Dr W. GAFFIELD
US Department of Agriculture, Western Regional Research Laboratory, Berkeley, Calif., USA

Dr L. E. GERSCHENSON
Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

Professor J. GIBSON
Department of Pathology, Queen Mary Hospital, Hong Kong

Mr. R. GINGELL
Eppley Institute for Research in Cancer, Omaha, Nebr., USA

Dr G. GIRALDO
Memorial Sloan-Kettering Cancer Center, New York, N.Y., USA

Professor N. GOLDBLUM
The Chanock Centre for Virology, Hebrew University, Hadassah Medical School, Jerusalem

Dr J. GOLDSMITH
Epidemiological Studies Laboratory, Berkeley, Calif., USA

Professor S. GRAHAM
Medical Sociology Department, Amherst, N.Y., USA

Mme J. GUERAIN
Laboratory of the National Union of Alcohol Distillers, Paris

Dr A. GRASSI
Regina Elena Institute, Rome

Dr D. E. HENSON
Laboratory of Pathology, National Cancer Institute, Bethesda, Md., USA

Dr B. HETZEL
Director, Division of Human Nutrition, CSIRO, Adelaide, Australia

Dr R. M. HICKS
Courtauld Institute of Biochemistry, London

Professor Y. HINUMA
Department of Microbiology, Kumamoto University Medical School, Kumamoto, Japan

Dr Y. HORN
Ministry of Health, Jerusalem

Mrs E. HOUZER
WHO Journal, WHO, Geneva, Switzerland

Mme W. HOUEI-KIAUN
Institute of Cancer Research, Academy of Medical Sciences, Peking

Dr W. P. T. JAMES
Dunn Nutritional Laboratory, Medical Research Council, Cambridge, UK

Mr P. S. JONES
Internal Audit, WHO, Geneva, Switzerland

Dr L. K. KEEFER
National Institutes of Health, Bethesda, Md., USA

Dr M. E. KNOWLES
Ministry of Agriculture, Fisheries and Food, London

Dr M. KODAMA
Curie Foundation, Paris

Dr G. KOLAR
German Cancer Research Centre, Heidelberg, Federal Republic of Germany

Mr. A. KOVALYOV
President, Executive Committee, Minsk, USSR

Dr K. KRLEZA JERID
School of Public Health, University of Zagreb, Zagreb, Yugoslavia

Dr Z. KULCAR
Institute of Public Health of Croatia, Zagreb, Yugoslavia

Dr P. D. LAWLEY
Chester Beatty Research Institute, Chalfont St Giles, UK

Professor L. Y. LE TALAER
Department of Biology, François Baclesse Regional Centre, Caen, France

Mr G. LINDEN
2968 Shasta Road, Berkeley, Calif., USA

Mr D. LIPPMAN
National Conservatory of Arts and Trades, Paris

Dr W. P. D. LOGAN
11, rue Butini, Geneva, Switzerland

Mr V. LOUKASHEVITCH
Deputy of the Soviet of Workers' Deputies of Minsk, Minsk, USSR

Dr A. O. LUCAS
Director, Special Programme for Research and Training in Tropical Diseases, WHO, Geneva, Switzerland

Dr M. MAKAGIANSAR
Ministry of Higher Education, Djakarta

Dr G. MANDARD
Department of Pathological Anatomy, François Baclesse Regional Centre, Caen, France

Mr H. MANSOOR
Ministry of Education, Djakarta

Professor P. MAUPAS
Microbiology Laboratory, Faculty of Medicine, Tours, France

Dr J. M. MCCUNE
1232 Daryl Lane, Northbrook, I11., USA

Dr J. MCHARDY
Greater Glasgow Health Board, Glasgow, UK

Dr N. MERANI
United Nations Environment Programme, Geneva, Switzerland

Professor L. MIN-HSIN
Institute of Cancer Research, Academy of Medical Sciences, Peking

Dr F. MODABBER
Teheran University, School of Public Health, Teheran

Dr N. MOHAGHEGHPOUR
University of Teheran, Institute of Public Health Research, Teheran

Dr J. B. MOLONEY
National Cancer Institute, Bethesda, Md., USA

Miss C. MOUGNE
School of Oriental and African Studies, London

Dr N. MOURALI
Salah Azaiz Institute, Tunis

Dr T. MUNKNER
International Atomic Energy Agency, Vienna

Dr R. MURRAY
Newton Green, Sudbury, Suffolk, UK

Dr S. NEUKOMM
University Institute of Social and Preventive Medicine, Le Mont-sur-Lausanne, Switzerland

Dr K. NILSSON
Wallenberg Laboratories, University of Uppsala, Uppsala, Sweden

Mr P. OANCEA
Cultural Attaché to the Romanian Embassy, Paris

Dr G. PARDOE
Chemistry Department, University of Birmingham, Birmingham, UK

Dr W. E. PAUL
National Institutes of Health, Bethesda, Md., USA

Dr F. T. PERKINS
Chief, Biological Standardization, WHO, Geneva, Switzerland

Professor C. J. PFEIFER
Memorial University of Newfoundland, St Johns, Canada

Miss J. POWELL
Birmingham Regional Cancer Registry, Queen Elizabeth Hospital, Birmingham, UK

Professor R. L. RALEIGH
Director, Laboratory of Health and Security, Eastman-Kodak, Rochester, N.Y., USA

Miss L. RAMIOUL
School of Public Health, Brussels

Dr H. RAPPAPORT
City of Hope National Medical Center, Duarte, Calif., USA

Mr L. RAYMOND
Geneva Tumour Registry, Geneva, Switzerland

Dr H. B. RICHTER-REICHHELM
Hanover Medical School, Department of Experimental Pathology, Hanover, Federal Republic of Germany

Dr R. ROCH
Geneva Tumour Registry, Geneva, Switzerland

Mr S. ROMANOV
Executive Committee, Minsk, USSR

Mr C. E. ROSSITER
Medical Research Council Pneumoconiosis Unit, Penarth, Glamorgan, UK

Professor B. H. RUEBNER
Department of Pathology, University of California, Davis, Calif., USA

Dr N. SABADIE-PIALOUX
Claude Bernard University, Lyon, France

Dr J. SANCHIS
Asturias General Hospital, Oviedo, Spain

Dr S. SAPIIE
Deputy Director, Kalibata National Memorial Project, Kalibata, Indonesia

Dr M. SCHNEIDERMAN
National Cancer Institute, Bethesda, Md., USA

Dr S. K. SETHI
Institute of Scientific Research on Cancer, Villejuif, France

Professor K. SHANMUGARATNAM
IARC Singapore Research Centre, University of Singapore, Singapore

Dr M. SHARIATY
Tadj Pahlavi Cancer Institute, University of Teheran, Teheran

Dr L. R. SIBAL
Viral Oncology Branch, National Cancer Institute, Bethesda, Md., USA

Dr A. SOMOGYI
Robert von Ostertag Institute, Berlin, Federal Republic of Germany

Dr H. SOOBADIO
Dean, Faculty of Letters, University of Indonesia, Djakarta

Dr T. SOUISSI
Salah Azaiz Institute, Tunis

Dr T. STEMATSKY
Virus Laboratory, Public Health Laboratories, Tel Aviv, Israel

Dr M. STUKONIS
Oncological Research Institute, Vilnius, USSR

Dr D. SWENSON
Chester Beatty Research Institute, Chalfont St Giles, UK

Dr S. TARKOWSKI
WHO Regional Office for Europe, Copenhagen

Professor Y. TATARINOV
Department of Biochemistry, 2nd Moscow Medical Institute, Moscow

Dr B. TERRACINI
Institute of Anatomy and Pathological Histology, University of Turin, Turin, Italy

