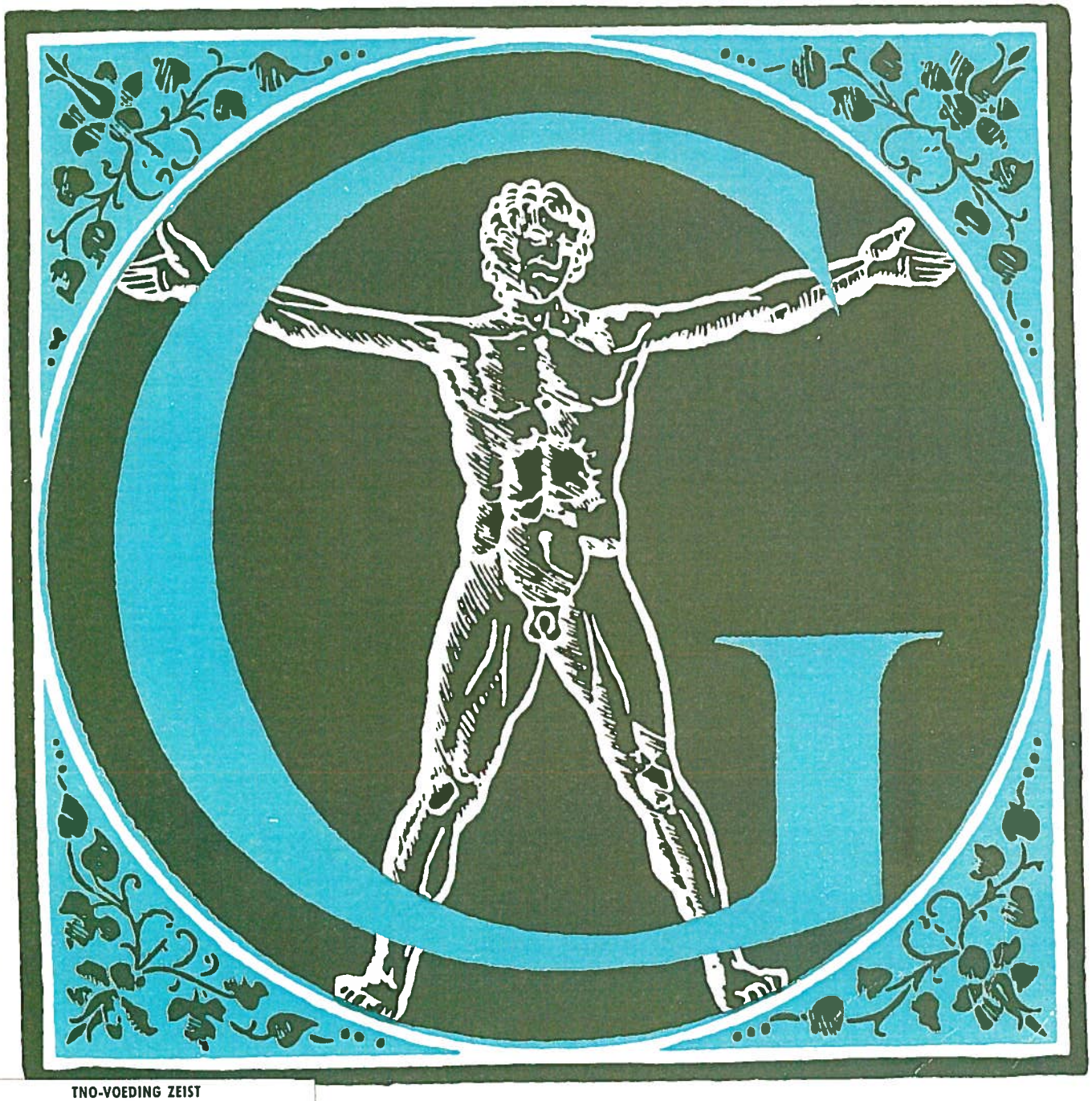
gezondheidsorganisatie tno



gezondheidsorganisatie tno

INHOUD

INTRODUCTION

In November of each year, TNO News is devoted to the coverage of a particular topic selected from the extensive research program of the Organization for Health Research TNO.

This time, various aspects of the Organization's activities in the field of cancer research are considered. It is the intention to provide the reader with up to date information regarding results in this field obtained in the various institutes and research units. Also included are progress reports on cooperative programs on cancer with University Hospitals and Institutes, as well as with independant researchers.

This issue begins with reports on probable causes of cancer, including viruses and carcinogenic chemicals, and a review of certain epidemiologic aspects of the cancer problem. Furthermore there are reports on immunology and aging in relation to cancer, followed by articles about early detection methods employing physical and biomedical techniques. Finally, various aspects of experimental tumor therapy are described and current approaches in chemotherapy and fast neutron therapy discussed. This publication may be regarded as an example of integrated efforts of the workers of the Organization for Health Research TNO in a specific field of biomedical research. Since cancer research is rapidly becoming a truly international effort, this issue is published in English, which should facilitate access by cancer researchers elsewhere to this program of the Organization for Health Research TNO.

M. A. Bleiker

Acting President

I. CAUSES AND EPIDEMIOLOGICAL FACTORS
Oorzaken en epidemiologische factoren

1. The role of viruses in the origin of cancer
   De rol van virussen bij het ontstaan van kanker

2. The occurrence of polynuclear aromatic hydrocarbons (PAH) in outdoor air
   Het voorkomen van polynucleaire aromaten (PNA) in de buitenlucht

3. Detection and measurement of the concentration of asbestos in air
   Bepaling van de asbestconcentratie in lucht

4. Asbestos and mesothelioma in The Netherlands
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The Role of Viruses in the Origin of Cancer

De rol van virussen bij het ontstaan van kanker

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Summary

Those tumour-inducing viruses, whose genome consists of RNA, are discussed. Evidence is accumulating that every mouse contains genetic information for a leukaemia virus and a mammary tumour virus. This information is expressed either spontaneously, during the aging process, after carcinogenic treatment, or after irradiation. It seems that some cells release virus spontaneously, or following irradiation, and that other cells become superinfected. This process, which seems to be necessary for the development of a tumour, can be intercepted by passive immunization. The animal's own immunological system can also be exploited to combat these viruses although they are partof the genetic make-up of an animal. The tumourous process can be considerably delayed by these interventions.

Some evidence has been obtained that certain forms of virus-induced leukaemia are hormone-dependent. This provides the perspective that this form of cancer can be controlled by natural regulating factors.

Samenvatting

In dit artikel worden die tumorvirussen besproken, waarvan het genoom uit RNA bestaat. Er zijn nu vele aanwijzingen dat iedere muis genetische informatie over een leukemievirus en een mammatumorvirus bevat. Deze informatie kan zowel spontaan, of gedurende het verouderingsproces, of na carcinogene behandeling of na bestraling vrijkomen. Waarschijnlijk produceren slechts enkele cellen spontaan virus of na bestraling en vindt daarna superinfectie van andere cellen plaats. Dit proces — dat nodig schijnt te zijn voor de ontwikkeling van een tumor — kan verhinderd worden door passieve immunisatie. Het immunologisch apparaat van een dier kan ook gestimuleerd worden tot afweer van deze virussen ondanks het feit dat zij onderdeel zijn van het erfelijk materiaal van het dier. Het ontstaan van enkele soorten tumoren kan op deze manier aanzienlijk vertraagd worden.

Uit ons werk met enige muizen-leukemievirussen kan geconcludeerd worden dat sommige vormen van leukemie hormoonafhankelijk zijn en daardoor eventueel onder controle gehouden kunnen worden door de hormonale regulatie van bloedcelvorming te beïnvloeden.

Introduction

In attempts to understand the genesis of human neoplasia, the cancer researcher usually still resorts to animal models. In experimental animals, cancers may be induced by a wide variety of agents. These include ionizing irradiation, a variety of chemical compounds, and certain viruses. Extrapolation to the human situation is a difficult task, in view of this complexity of etiological factors. Another problem is that cancers also develop spontaneously in animals, that is without any interference by the researcher apart from keeping them in good condition for as long as possible. There is little evidence that natural irradiation or carcinogenic chemicals in the animal food are responsible for the development of spontaneous tumours. This has led to the concept that such tumours represent an hereditary disease. This is supported by the finding of the familial occurrence of different kinds of cancer. From various spontaneous malignancies, however, a virus could be retrieved that gave rise to a similar tumour when inoculated into other animals. Although during the last decade many tumour viruses have been isolated from various animal species, the list of cancers with an unproved viral etiology is considerable longer. It should be borne in mind, however, that, upon inoculation and subsequent tumour induction, a virus often remains hidden in the developing cancer. Techniques, such as the immunological detection of some virus specific proteins, have been developed to recognize "footprints" of the virus. However, if a spontaneous tumour is caused by a hitherto unknown agent, such techniques will fail.

Up to now, the most important group of natural oncogenic viruses are those whose genome consists of RNA (*oncorna* viruses) - (Fig. 1, 2). In our laboratory, most studies are done with this group of agents. We work mainly with viruses that induce leukaemia in mice, but also to some extent with murine mammary tumour viruses (MTV). The latter group of agents is investigated because it has so many problems in common with the genus of murine leukaemia viruses such as ubiquity, ex-