Dr C. THILLY
Secretary, School of Public Health, Brussels

Dr M. K. TOLBA
United Nations Environment Programme, Geneva, Switzerland

Dr G. TORRIGIANI
Chief, Immunology Unit, WHO, Geneva, Switzerland

Dr P. TUKEI
Director, East African Virus Research Institute, Entebbe, Uganda

Dr T. B. TURNER
Dean Emeritus, Johns Hopkins University School of Medicine, Baltimore, Md., USA

Dr V. S. TURUSOV
Cancer Research Centre, USSR Academy of Medical Sciences, Moscow

Dr S. J. VAN RENSBURG
Head, Division of Toxicology, National Research Institute for Nutritional Diseases, Tiervlei, South Africa

Dr R. J. VAN ZONNEVELD
Director, Bureau of the Council for Health Research TNO, The Hague

Dr A. VOLLER
The Zoological Society of London, Nuffield Institute of Comparative Medicine, London

Dr C. F. VON ESSEN
Department of Radiation Oncology, University Hospital, San Diego, Calif., USA

Dr VU-DUC
University Institute of Social and Preventive Medicine, Le Mont-sur-Lausanne, Switzerland

Mr G. VULLIOUD
Division of Health Statistics, WHO, Geneva, Switzerland

Professor G. WAGNER
Director, Institute of Information, Statistics and Documentation, German Cancer Research Centre, Heidelberg, Federal Republic of Germany

Dr M. WATANABE
Tohoku University, Sendai, Japan

Professor J. A. H. WATERHOUSE
Director, Birmingham Regional Cancer Registry, Queen Elizabeth Medical Centre, Birmingham, UK

Mr A. R. K. WATKINSON
Academic Press, London

Dr B. WEINSTEIN
College of Physicians and Surgeons of Columbia University, New York, N.Y., USA

Dr T. WONE
President of the Special Committee on Health Conditions of the Population in Occupied Territories, WHO, Geneva, Switzerland

Dr E. L. WYNDER
Naylor Dana Institute for Disease Prevention, Valhalla, N.Y., USA

Professor A. YAKER
Central Laboratory of Pathological Anatomy, Algiers

Mr M. YAMAMOTO
President of Medical Care, Facilities Finance Cooperation, Tokyo

Mrs R. YARED
WHO Journal, WHO, Geneva, Switzerland

Dr T. O. YOSHIDA
Department of Microbiology, Hamamatsu University, Shizuoka, Japan

Annex 6

INTERNAL TECHNICAL REPORTS, 1975–76

IARC Internal Technical Report No.	
75/003	Proceedings of a workshop on Epstein–Barr virus production, concentration and purification (Frederick, Md., USA, 10–12 February 1975)
75/004	Report of a workshop on childhood cancer (Freudenstadt, Federal Republic of Germany, 22 September 1975)
75/005	Report of a working group on testing of faeces for carcinogenicity (Lyon, 28 April 1975)
76/001	Report of the meeting of the European sub-committee on methods of analysis for *N*-nitroso compounds and for guidance of collaborative studies (Lyon, 29–30 March 1976)

Annex 7

PAPERS PUBLISHED OR SUBMITTED FOR PUBLICATION BY IARC STAFF AND FELLOWS

IARC staff:

Agthe, C. & Tomatis, L. (1976) *Evaluation of the carcinogenicity of environmental chemicals and the possibility of cancer prevention.* In: Homburger, E., ed., *Physiopathology of Cancer*, Basel, Karger, pp. 345–358

Audigier, J. C., Tuyns, A. J. & Lambert, R. (1975) Epidemiology of oesophageal cancer in France. Increasing mortality and persistent correlation with alcoholism. *Digestion*, **13**, 209–219

Barbin, A., Brésil, H., Croisy, A., Jacquignon, P., Malaveille, C., Montesano, R. & Bartsch, H. (1975) Liver-microsome-mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem. biophys. Res. Commun.*, **67**, 596–603

Bartsch, H. (1976) Predictive value of mutagenicity tests in chemical carcinogenesis. *Mutation Res.*, **38**, 177–190

Bartsch, H. (1976) The use of microbial mutagenicity assays for the detection of chemical carcinogens — a critique. *Colloque internat. CNRS* (in press)

Bartsch, H. (1976) *The predictive value of mutagenicity assays to assess the possible carcinogenic risk of chemicals.* In: Rosenfeld, C. & Davis, W., eds, *Environmental Pollution and Carcinogenic Risks*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 13**) (in press)

Bartsch, H., Camus, A. & Malaveille, C. (1976) Comparative mutagenicity of *N*-nitrosamines in semi-solid and in a liquid incubation system in the presence of rat or human tissue fractions. *Mutation Res.* (in press)

Bartsch, H., Malaveille, C., Barbin, A., Brésil, H., Tomatis, L. & Montesano, R. (1976) Mutagenicity and metabolism of vinyl chloride and related compounds. *Environm. Hlth Persp.* (in press)

Bartsch, H., Malaveille, C., Barbin, A., Planche, G. & Montesano, R. (1976) Alkylating and mutagenic metabolites of halogenated olefins produced by human and animal tissues. *Proc. Amer. Assoc. Cancer Res.*, **17**, 17

Bartsch, H., Malaveille, C. & Montesano, R. (1976) *The predictive value of tissue-mediated mutagenicity assays to assess the carcinogenic risk of chemicals.* In: Montesano, R., Bartsch, H. & Tomatis, L., eds, *Screening Tests in Chemical Carcinogenesis*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 12**), pp. 467–486

Bartsch, H. & Montesano, R. (1975) Mutagenic and carcinogenic effects of vinyl chloride. *Mutation Res.*, **32**, 93–114

Biancifiori, C., Montesano, R. & Bolis, G. B. (1975) Indagini sull'azzione cancerogenesi da sodio nitrito e/o etambutolo in topi BALB/c/Cb/Se. *Lav. Anat. Pat.*, **35**, 45–79

Breslow, N. (1975) Analysis of survival data under the proportional hazards model. *Int. Stat. Rev.*, **43**, 45–58

Brown, C. & Muenz, L. (1976) Reduced mean square error estimation in R × C contingency tables. *J. Amer. Stat. Ass.*, **71**, 176–182

Camus, A., Bertram, B., Kruger, F. W., Malaveille, C. & Bartsch, H. (1976) Mutagenicity of β-oxidized *N,N*-di-*n*-propylnitrosamine derivatives in *S. typhimurium* mediated by rat and hamster tissues. *Z. Krebsforsch.* (in press)

Castegnaro, M. & Walker, E. A. (1976) *Some developments in nitrosamine analysis.* In: *Proceedings of the II International Symposium on Nitrite in Meat Products, Zeist, September 1976* (in press)

Crowther, J. S., Drasar, B. S., Hill, M. J., MacLennan, R., Magnin, D., Peach, S. & Teoh-Chan, C. H. (1976) Faecal steroids and bacteria and large bowel cancer mortality in Hong Kong by socio-economic indicators. *Brit. J. Cancer* (in press)

Dambuyant, C. & Sizaret, P. (1975) Non-specific interference of certain components of tissue culture media with the radioimmunoassay of alpha-foetoprotein. *J. immunol. Methods*, **7**, 387–392

Dambuyant, C. & Sizaret, P. (1975) Increased reactivity of rat alpha-foetoprotein with corresponding antiserum after ^{125}I labelling. *J. immunol. Methods*, **8**, 289-294

Davis, W. & Maltoni, C., eds (1976) *Advances in Tumour Prevention, Detection and Characterization. 3. Biological Characterization of Human Tumours*, Amsterdam, Excerpta Medica

Day, N. E. (1975) Some aspects of the epidemiology of oesophageal cancer. *Cancer Res.*, **35**, 3304–3307

Day, N. E. (1976) *Multiple case-family studies and disease susceptibility genes.* In: *HL-A and Disease*, Paris, INSERM (*INSERM Publications* **No. 58**), p. 221

Day, N. E. (1976) High risk for cancer. *Cancer Detect. Prev.*, **1** (in press)