*) Kindly provided by the Institute for Experimental Gerontology TNO

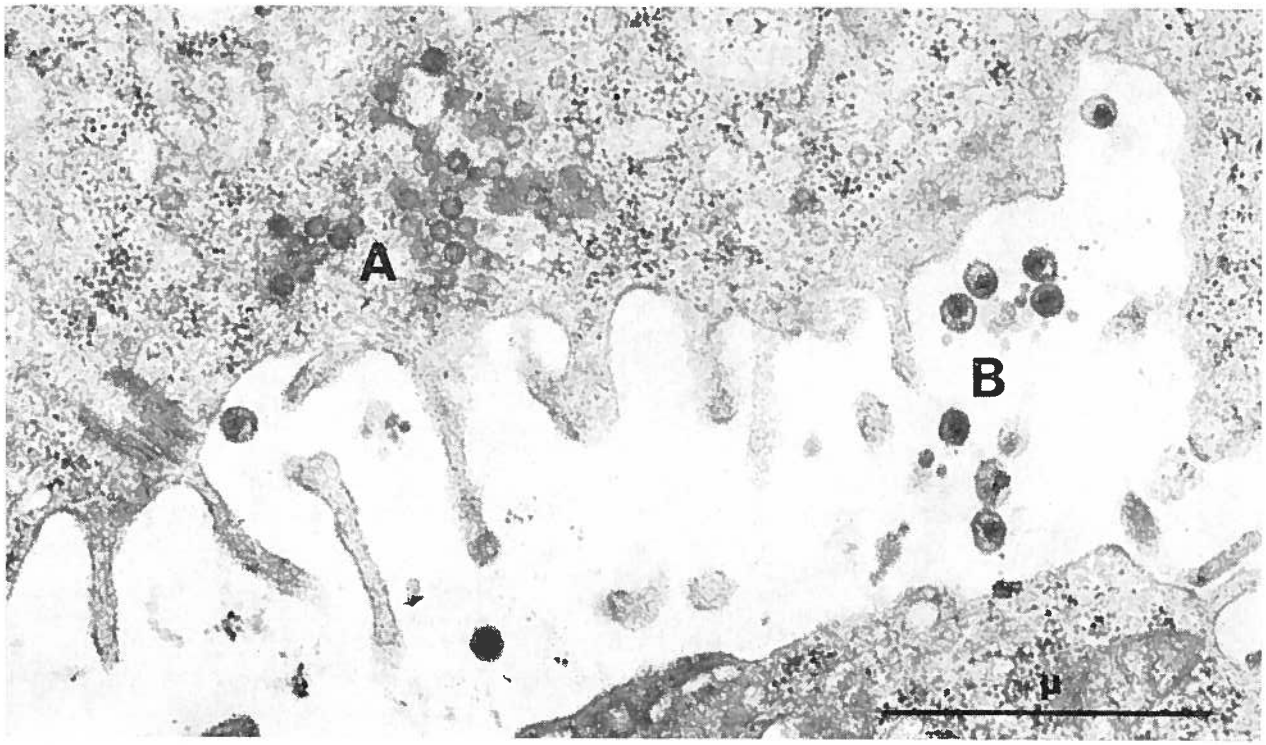


Fig. 1. Murine mammary tumour virus particles (B particles) in a tumour of BALB/c mice induced by the endogenous virus. In cytoplasm cluster of precursor stage (A particles) (43.000 ×).

pression in relation to aging, induction by irradiation. Furthermore, in experiments designed to detect avirulent leukaemia viruses in mice, we occasionally encountered low-oncogenic mammary tumour viruses. If possible, we now check for the presence of both viruses in an animal.

Cell differentiation and viral leukaemogenesis

There is now considerable evidence that *oncorna* viruses are capable of replication in various tissues, but will transform only a single cell type. For instance, leukaemia viruses replicate well in fibroblasts, but, unlike the sarcoma viruses, cannot transform them into tumour cells. It follows that cell differentiation has a considerable impact on the oncogenic activity of an *oncorna* virus. Leukaemogenesis offers a unique opportunity for the study of differentiation in relation to viral carcinogenesis, because so much is known about differentiation of the haematopoietic apparatus. The long standing interest in bone marrow transplantation at the Radiobiological Institute has resulted in the accumulation of experience in the field of haemopoiesis, which is extremely helpful in our studies. So far, investigations at the Radiobiological Institute concentrated on the Rauscher leukaemia virus (RLV). This agent induces a rapidly developing erythroblastosis in mice, which is characterized by splenomegaly and an excess of erythroblasts in the peripheral blood (fig. 3 and 4). Animals die from this disease within a few weeks after inoculation. For some years, we have attempted to test the hypothesis that haemopoietic stem cells play an important role in the genesis of this disease. A considerable increase of stem cells in the spleen during the leukaemic process has been observed by Brommer [1]. These stem cells are able to overcome the bone marrow syndrome in lethally irradiated mice by proliferation and differentiation, but in such recipients a leukaemia gradually develops. This leads to death after some months. With highly specific antisera to the virus, it could be

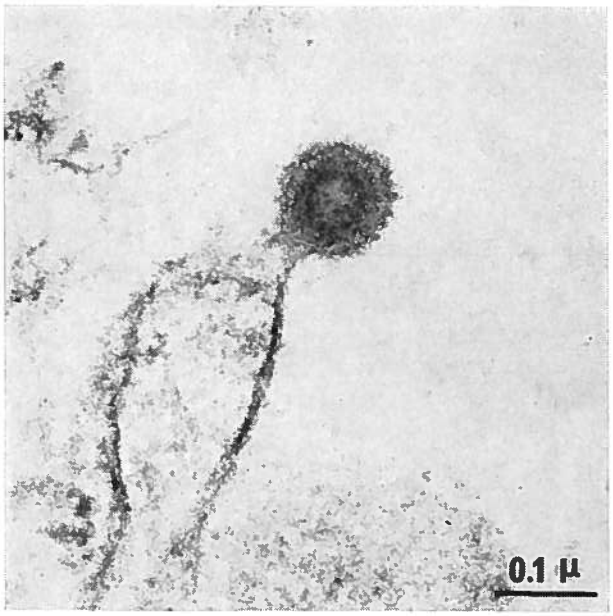


Fig. 2. Murine sarcoma virus particles (C particles) produced by sarcoma cells infected with a leukaemia virus (138.000 x).

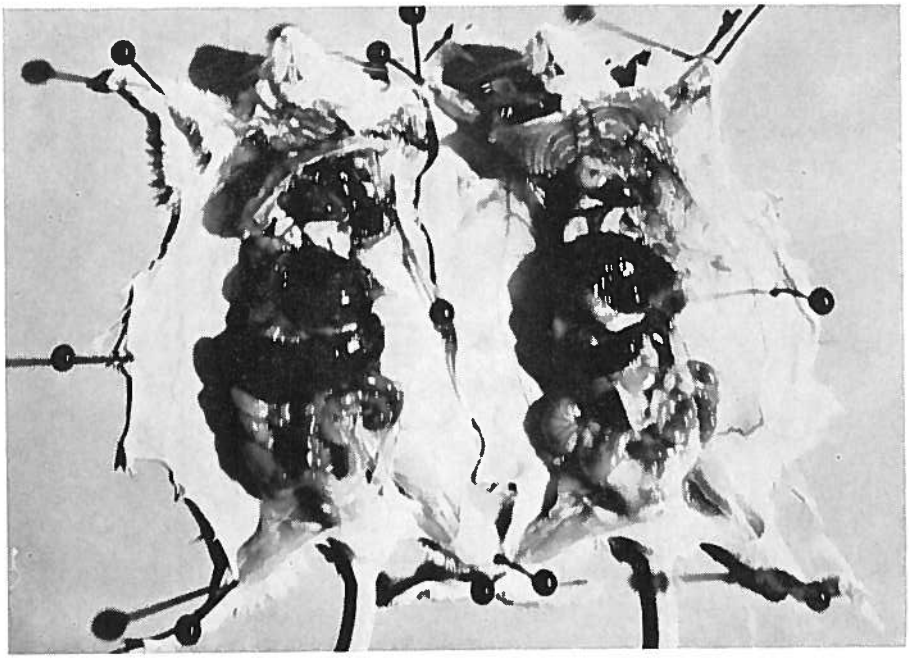
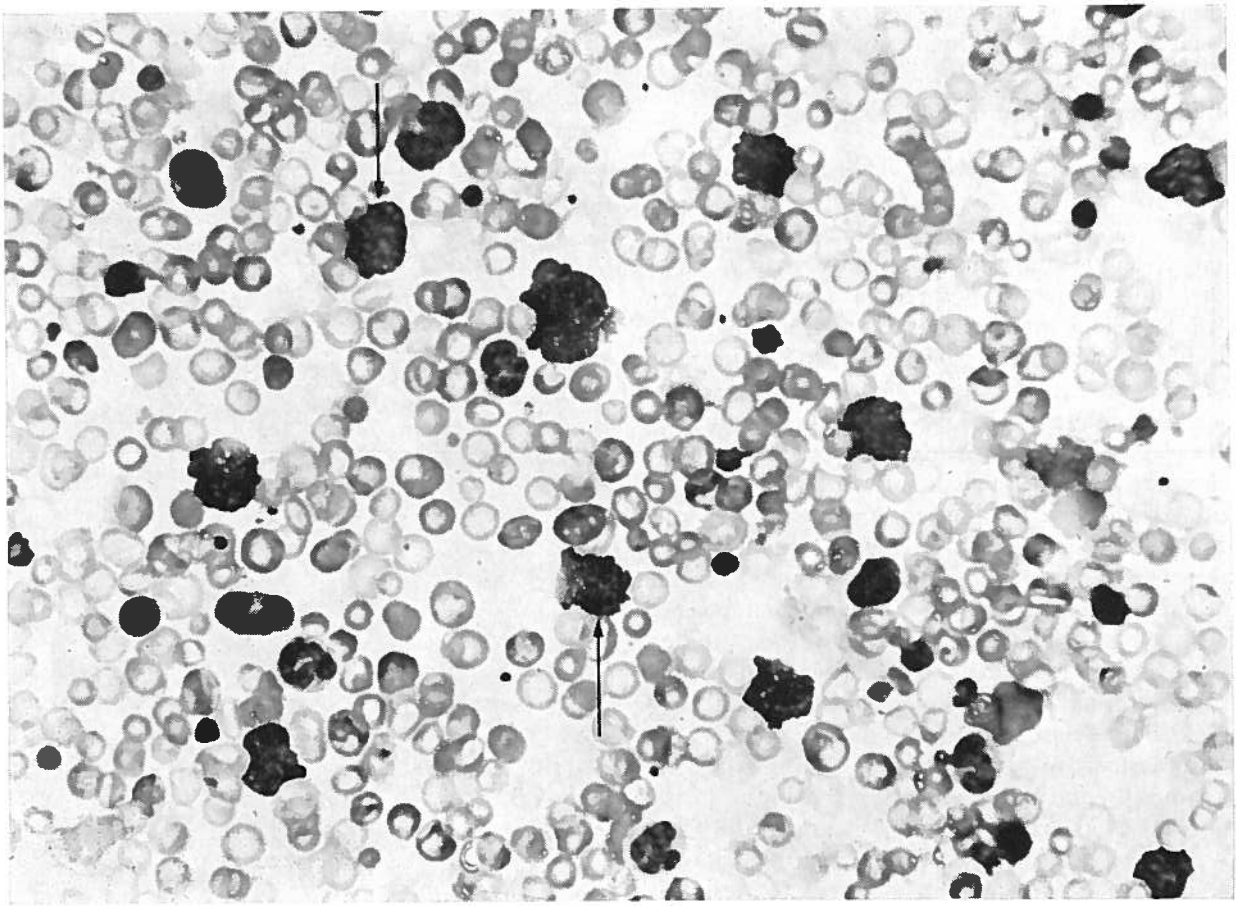