Day, N. E. & Charnay, B. A note on a test for clustering. *Brit. J. prev. soc. Med.* (in press)

Day, N. E., Muenz, L. & Tulinius, H. (1976) *Some aspects of familial breast cancer.* In: *Proceedings of the VIII International Symposium of the Gesellschaft zur Bekämpfung der Krebskrankheiten Nordrhein-Westfalen, Düsseldorf, June 1976* (in press)

Day, N. E. & Simons, M. J. (1976) Disease susceptibility genes — their identification by multiple case-family studies. *Tissue Antigens* (in press)

Desgranges, C., de-Thé, G., Wolf, H. & zur Hausen, H. (1975) *Further studies in the detection of the Epstein–Barr virus DNA in nasopharyngeal carcinoma biopsies from different parts of the world.* In: de-Thé, G., Epstein, M. A. & zur Hausen, H., eds, *Oncogenesis and Herpesviruses II*, Vol. 2, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 11**), pp. 191–193

Desgranges, C., Lavoué, M. F. & de-Thé, G. Lymphoblastoid immortalization and kinetics of appearance of early and viral capsid EB antigens in cord blood lymphocytes infected by different strains of Epstein–Barr virus (EBV) (submitted for publication)

Desgranges, C., Lenoir, G., de-Thé, G., Seigneurin, J. M., Hilgers, J. & Dubouch, P. *In vitro* transforming activity of EBV. I. Establishment and properties of two EBV strains (M81 and M72) produced by immortalized *Callithrix jacchus* lymphocytes (submitted for publication)

de-Thé, G. (1975) EBV behaviour in different populations — implications for control of EBV-associated tumours. *Cancer Res.*, **36**, 692–695

de-Thé, G. (1976) *Viruses and human cancers : achievements, problems, prospects.* In: *Proceedings of the 11th Canadian Cancer Research Conference, Toronto, May 1976* (in press)

de-Thé, G., Day, N., Geser, A., Ho, J. H. C., Simons, M. J., Sohier, R., Tukei, P. & Vonka, V. (1975) *Epidemiology of the Epstein–Barr virus infection and associated tumours in man, a tool for aetiology and control.* In: Clemmesen, J. & Yohn, D. S., eds, *Comparative Leukemia Research*, Basel, Karger, pp. 216–220

de-Thé, G., Day, N. E., Geser, A., Ho, J. H. C., Lavoué, M. F., Simons, M. J., Sohier, R., Tukei, P., Vonka, V. & Zavadova, H. (1975) *Sero-epidemiology of the Epstein–Barr virus : preliminary analysis of an international study — a review.* In: de-Thé, G., Epstein, M. A. & zur Hausen, H., eds, *Oncogenesis and Herpesviruses II*, Vol. 2, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 11**), pp. 3–16

de-Thé, G. & Geser, A. (1976) Etudes épidémiologiques prospectives et nature de l'association entre le virus d'Epstein–Barr (EBV) et le lymphôme de Burkitt (BL). *C.R. Acad. Sci. (Paris)*, **282**, 1387

de-Thé, G., Ho, J. H. C., Ablashi, D. V., Day, N. E., Macario, A. J. L., Pearson, G., Sohier, R. & Martin-Berthelon, M. C. (1975) Nasopharyngeal carcinoma. IX. Antibodies to EBNA and correlations with other EBV reactivities. *Int. J. Cancer*, **16**, 713–721

Didier, M. L., Ablashi, D. V., Oie, H. K., Armstrong, G. R., Easton, J. M., Chu, E. W. & Rabson, A. S. (1975) *Some biological properties of herpesviruses saimiri from chronically infected monolayer and suspension cultures.* In: de-Thé, G., Epstein, M. A. & zur Hausen, H., eds, *Oncogenesis and Herpesviruses II*, Vol. 1, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 11**), pp. 491–495

Falk, L., Deinhardt, F., Wolfe, L., Johnson, D., Hilgers, J. & de-Thé, G. (1976) Epstein–Barr virus: experimental infection of *Callithrix jacchus* marmosets. *Int. J. Cancer*, **17**, 785–788

Glaser, R., de-Thé, G., Lenoir, G. & Ho, J. H. C. (1976) Superinfection of epithelial nasopharyngeal carcinoma cells with Epstein–Barr virus. *Proc. nat. Acad. Sci. (Wash.)*, **23**, 468–469

Glaser, R., Lenoir, G., Desgranges, C. & de-Thé, G. Superinfection of epithelial Burkitt hybrid cells with Epstein–Barr virus (submitted for publication)

Glaser, R., Lenoir, G., Ho, J. H. C. & de-Thé, G. (1975) *In vitro* induction of EBV early antigens in NPC epithelial tumour cells by IUDR or superinfection. *Biomed. Express*, **23**, 468–469

Griciute, L. (1976) N-*Nitroso compounds — their analysis and possible significance as human carcinogens.* In: Rosenfeld, C. & Davis, W., eds, *Environmental Pollution and Carcinogenic Risks*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 13**) (in press)

Griciute, L. (1976) *Measurement of chemical carcinogens in the human environment — the objectives and problems encountered.* In: *Proceedings of the International Conference on Ecological Perspectives on Carcinogens and Cancer Control, Cremona, September 1976* (in press)

Griciute, L. & Shabad, L. (1976) Le centenaire de la première transplantation de tumeur. *Bull. Cancer* (in press)

Gross, R. L., Levin, A. G., Peers, F. G., Steel, C. M. & Tsu, T. (1975) Spontaneous rosette formation and rosette-inhibition tests on fresh and preserved lymphocytes. *J. Cryobiol.*, **12**, 455–462

Hampar, B., Lenoir, G., Nonoyama, M., Derge, J. G. & Chang, S. Y. (1976) Cell cycle dependence for activation of Epstein–Barr virus by inhibitors of protein synthesis or medium deficient in arginine. *Virology*, **69**, 660–668

Higginson, J. (1975) *Developments in cancer prevention through environmental control.* In: Maltoni, C., ed., *Proceedings of the Second International Symposium on Cancer Detection and Prevention*, Amsterdam, Excerpta Medica, pp. 3–18

Higginson, J. (1975) *Cancer etiology and prevention.* In: Fraumeni, J. F., Jr., ed., *Persons at High Risk of Cancer — an Approach to Cancer Etiology and Control*, New York, San Francisco & London, Academic Press, pp. 385–398

Higginson, J. (1976) *Chronic toxicology — an epidemiologist's approach to the problem of carcinogenesis.* In: Hayes, W. J., ed., *Essays in Toxicology*, Vol. 7, New York, San Francisco & London, Academic Press, pp. 29–72

Higginson, J. (1976) Les progrès de la recherche sur le cancer. *Le Concours méd.*, **22**, 3212–3214

Higginson, J. (1976) *The role of epidemiology in evaluating potential toxicological hazards for man.* In: Duncan, W. A. M., ed., *Drug Toxicity*, Amsterdam, Excerpta Medica, pp. 104–111

Higginson, J. (1976) *Primary carcinoma of the liver as a pathologist's problem (addendum).* In: Sommers, S. C., ed., *Gastrointestinal & Hepatic Pathology, Decennial 1966–1975*, New York, Appleton-Century-Crofts, pp. 359–390

Higginson, J. (1976) A hazardous society? Individual *versus* community responsibility in cancer prevention. *Amer. J. publ. Hlth*, **66**, 359–366

Higginson, J. (1976) *Importance of environmental factors in cancer.* In: Rosenfeld, C. & Davis, W., eds, *Environmental Pollution and Carcinogenic Risks*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 13**) (in press)

Higginson, J. (1976) *The future role of epidemiology in food safety evaluation.* In: *Proceedings of a Symposium on Health Effects of Chemicals in Foods, Ottawa, 1975* (in press)