Fig. 3. Enlarged spleen of a BALB/c mouse three weeks after inoculation with Rauscher leukaemia virus (left); control mouse (right).

Fig. 4. Excess of erythroblasts in the peripheral blood of a BALB/c mouse infected with Rauscher leukaemia virus.



demonstrated that virus is produced in stem cells of leukaemic mice. These cells have been altered, in that they are significantly less susceptible to the cytotoxic action of normal rabbit serum, but are more sensitive to the plant lectin, Concanavalin A. They, nevertheless, seem to be capable of normal differentiation, except for the erythroid series.

It was also found that various treatments, which promote the proliferation of stem cells but do not effect the erythroid cell compartment, strongly enhance the leukaemic process. For instance, various antigens or antiplatelet serum, which affect the stem cell pool, accelerate development of the leukaemic disease. On the other hand, partial eradication of the stem cell pool (e.g. by irradiation) leads to a manifold reduced leukaemic response. These results suggest that the target cell for the virus is the haemopoietic stem cell [2]. It is interesting to note that manipulation of the stem cell compartment after virus inoculation also affects the development of the disease. This indicates that leukaemic cells are continuously being recruited from this pool.

We found the Rauscher leukaemia virus to be a mixture of several virus types. The erythroblastosis-inducing principle proves to be defective, *i.e.* it needs another type of leukaemia virus to induce the erythroblastic disease. We have obtained indications that the helper virus is needed for the reproduction of the erythroblastosis agent. It seems that continuous infection of new cells is necessary for the development of the disease.

With the aid of chromosomal markers, among other things, we have established that Rauscher leukaemic cells cannot be transplanted, since the resulting leukaemia had the host karyotype (fig. 5). It must have been induced, therefore, by the virus harboured by the inoculated cells. This indicated

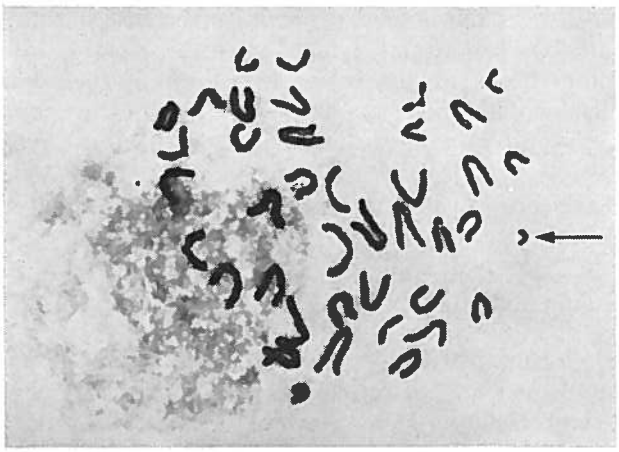


Fig. 5. T6 chromosomal marker (arrow) in a cell of a leukemia induced in (CBA/T6 x BALB/c) F1 mice by inoculation with BALB/c Rauscher virus leukaemic cells (1000 x) (Courtesy of Dr. E. J. P. Brommer)

that the leukaemic cells are not truly autonomous neoplastic cells *i.e.* they are not able to proliferate independently from regulatory systems. We do not exclude the possibility, however, that such cells emerge in a late stage of the disease.