Higginson, J. (1976) *The role of epidemiology in identifying environmental carcinogens.* In: Mehlman, M. A., ed., *Advances in Modern Toxicology*, Vol. I, Part 2, Washington, D.C., Hemisphere Publishing Corporation (in press)

Higginson, J. & Jensen, O. (1976) *Epidemiological review of lung cancer in man.* In: Mohr, U., Schmähl, D. & Tomatis, L., eds, *Air Pollution and Cancer in Man*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 16**) (in press)

Higginson, J., MacLennan, R. & Muir, C. S. (1976) *Etiological factors in human cancer.* In: Homburger, F., Brennan, M. J. & Krakoff, I. H., eds, *The Physiopathology of Cancer*, Vol. 2, *Diagnosis, Treatment, Prevention*, Basel, Karger, pp. 281–299

Higginson, J. & Muir, C. S. (1976) *Geographical variation in cancer distribution.* In: Homburger, F., Brennan, M. J. & Krakoff, I. H., eds, *The Physiopathology of Cancer*, Vol. 2, *Diagnosis, Treatment, Prevention*, Basel, Karger, pp. 300–322

Higginson, J. & Muir, C. S. (1976) The role of epidemiology in elucidating the importance of environmental factors in human cancer. *Cancer Detect. Prev.*, **1**, 79–105

Hilfrich, J., Haas, H., Montesano, R., Mohr, U. & Magee, P. N. (1975) The modification of the renal carcinogenicity of dimethylnitrosamine by actinomycin D and a protein deficient diet. *Brit. J. Cancer*, **32**, 578–587

Hormozdiari, H., Day, N. E., Aramesh, B. & Mahboubi, E. (1975) Dietary factors and oesophageal cancer in the Caspian littoral of Iran. *Cancer Res.*, **35**, 3493–3498

Huberman, E., Bartsch, H. & Sachs, L. (1975) Mutation induction in Chinese hamster V79 cells by two vinyl chloride metabolites, chloroethylene oxide and 2-chloroacetaldehyde. *Int. J. Cancer*, **16**, 639–644

Kuroki, T., Drevon, C., Saint Vincent, L., Tomatis, L. & Montesano, R. (1976) Studies on the use of liver parenchymal cells in *in vitro* carcinogenesis. *Colloque internat. CNRS* (in press)

Lamelin, J. P., Ho, J. H. C., Souissi, T., de-Thé, G. & Gabbiani, G. Smooth muscle antibody in Burkitt's lymphoma and nasopharyngeal carcinoma (submitted for publication)

Lamelin, J. P., Revillard, J. P., Chalopin, J. M., Souissi, T., Ho, J. H. C. & de-Thé, G. Cold lymphocytotoxins in nasopharyngeal carcinoma (submitted for publication)

Lamelin, J. P., Revillard, J. P., Samarut, C., Souissi, T. & de-Thé, G. Alteration of T lymphocyte population and mitogenic responses in nasopharyngeal carcinoma (submitted for publication)

Law, C. H., Day, N. E. & Shanmugaratnam, K. (1976) Incidence rates of specific histological types of lung cancer in Singapore Chinese and their aetiological significance. *Int. J. Cancer*, **17**, 304–309

Lenoir, G., Berthelon, M. C., Favre, M. C. & de-Thé, G. (1975) Characterization of Epstein–Barr virus (EBV) antigens. II. Detection of early antigens (S) using anticomplement immunofluorescence (ACIF) and complement fixation (CF) tests. *Biomed.*, **23**, 461–464

Lenoir, G., Martin-Berthelon, M. C., Favre, M. C. & de-Thé, G. (1976) Characterization of EBV antigens. I. Biochemical analysis of the complement-fixing soluble antigen and relationship with EBNA. *J. Virol.*, **17**, 672–674

Levine, P. H., de-Thé, G., Ambrosioni, J. C., Brugère, J., Schwaab, G., Cammoun, N. & Ellouz, R. (1976) Immunity to antigens associated with a cell line derived from nasopharyngeal cancer (NPC) in non-Chinese NPC patients. *Int. J. Cancer*, **17**, 155–160

Linsell, C. A. (1976) *Aflatoxins.* In: Lenihan, J., ed., *Man and the Environment. 5. The Chemical Environment*, London & Glasgow, Blackie (in press)

Linsell, C. A. & Higginson, J. (1976) *The geographical pathology of liver cell cancer.* In: Cameron, H. M., Linsell, C. A. & Warwick, G. P., eds, *Liver Cell Cancer*, Amsterdam, Excerpta Medica (in press)

Loprieno, N., Barale, R., Barnoncelli, S., Bartsch, H., Bronzetti, G., Cammellini, A., Corsi, C., Frezza, D., Nieri, R., Leporini, C., Rossellini, D. & Rossi, A. M. (1976) Induction of gene mutations and gene conversions by vinyl chloride metabolites in yeast. *Cancer Res.* (in press)

MacLennan, R. (1975) Epidémiologie du cancer colo-rectal. *Bull. Cancer (Paris)*, **62**, 411-418

MacLennan, R. & Gaitan, E. (1974) *Measurement of thyroid size in epidemiologic surveys.* In: *Endemic Goiter and Cretinism: Continuing Threats to World Health*, Washington (*PAHO Scientific Publications* **No. 292**), pp. 195–197

MacLennan, R., Levine, L., Newell, K. W. & Edsall, G. (1973) The early primary immune response to absorbed tetanus toxoid in man. *Bull. Wld Hlth Org.*, **49**, 615–626

MacLennan, R. & Walsh, R. J. (1973) Some haematological and biochemical effects of a malaria control programme in New Guinea. *Human Biology in Oceania*, **2**, 34–41

Magee, P. N., Montesano, R. & Preussmann, R. (1976) N-*Nitroso compounds and related carcinogens.* In: Searle, C. E., ed., *Chemical Carcinogens*, ACS Monogr. (in press)

Mahboubi, E., Day, N. E., Ghadirian, P. & Salmasizadeh, S. (1976) The negligible role of alcohol and tobacco in the etiology of oesophageal cancer in Iran — a case-control study. *Cancer Detect. Prev.*, **1** (in press)

Malaveille, C., Kolar, C. F. & Bartsch, H. (1976) Rat and mouse tissue-mediated mutagenicity of ring-substituted 3,3-dimethyl-1-phenyltriazenes in *Salmonella typhimurium. Mutation Res.*, **36**, 1–10

Margison, G. P., Brésil, H., Margison, J. M. & Montesano, R. (1976) Effect of chronic administration of dimethylnitrosamine of the excision of O^6-methylguanine from rat liver DNA. *Cancer Lett.* (in press)

Margison, G. P., Margison, J. M. & Montesano, R. (1976) Methylated purines in the deoxyribonucleic acid of various Syrian golden hamster tissues after administration of a hepatocarcinogenic dose of dimethylnitrosamine. *Biochem. J.*, **157**, 627–634

Mohr, U., Schmähl, D. & Tomatis, L., eds (1976) *Air Pollution and Cancer in Man*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 16**) (in press)

Montesano, R. (1976) Cancérogénicité des composés *N*-nitrosés. *Ann. Nutr. Alim.* (in press)

Montesano, R. & Bartsch, H. (1976) *Mutagenicity and metabolism of vinyl chloride.* In: Davis, W. & Maltoni, C., eds, *Advances in Tumour Prevention, Detection and Characterization. 3. Biological Characterization of Human Tumours*, Amsterdam, Excerpta Medica, pp. 242–245

Montesano, R. & Bartsch, H. (1976) Mutagenic and carcinogenic *N*-nitroso compounds: possible environmental hazards. *Mutation Res.*, **32**, 179–228

Montesano, R., Bartsch, H. & Tomatis, L., eds (1976) *Screening Tests in Chemical Carcinogenesis*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 12**)

Montesano, R., Bartsch, H. & Tomatis, L. (1976) *Carcinogenesis and mutagenesis.* In: *Principles and Guidelines for the Evaluation of the Toxicity of Chemicals*, Geneva, WHO (in press)

Montesano, R., Saint Vincent, L., Drevon, C. & Tomatis, L. (1975) Production of epithelial and mesenchymal tumours with rat liver cells transformed *in vitro*. *Int. J. Cancer*, **16**, 550–558

Montesano, R. & Tomatis, L. Considerations on legislation concerning chemical carcinogens in several industrialized countries (submitted for publication)

Muir, C. S. (1975) L'épidémiologie du cancer du cavum. *Bull. Cancer (Paris)*, **62**, 261–264

Muir, C. S. (1975) *International variations in high risk populations.* In: Fraumeni, J. F., Jr., ed., *Persons at High Risk of Cancer : An Approach to Cancer Etiology and Control,* New York, San Francisco & London, Academic Press, pp. 293–305

Muir, C. S. (1976) *The evidence from epidemiology.* In: Lenihan, J. & Fletcher, W. W., eds, *Environment and Man. 3. Health and the Environment,* London & Glasgow, Blackie, pp. 88–115

Muir, C. S. (1976) The programs of the International Agency for Research on Cancer (IARC) in Asia. *Gann Monogr.,* **18**, 303–314

Muir, C. S. (1976) Epidemiological identification of cancer hazards. *Cancer Detect. Prevent.,* **1** (in press)

Muir, C. S. (1976) *Users' needs for information : the needs of the cancer epidemiologist.* In: *Proceedings of the 2nd International Conference of National Committees on Vital and Health Statistics, Copenhagen, October 1973,* Geneva, WHO (in press)

Muir, C. S., MacLennan, R., Waterhouse, J. A. H. & Magnus, K. (1976) *Feasibility of monitoring populations to detect environmental carcinogens.* In: Rosenfeld, C. & Davis, W., eds, *Environmental Pollution and Carcinogenic Risks,* Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 13**) (in press)

Muir, C. S. & Nectoux, J. (1976) *The role of the cancer registry.* In: *Proceedings of the Symposium on Epidemiology and Cancer Registries in the Pacific Basin, Hawaii, November 1975* (in press)

Muir, C. S. & Péron, Y. (1976) Special demographic situations. *Seminars in Oncology,* **3**, 35–47

Muñoz, N. (1976) *Geographical distribution of pediatric tumours.* In: Bucalossi, P., Veronesi, U., Emmanuelli, H. & Fossati-Bellani, F., eds, *I Tumori Infantili,* Milan, Ambrosiana, pp. 5–12

Muñoz, N. (1976) *Prenatal exposure and carcinogenesis.* In: Bucalossi, P., Veronesi, U., Emmanuelli, H. & Fossati-Bellani, F., eds, *I Tumori Infantili,* Milan, Ambrosiana, pp. 27–32

Muñoz, N. (1976) Model systems for cervical cancer. *Cancer Res.,* **36**, 792–793

Muñoz, N. & de-Thé, G. (1976) L'épidémiologie et la recherche étiologique dans les hématosarcomes de l'enfant. *C.R. Acad. Sci. (Paris),* **282**, 1329–1332

Muñoz, N., de-Thé, G., Aristazabal, N., Yee, C., Rabson, A. & Pearson, G. (1975) *Antibodies to herpesviruses in patients with cervical cancer and controls.* In: de-Thé, G., Epstein, M. A. & zur Hausen, H., eds, *Oncogenesis and Herpesviruses II,* Vol. 2, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 11**), pp. 45-51

Nishioka, K., Levin, A. G. & Simons, M. J. (1975) Hepatitis B antigen, antigen sub-types, and hepatitis B antibody in normal subjects and in patients with liver disease. Results of a collaborative study. *Bull. Wld Hlth Org.,* **52**, 293–300

Pagano, J. S., Huang, C. H., Klein, G., de-Thé, G., Shanmugaratnam, K. & Yang, C. S. (1975) *Homology of Epstein–Barr virus DNA in nasopharyngeal carcinomas from Kenya, Taiwan, Singapore and Tunisia.* In: de-Thé, G., Epstein, M. A. & zur Hausen, H., eds, *Oncogenesis and Herpesviruses II,* Vol. 2, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 11**), pp. 179–190

Peers, F. G., Gilman, G. A. & Linsell, C. A. (1976) Dietary aflatoxins and human liver cancer. A study in Swaziland. *Int. J. Cancer,* **17**, 167–176

Peers, F. G. & Linsell, C. A. (1975) Aflatoxin contamination and its heat stability in Indian cooking oils. *Tropical Sci.,* **17**, 229–232

Peers, F. G. & Linsell, C. A. (1976) Acute toxicity of aflatoxin B_1 for baboons. *Fd Cosmet. Toxicol.*, **14**, 227–229

Péquignot, G. & Tuyns, A. J. (1975) *Rations d'alcool consommées 'déclarées' et risques pathologiques.* In: *Alcoolisme*, Paris, INSERM (*INSERM Publications* **No. 54**), pp. 23–29

Pignatelli, B., Castegnaro, M. & Walker, E. A. (1976) *Effects of gallic acid and of ethanol on formation of nitrosodiethylamine.* In: Walker, E. A., Bogovski, P. & Griciute, L., eds, *Environmental N-Nitroso Compounds — Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 14**) (in press)

Planche, G., Malaveille, C. & Bartsch, H. (1976) Factors for efficiency of the *Salmonella*/microsome mutagenicity assay. *Chem.-biol. Interact.* (in press)

Plato, C. C. & MacLennan, R. (1975) The dermatoglyphics of the Maprik sub-district of the Sepic district of New Guinea. *Z. Morph. Anthrop.*, **66**, 208–216

Ponomarkov, V. & Mackey, L. J. (1976) Tumours of the liver and biliary system. *Bull. Wld Hlth Org.* (in press)

Ponomarkov, V., Tomatis, L. & Turusov, V. (1976) The effect of long-term administration of phenobarbitone in CF-1 mice. *Cancer Lett.*, **1**, 165–172

Rosenfeld, C. & Davis, W., eds (1976) *Environmental Pollution and Carcinogenic Risks*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 13**) (in press)

Rudali, G., Apiou, F., Boyland, E. & Castegnaro, M. (1976) Cancérologie — à propos de l'action cancérigène de la γ-butyrolactone chez les souris. *C.R. Acad. Sci. (Paris)*, **282**, 799–802

Saracci, R. (1976) *Epidemiology of lung cancer in Italy.* In: Mohr, U., Schmähl, D. & Tomatis, L., eds, *Air Pollution and Cancer in Man*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 16**) (in press)

Seigneurin, J. M., Desgranges, C., Lavoué, M. F. & de-Thé, G. (1976) Herpes simplex-Raji (A44), a new cell line for serologic testing by immunofluorescence. *J. Immunol.* (in press)

Shabad, L. M. & Ponomarkov, V. (1976) Mstislav Novinski, pioneer of tumour transplantation. *Cancer Lett.* (in press)

Simons, M. J., Chan, S. H., Ho, J. H. C., Chau, J. C. W., Day, N. E. & de-Thé, G. (1976) *A Singapore 2-associated LD antigen in Chinese patients with nasopharyngeal carcinoma.* In: *Proceedings of the Sixth International Histocompatibility Workshop Conference, Aarhus, Denmark, October 1975* (in press)

Simons, M. J. & Day, N. E. (1976) *HL-A patterns and NPC.* In: *Proceedings of the Symposium on Epidemiology and Cancer Registries in the Pacific Basin, Hawaii, November 1975* (in press)

Simons, M. J., Wee, G. B., Chan, S. H., Shanmugaratnam, K., Day, N. E. & de-Thé, G. (1976) *Immunogenetic aspects of nasopharyngeal carcinoma (NPC). III. HL-A type as a genetic marker of NPC predisposition to test the hypothesis that Epstein–Barr virus is an etiological factor in NPC.* In: de-Thé, G., Epstein, M. A. & zur Hausen, H., eds, *Oncogenesis and Herpesviruses II*, Vol. 2, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 11**), pp. 249–258