On the basis of these results the hypothesis was advanced that erythropoietic stimulation of RLV-infected stem cells would give rise to abnormal erythroblasts whose maturation has been arrested. The lack of physiologically normal cells would result in continuous erythropoietic stimulation and, in this way, in the perpetuation of the disease (fig. 6). The hormone-dependency of the disease was shown by the fact that after RLV-infection, blood transfusion-induced polycythemia strongly suppresses the erythroblastosis, while erythropoietic stimuli accelerate the disease.

We expect several other virus-induced leukaemias

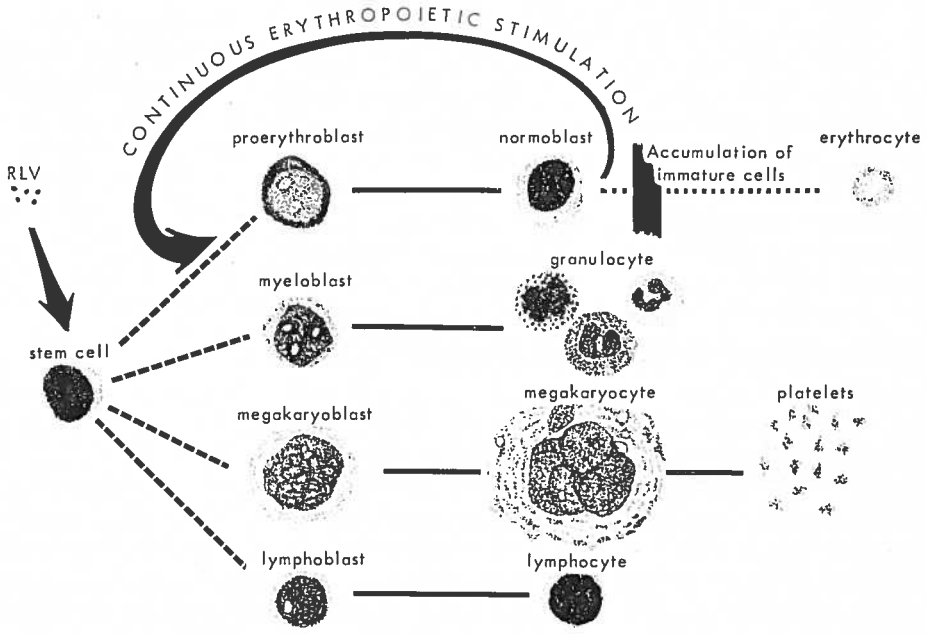


Fig. 6. Schematic representation of the role of the haemopoietic stem cell in leukaemogenesis by Rauscher virus.

also to be hormone-dependent. In the case of autonomous leukaemias, which are transplantable into other hosts, an interesting hypothesis is that they also would find their origin in pluripotent stem cells and, that upon differentiation into a given direction, malignant derailment takes place. We have initiated investigations into this problem with virus strains other than RLV.

Aging and viral oncogenesis

Understandably, most tumour virologists prefer to work with fast acting viruses such as the Rauscher erythroblastosis virus. However, cancer is mostly a disease of old age. In several late occurring tumours we could demonstrate an *oncorna* virus. As will be discussed below, three separate reasons may be cited for the late appearance of virus-induced tumours:

a. host-dependent resistance to the virus,

b. low virulence of the virus,

c. late emergence of an endogenous *oncorna* virus.

(Ad. *a.*): Despite neonatal infection with an *oncorna* virus, it often takes several months, and sometimes more than a year, before a tumour appears. In the case of the mammary tumour virus (MTV), we have obtained evidence that immunological resistance against the virus plays an important role in this phenomenon [3]. This has recently been demonstrated also to be the case for viral leukaemogenesis [4]. We have some indications that a tumour finally emerges because of the production of so-called enhancing antibodies. These react with the virus, but prevent the more effective reactions of other immunological systems such as cell-mediated immunity. It is not completely clear, however, how these effective systems interfere with the virus in the latency stage.
Upon inoculation with an *oncorna* virus some inbred mouse strains, develop a few tumours which appear only late in life. In some cases, this may be due to an excellent immunological system, but, in general, it proves to be of a more specific nature: a mouse strain can be susceptible to MTV but resistant to a leukaemia virus. Among the various possible mechanisms, intracellular interference with the replication of the virus seems to be important. We have observed this phenomenon in several instances [5].