Simons, M. J., Wee, G. B., Chan, S. H., Shanmugaratnam, K., Day, N. E. & de-Thé, G. (1976) Increased risk in Chinese for nasopharyngeal carcinoma associated with the Chinese-related HL-A profile (HL-A2, Singapore 2). *J. nat. Cancer Inst.* (in press)

Simons, M. J., Wee, G. B., Singh, D., Dharmalingham, S., Yong, N. K., Chau, J. W., Ho, J. H. C., Day, N. E. & de-Thé, G. B. (1976) *Immunogenetic aspects of nasopharyngeal carcinoma (NPC). V. Confirmation of a Chinese-related HL-A halo-type (HL-A2 — Singapore 2) associated with an increased risk in Chinese for NPC.* In: *Proceedings of the Symposium on Epidemiology and Cancer Registries in the Pacific Basin, Hawaii, November 1975* (in press)

Sizaret, P., Martel, N., Tuyns, A., Jouvenceaux, A., Levin, A., Ong, Y. W., Rive, J. & Reynaud, S. (1976) Mean AFP values of 1,333 males over 15 years of age by age groups. *Digestion* (in press)

Sizaret, P., Tuyns, A., Martel, N., Jouvenceaux, A., Levin, A., Ong, Y. W. & Rive, J. (1975) Alpha-foetoprotein levels in normal males from seven ethnic groups with different hepatocellular carcinoma risks. *Ann. N.Y. Acad. Sci.*, **259**, 136–155

Tomatis, L. (1976) The IARC program on the evaluation of the carcinogenic risk of chemicals to man. *Ann. N.Y. Acad. Sci.*, **271**, 396–409

Tomatis, L. (1976) *Cancerogenesi ambientale e possibilità di prevenzione.* In: *Cancerogenesi Chimica*, Milan, Ambrosiana, pp. 3–8

Tomatis, L. (1976) *Validity and limitations of long-term experimentation in cancer research.* In: Mohr, U., Schmähl, D. & Tomatis, L., eds, *Air Pollution and Cancer in Man*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 16**) (in press)

Tomatis, L. (1976) Prenatal exposure to chemical carcinogens and its effect on subsequent generations. *Nat. Cancer Inst. Monogr.* (in press)

Tomatis, K., Ponomarkov, V. & Turusov, V. Effects of ethylnitrosourea administered during pregnancy on three subsequent generations (submitted for publication)

Toussaint, G., Lafaverges, F. & Walker, E. A. (1976) *The use of high pressure liquid chromatography for determination of aflatoxins in olive oil.* In: *Proceedings of the III International Symposium on Mycotoxins in Foodstuffs, Paris, September 1976* (in press)

Tuyns, A. J. (1976) Le choix difficile d'un bon groupe de témoins dans une enquête rétrospective. *Rev. fr. Epid.* (in press)

Tuyns, A. J. & Audigier, J. C. (1976) Double wave cohort increase for oesophageal and laryngeal cancer in France in relation to reduced alcohol consumption during the Second World War. *Digestion* (in press)

Tuyns, A. J. & Massé, G. (1975) Le cancer de l'œsophage en Ille-et-Vilaine. Etude de l'incidence de la maladie, de ses aspects cliniques et histologiques et de sa distribution géographique. *Ouest méd.*, **28**, 1757–1770

Tuyns, A. J., Péquignot, G., Jensen, O. M. & Pomeau, Y. (1975) La consommation individuelle de boissons alcoolisées et de tabac dans un échantillon de la population en Ille-et-Vilaine. *Rev. Alcool.*, **21**, 105–150

Walker, E. A., Bogovski, P. & Griciute, L., eds (1976) *Environmental N-Nitroso Compounds — Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 14**), (in press)

Walker, E. A. & Castegnaro, M. (1976) *Problems in trace analysis of nitrosamines in foodstuffs.* In: *Proceedings of the 7th International Materials Research Symposium, Gaithersburg, Md., October, 1974,* National Bureau of Standards Special Publication **No. 422**

Walker, E. A. & Castegnaro, M. (1976) *New data on collaborative studies on analysis of volatile nitrosamines.* In: Walker, E. A., Bogovski, P. & Griciute, L., eds, *Environmental* N-*Nitroso Compounds — Analysis and Formation,* Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 14**) (in press)

Walker, E. A., Castegnaro, M. & Pignatelli, B. (1975) Use of a clean-up method to improve specificity in nitrosamine analysis. *Analyst,* **100**, 817–821

Walker, E. A., Pignatelli, B. & Castegnaro, M. (1975) Effects of gallic acid on nitrosamine formation. *Nature (Lond.),* **258**, 176

Wassermann, M., Nogueira, D. P., Tomatis, L., Mirra, A. P., Shibata, H., Arie, G., Cucos, S. & Wasserman, D. (1976) Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. *Bull. environm. Contam. Toxicol.,* **15**, 478-484

Waterhouse, J. A. H., Muir, C. S., Correa, P. & Powell, J., eds (1976) *Cancer Incidence in Five Continents,* Vol. 3, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* **No. 15**) (in press)

Zardi, L., Saint Vincent, L., Barbin, A., Montesano, R. & Margison, G. Effect of split doses of *N*-methyl-*N*-nitrosourea on DNA repair synthesis in cultured mammalian cells (submitted for publication)

IARC Fellows [1] *:*

Albrecht-Buehler, G. & Goldman, R. D. (1976) Microspike-mediated particle transport towards the cell body during early spreading at 3T3 cells. *Exptl Cell Res.,* **97**, 329–339

Alexandrov, K., Brookes, P., King, H. W. S., Osborne, M. R. & Thompson, M. H. (1976) Comparison of the metabolism of benzo[*a*]pyrene and binding to DNA caused by rat liver nuclei and microsomes. *Chem.-biol. Interact.,* **12**, 269–277

Bataillon, G., Pross, H. & Klein, G. (1975) Comparative *in vitro* sensitivity of two methylcholanthrene-induced murine sarcoma lines to humoral and cellular immune cytotoxicity. *Int. J. Cancer,* **16**, 255–265

Björk, G. R. (1975) Transductional mapping of gene *trmA* responsible for the production of 5-methyluridine in transfer ribonucleic acid of *Escherichia coli. J. Bacteriol.,* **124**, 92–98

Björk, G. R. & Deinhardt, F. C. (1975) Physiological and biochemical studies on the function of 5-methyluridine in the transfer ribonucleic acid of *Escherichia coli. J. Bacteriol.,* **124**, 99–111

Forni, G., Rhim, J. S., Pickeral, S., Shevach, E. M. & Green, I. (1975) Antigenicity of carcinogen and viral induced sarcomas in inbred and random bred guineapigs. *J. Immunol.,* **115**, 204–210

Goth, R. & Cleaver, J. E. Metabolism of caffeine to nucleic acid precursors in mammalian cells (submitted for publication)

Karasek, J. (1975) Ultrastructural nuclear changes of extranucleolar ribonucleoprotein structures during autolysis of normal liver cells. *Cell. Path.,* **18**, 337–346

[1] The names of Fellows are given in bold type.