(Ad. *b.*): If a purified *oncorna* virus does not induce a tumour within a few months, many tumour virologists are tempted to call it non-oncogenic.
It is to be expected, however, that most naturally occurring tumour viruses (also in man) are of a low-virulent nature. It is worthwhile, therefore, also to study the lesser oncogenic virus strains. We have some evidence that low-virulence is often associated with a poor rate of replication; but, this is probably not the sole mechanism.
It sometimes occurs that even inoculation of high doses of virus and some years of patience are not sufficient for the demonstration of oncogenic potential. After repeated experimentation we failed to detect an MTV by bioassay in the late occurring mammary tumours of the ND2 mouse strains. This was despite the fact that we had serological evidence for the presence of the virus. However, when spleen cells of one year old mice were injected into young ND2 mice, several mammary tumours were obtained at a comparatively young age; cellfree preparations did not give this result. It is known that MTV replicates in the spleen, and that infectivity is greatly reduced if cellfree preparations are made [6].
In the experiment mentioned above many recipients also developed leukaemia. This could be due to the presence of some dormant leukaemic cells in the spleen cell inoculum which gradually start proliferating in the young hosts. By making use of a chromosomal marker, this possibility could be excluded. The resultant leukaemia did not have the karyotype of the donor cells. Obviously the leukaemia was induced by a virus present in the old spleen cells which cannot be transferred acellularily. In the meantime, it was found that intact cells also provide considerable protection to virulent leukaemia viruses such as RLV. It seems that transplantation of intact cells from old animals provides a good means of detecting avirulent cancer viruses.

(Ad. *c.*): We observed in several old mice (more than 2 years) of the low-cancer strain C57BL*), that spleen cells produce antigens of a mammary tumour virus, or a leukaemia virus (MLV). This was achieved by using the indirect immunofluorescence method employing specific antisera directed against either virus group.
In collaboration with Dr. J. H. Daams of the Netherlands Cancer Institute, many so-called MTV-free strains were then investigated for the appearance of virus-specific proteins in relation to aging. The average age at which animals of such a strain become positive correlated well with the spontaneous mammary tumour incidence of that strain. This suggests an all-viral etiology for this disease in mice [7]. Preliminary results with the murine leukaemia virus suggest the same for the lymphoreticular malignancies.
In collaboration with the group of Dr. S. Spiegelman of the Institute of Cancer Research, Columbia University, New York, a search is being made for virus-specific RNA in tissues of young and old mice from various strains. The procedure is as follows: with the aid of the virus' own reverse transcriptase a radioactively labelled DNA copy of the viral RNA is made. This DNA product is then used as a probe for finding virus-specific RNA by

hybridizing it with cytoplasmic RNA [8]. After having assessed the specificity of this reaction, it was employed with tissues of low-cancer strains. No virus-specific RNA (MTV or MLV) is found in tissues of young mice from such strains. In several instances, however, it was found in those of old mice. Sometimes viral RNA could be detected where virus-specific antigens were not. It is (obvious) that this method is a very efficient tool in the search for *oncorna* viruses.

Viral aspects of radiation and chemical carcinogenesis

There has lately been a revival of the theory that all tumours are caused by viral factors, even when the initial stimulus is a chemical compound or ionizing irradiation [9]. Up to now, convincing evidence for such an hypothesis has only been demonstrated in mouse leukaemia [10] and the mammary tumour system in mice [11]. From these sources, *oncorna* viruses, that could reproduce the disease, were recovered from "non-virally" induced tumours. In addition, we found that, after irradiation or urethane-treatment, *oncorna* virus antigens could be found in mice that ordinarily do not show the presence of such viruses [7]. With the molecular hybridization method of Spiegelman [8], MTV RNA was found in spleens of carcinogen treated mice while the controls were negative. The results are compatible with the hypothesis we advanced four years ago [12], that every mouse would contain genetic information for both MTV and MLV; and, that such information can be expressed either spontaneously, as in the high-cancer strains, or after carcinogenic treatments (fig. 7). It should be noted here that this hypothesis comprises elements of the genetic concept of cancer and the all-viral theory on the origin of the disease.

In the course of another series of investigation Balner [13] observed that, contrary to expectations, a particular anti-mouse lymphocyte serum inhibited the radiation-induction of leukaemia in C57BL mice. We have found that this serum contained antibodies which could precipitate MLV virus particles and which neutralize RLV. This was in contrast to other antilymphocyte sera which did not contain detectable levels of anti-viral antibodies and which do not interfere with radiation-leukaemogenesis. These results suggest that the leukaemia inhibition was achieved by the anti-viral properties of the serum. The most likely explanation for this phenomenon is that the antibodies prevent the spread of virus from cells that release virus after irradiation. Such a spread of virus also seems to play an important role in mouse strains having a high incidence of spontaneous leukaemia. It is not completely understood why generalization of the virus infection would be necessary. One could envisage that the few cells which are induced to produce virus can become malignant themselves and induce the leukaemia.