Kavenoff, R. & Ryder, O. A. (1976) Electron microscopy of membrane-associated folded chromosomes of *Escherichia coli*. *Chromosoma*, **55**, 13–25

Kavenoff, R., Talcove, D. & Mudd, J. A. (1975) Genome-sized RNA from reovirus particles. *Proc. nat. Acad. Sci. (Wash.)*, **72**, 4317–4321

Kroes, R., Berkvens, J. M. & Weisburger, J. (1975) Immunosuppression in primary liver and colon tumour inductions with *N*-hydroxy-*N*-2-fluorenylacetamide and azoxymethane. *Cancer Res.*, **35**, 2651–2656

Kroes, R., Weiss, J. W. & Weisburger, J. H. (1975) Immune suppression and chemical carcinogenesis. *Recent Results Cancer Res.*, **52**, 65–75

Kuroki, T. (1975) International Agency for Research on Cancer. *Cancer Chemother.*, **2**, 147–150

Laerum, O. D. & Rajewsky, M. F. (1975) Neoplastic transformation of fetal rat brain cells in culture after exposure to ethylnitrosourea *in vivo*. *J. nat. Cancer Inst.*, **55**, 1177–1187

Lejeune, F. J. (1975) Role of macrophages in immunity, with special reference to tumour immunology. A review. *Biomed.*, **22**, 25–34

Lever, J. E. (1976) Regulation of amino-acid and glucose transport activity expressed in isolated membranes from untransformed and SV40-transformed mouse fibroblasts. *J. cell. Physiol.* (in press)

Lever, J. E. (1976) Regulation of active alpha-aminoisobutyric acid transport expressed in membrane vesicles from mouse fibroblasts. *Proc. nat. Acad. Sci. (Wash.)* (in press)

Lever, J. E., Clingan, D. & Jimenez de Asua, L. (1976) Prostaglandin F2 alpha and insulin stimulate phosphate uptake and (Na-, K-) ATPase activity in resting mouse fibroblast cultures. *Biochem. biophys. Res. Comm.* (in press)

Delius, H., Duesberg, P. H. & **Mangel, W. F.** (1975) Electron microscope measurements of rous sarcoma virus RNA. *Cold Spring Harbor Symp. Quant. Biol.*, **39**, 835–843

Mangel, W. F., Delius, H. & Duesberg, P. H. (1974) Structure and molecular weight of the 60-70S RNA and the 30-40S RNA of the rous sarcoma virus. *Proc. nat. Acad. Sci. (Wash.)*, **71**, 4541–4545

Pinto, J. M., Bhalavat, R. L., **Nagaraj Rao, D.** & Gangadharan, P. (1975) Radiotherapy in carcinoma of the oesophagus. *Indian J. Cancer*, **12**, 380–388

Nagaraj Rao, D., Gangadharan, P., Jussawalla, D. J. & Paymaster, J. C. (1974) Multiple study approaches (for cancer) to a well-defined population of Parsis. *Excerpta Medica Intern. Congress Series*, **3**, 18–25

Révesz, T. & Greaves, M. F. (1975) Interaction of cholera toxin with lymphocyte membranes and gangliosides. In: *Proceedings of the International Symposium on Membrane Receptors of Lymphocytes, Paris, May 1975*

Hampton, A., Harper, P. J., **Sasaki, T.,** Howgate, P. & Preston, R. K. (1975) Carboxylic-phosphoric mixed anhydrides isosteric with AMP and ATP as reagents for enzymic AMP and sites. *Biochem. biophys. Res. Comm.*, **65**, 945–950

Sela, B. A., Wang, J. L. & Edelman, G. M. (1975) Antibodies reactive with cell surface carbohydrates. *Proc. nat. Acad. Sci. (Wash.)*, **72**, 1127-1131

Sela, B. A., Wang, J. L. & Edelman, G. M. (1975) Isolation of lectins of different specificities on a single affinity adsorbent. *J. biol. Chem.*, **250**, 7535–7538

Sela, B. A., Wang, J. L. & Edelman, G. M. (1976) Lymphocyte activation by monovalent fragments of antibodies reactive with cell surface carbohydrates. *J. exptl Med.*, **143**, 665–671

Rutishauser, U., **Thiery, J. P.,** Brackenbury, R., **Sela, B. A.** & Edelman, G. M. (1976) Mechanisms of adhesion among cells from neural tissues of the chick embryo. *Proc. nat. Acad. Sci. (Wash.)*, **73**, 577–581

Rapp, F. & **Westmoreland, D.** (1975) Do viruses cause cancer in man? *CA Cancer Journal for Clinicians*, **25**, 215–229

WHO publications may be obtained, direct or through booksellers, from :

ALGERIA	Société nationale d'Edition et de Diffusion, 3 bd Zirout Youcef, ALGIERS.
ARGENTINA	Librería de las Naciones, Cooperativa Ltda, Alsina 500, BUENOS AIRES — Editorial Sudamericana S.A., Humberto 1º 545, BUENOS AIRES.
AUSTRALIA	Mail Order Sales, Australian Government Publishing Service, P.O. Box 84, CANBERRA, A.C.T. 2600 ; *or over the counter from* Australian Government Publications and Inquiry Centres at : 113 London Circuit, CANBERRA CITY ; 347 Swanston Street, MELBOURNE ; 309 Pitt Street, SYDNEY ; Mr. Newman House, 200 St. George's Terrace, PERTH ; Industry House, 12 Pirie Street, ADELAIDE ; 156-162 Macquarie Street, HOBART — Hunter Publications, 58A Gipps Street, COLLINGWOOD, Vic. 3066.
AUSTRIA	Gerold & Co., I. Graben 31, VIENNA 1.
BANGLADESH	The WHO Representative, G.P.O. Box 250, DACCA 5.
BELGIUM	Office international de Librairie, 30 avenue Marnix, BRUSSELS.
BRAZIL	Biblioteca Regional de Medicina OMS/OPS, Unidad de Venta de Publicaciones, Caixa Postal 20.381, Vila Clementino, 01000 SÃO PAULO, S.P.
BURMA	*see* India, WHO Regional Office.
CANADA	Information Canada Bookstore, 171 Slater Street, OTTAWA, Ontario K1A 0S9 ; Main Library, University of Calgary, CALGARY, Alberta ; 1683 Barrington Street, HALIFAX, N.S. B3J 1Z9 ; 640 Ste Catherine West, MONTREAL, Quebec H3B 1B8 ; 221 Yonge Street, TORONTO, Ontario M5B 1NA ; 800 Granville Street, VANCOUVER, B.C. V6Z 1K4 ; 393 Portage Avenue, WINNIPEG, Manitoba R3B 2C6. *Mail orders to* 171 Slater Street, OTTAWA, Ontario K1A 0S9.
CHINA	China National Publications Import Corporation, P.O. Box 88, PEKING.
COLOMBIA	Distrilibros Ltd, Pío Alfonso García, Carrera 4a, Nos 36-119, CARTAGENA.
COSTA RICA	Imprenta y Librería Trejos S.A., Apartado 1313, SAN JOSÉ.
CYPRUS	MAM, P.O. Box 1674, NICOSIA.
CZECHOSLOVAKIA	Artia, Smecky 30, 111 27 PRAGUE 1.
DENMARK	Ejnar Munksgaard, Ltd, Nørregade 6, COPENHAGEN.
ECUADOR	Librería Científica S.A., P.O. Box 362, Luque 223, GUAYAQUIL.
EGYPT	Nabaa El Fikr Bookshop, 55 Saad Zaghloul Street, ALEXANDRIA — Anglo Egyptian Bookshop, 165 Mohamed Farid Street, CAIRO.
EL SALVADOR	Librería Estudiantil, Edificio Comercial B No 3, Avenida Libertad, SAN SALVADOR.
FIJI	The WHO Representative, P.O. Box 113, SUVA.
FINLAND	Akateeminen Kirjakauppa, Keskuskatu 2, HELSINKI 10.
FRANCE	Librairie Arnette, 2 rue Casimir-Delavigne, PARIS 6e.
GERMAN DEMOCRATIC REPUBLIC	Buchhaus Leipzig, Postfach 140, 701 LEIPZIG.
GERMANY, FEDERAL REPUBLIC OF	Govi-Verlag GmbH, Ginnheimerstrasse 20, Postfach 5360, 6236 ESCHBORN — W. E. Saarbach, Postfach 1510, Follerstrasse 2, 5 COLOGNE 1 — Alex. Horn, Spiegelgasse 9, Postfach 3340, 62 WIESBADEN.
GREECE	G. C. Eleftheroudakis S.A., Librairie internationale, rue Nikis 4, ATHENS (T. 126).
HAITI	Max Bouchereau, Librairie " A la Caravelle ", Boite postale 111-B, PORT-AU-PRINCE.
HUNGARY	Kultura, P.O.B. 149, BUDAPEST 62 — Akadémiai Könyvesbolt, Váci utca 22, BUDAPEST V.
ICELAND	Snaebjørn Jonsson & Co., P.O. Box 1131, Hafnarstraeti 9, REYKJAVIK.
INDIA	WHO Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, NEW DELHI 1 — Oxford Book & Stationery Co., Scindia House, NEW DELHI ; 17 Park Street, CALCUTTA 16 (Sub-agent).
INDONESIA	*see* India, WHO Regional Office.
IRAN	Iranian Amalgamated Distribution Agency, 151 Khiaban Soraya, TEHERAN.
IRELAND	The Stationery Office, DUBLIN.
ISRAEL	Heiliger & Co., 3 Nathan Strauss Street, JERUSALEM.
ITALY	Edizioni Minerva Medica, Corso Bramante 83-85, TURIN ; Via Lamarmora 3, MILAN.
JAPAN	Maruzen Co. Ltd. P.O. Box 5050, TOKYO International, 100-31.
KENYA	The Caxton Press Ltd, Head Office : Gathani House, Huddersfield Road, P.O. Box 1742, NAIROBI.
KUWAIT	The Kuwait Bookshops Co. Ltd, Thunayan Al-Ghanem Bldg, P.O. Box 2942, KUWAIT.
LAO PEOPLE'S DEMOCRATIC REPUBLIC	The WHO Representative, P.O. Box 343, VIENTIANE.
LEBANON	Documenta Scientifica/Redico, P.O. Box 5641, BEIRUT.
LUXEMBOURG	Librairie du Centre, 49 bd Royal, LUXEMBOURG.
MALAYSIA	The WHO Representative, Room 1004, Fitzpatrick Building, Jalan Raja Chulan, KUALA LUMPUR 05-02 — Jubilee (Book) Store Ltd, 97 Jalan Tuanku Abdul Rahman, P.O. Box 629, KUALA LUMPUR — Parry's Book Center, K.L. Hilton Hotel, KUALA LUMPUR.

Price : Sw. fr. 12.—

Prices are subject to change without notice.

WHO publications may be obtained, direct or through booksellers, from :

MEXICO	La Prensa Médica Mexicana, Ediciones Científicas, Paseo de las Facultades 26, MEXICO CITY 20, D.F.
MONGOLIA	*see* India, WHO Regional Office.
MOROCCO	Editions La Porte, 281 avenue Mohammed V, RABAT.
NEPAL	*see* India, WHO Regional Office.
NETHERLANDS	N.V. Martinus Nijhoff's Boekhandel en Uitgevers Maatschappij, Lange Voorhout 9, THE HAGUE.
NEW ZEALAND	Government Printing Office, Government Bookshops at : Rutland Street, P.O. Box 5344, AUCKLAND ; 130 Oxford Terrace, P.O. Box 1721, CHRISTCHURCH ; Alma Street, P.O. Box 857, HAMILTON ; Princes Street, P.O. Box 1104, DUNEDIN ; Mulgrave Street, Private Bag, WELLINGTON — R. Hill & Son Ltd, Ideal House, Cnr. Gilles Avenue & Eden Street, Newmarket, AUCKLAND S.E. 1.
NIGERIA	University Bookshop Nigeria, Ltd, University of Ibadan, IBADAN.
NORWAY	Johan Grundt Tanum Bokhandel, Karl Johansgt. 43, OSLO 1.
PAKISTAN	Mirza Book Agency, 65 Shahrah Quaid-E. Azam, P.O. Box 729, LAHORE 3.
PARAGUAY	Agencia de Librerías Nizza S.A., Estrella No. 721, ASUNCIÓN.
PERU	Distribuidora Inca S.A., Apartado 3115, Emilio Althaus 470, LIMA.
PHILIPPINES	World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, MANILA — The Modern Book Company Inc., P.O. Box 632, 926 Rizal Avenue, MANILA.
POLAND	Składnica Księgarska, ul. Mazowiecka 9, WARSAW (*except periodicals*) — BKWZ Ruch, ul. Wronia 23, WARSAW (*periodicals only*).
PORTUGAL	Livraria Rodrigues, 186 Rua Aurea, LISBON.
REPUBLIC OF KOREA	The WHO Representative, Central P.O. Box 540, SEOUL.
SINGAPORE	The WHO Representative, 144 Moulmein Road, G.P.O. Box 3457, SINGAPORE 1.
SOUTH AFRICA	Van Schalk's Bookstore (Pty) Ltd. P.O. Box 724, PRETORIA.
SPAIN	Comercial Atheneum S.A., Consejo de Ciento 130-136, BARCELONA 15 ; General Moscardó 29, MADRID 20 — Librería Díaz de Santos, Lagasca 95, MADRID 6 ; Balmes 417 y 419, BARCELONA 6.
SRI LANKA	*see* India, WHO Regional Office.
SWEDEN	Aktiebolaget C.E. Fritzes Kungl. Hovbokhandel, Fredsgatan 2, STOCKHOLM 16.
SWITZERLAND	Medizinischer Verlag Hans Huber, Länggass Strasse 76, 3012 BERNE 9.
THAILAND	*see* India, WHO Regional Office.
TUNISIA	Société Tunisienne de Diffusion, 5 avenue de Carthage, TUNIS.
TURKEY	Librairie Hachette, 469 avenue de l'Indépendance, ISTANBUL.
UGANDA	*see address under* KENYA.
UNITED KINGDOM	H.M. Stationery Office : 49 High Holborn, LONDON WC1V 6HB ; 13a Castle Street, EDINBURGH EH2 3AR ; 41 The Hayes, CARDIFF CF1 1JW ; 80 Chichester Street, BELFAST BT1 4JY ; Brazennose Street, MANCHESTER M60 8AS ; 258 Broad Street, BIRMINGHAM B1 2HE ; Southey House, Wine Street, BRISTOL BS1 2BQ. *All mail orders should be sent to* P.O. Box 569, London SE1 9NH.
UNITED REP. OF TANZANIA	*see address under* KENYA.
UNITED STATES OF AMERICA	*Single and bulk copies of individual publications (not subscriptions) :* Q Corporation, 49 Sheridan Avenue, ALBANY, NY 12210. *Subscriptions :* Subscription orders, accompanied by check made out to the Chemical Bank, New York, Account World Health Organization, should be sent to the World Health Organization, P.O. Box 5284, Church Street Station, NEW YORK, NY 10249. Correspondence concerning subscriptions should be forwarded to the World Health Organization, Distribution and Sales Service, 1211 GENEVA 27, Switzerland. *Publications are also available from the* United Nations Bookshop, NEW YORK, NY 10017 (*retail only*).
USSR	*For readers in the USSR requiring Russian editions :* Komsomolskij prospekt 18, Medicinskaja Kniga, MOSCOW — *For readers outside the USSR requiring Russian editions :* Kuzneckij most 18, Meždunarodnaja Kniga, MOSCOW G-200.
VENEZUELA	Editorial Interamericana de Venezuela C.A., Apartado 50785, CARACAS — Librería del Este, Av. Francisco de Miranda 52, Edificio Galipán, CARACAS.
YUGOSLAVIA	Jugoslovenska Knjiga, Terazije 27/II, BELGRADE.

Orders from countries where sales agents have not yet been appointed may be addressed to: World Health Organization, Distribution and Sales Service, 1211 Geneva 27, Switzerland, but must be paid for in pounds sterling, US dollars, or Swiss francs.