So far, chemically induced sarcomas have not yielded sarcomatogenic virus. Klein [14] at this institute developed a continuous cell line growing *in vitro* which does not transform spontaneously

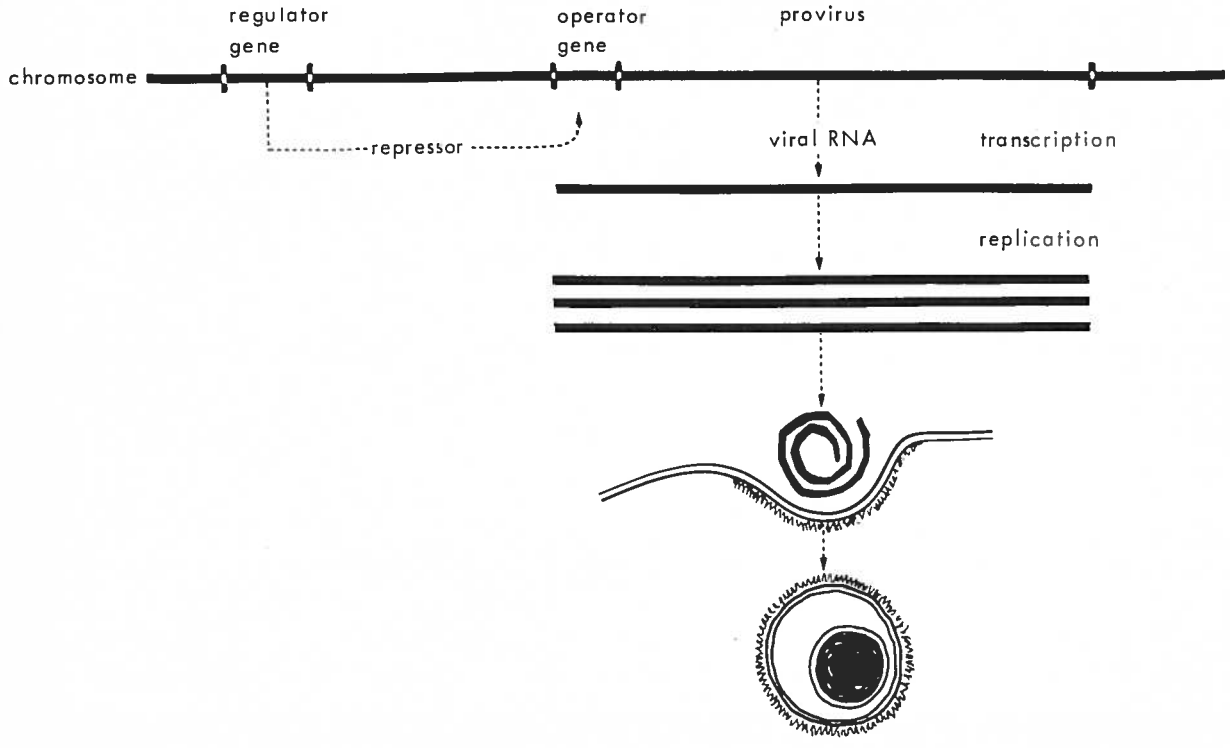


Fig. 7. Schematic representation of the hypothesis on the presence of genetic information for oncorna viruses.

under the culture conditions employed, but which is readily tranformed by methylchlolanthrene [14]. From such transformed cells, no tumour virus could be retrieved. We found that, following infection with a leukaemia virus these cells sometimes produce a virus that induces sarcomas in mice and which can transform mouse and rat cells *in vitro* (fig. 8). Our tentative explanation for this phenomenon is as follows (fig. 9): methylcholanthrene induces the expression of the genetic information for sarcomatous transformation. The released viroid can perpetuate itself, but does not contain information for the production of complete virus particles. The superinfecting MLV provides the genes required for these functions. A new viral entity is subsequently produced which contains the sarcoma-inducing genes and the leukaemia virus genes for virion production.

Immunity to *oncorna* viruses

As the spread of virus in the organism seems to be an important factor in various systems of leukaemogenesis and can be intercepted by passive immunization, the question rises whether the animal's own immune system cannot be employed in the control of the disease. Mice can be successfully vaccinated against artificial infections with *oncorna* viruses, but this has little relevance to the natural disease since horizontal transmission seldom takes place. In view of the genetic transmission of *oncorna* viruses, it was believed by many that an animal would be immunologically tolerant to its endogenous tumour viruses. This often led to the pessimistic opinion that active immunization to natural *oncorna* viruses would be virtually impossible. Fortunately, this tolerance concept proved to be wrong. Spontaneous antibodies to *oncorna* viruses, or their internal proteins, can often be detected in old animals.

We have attempted to immunize the high-leukaemia mouse strain AKR, and the high-mammary cancer strain GR, against tumour development. Vaccination with their own virus strains had no effect whatsoever, but the use of related but antigenically different virus strains, gave a significant delay in the development of either one of the predominant malignancies. In both cases, tumours appeared about two months later than normally. However, in a radical attempt to render these mouse strains tumour-free by repeated immunization, we obtained the reverse effect, namely that tumours appeared signifcantly earlier. This was associated with the production of antibodies that could precipitate intact virions, and virus-specific proteins, in double immunodiffusion tests (fig. 10).

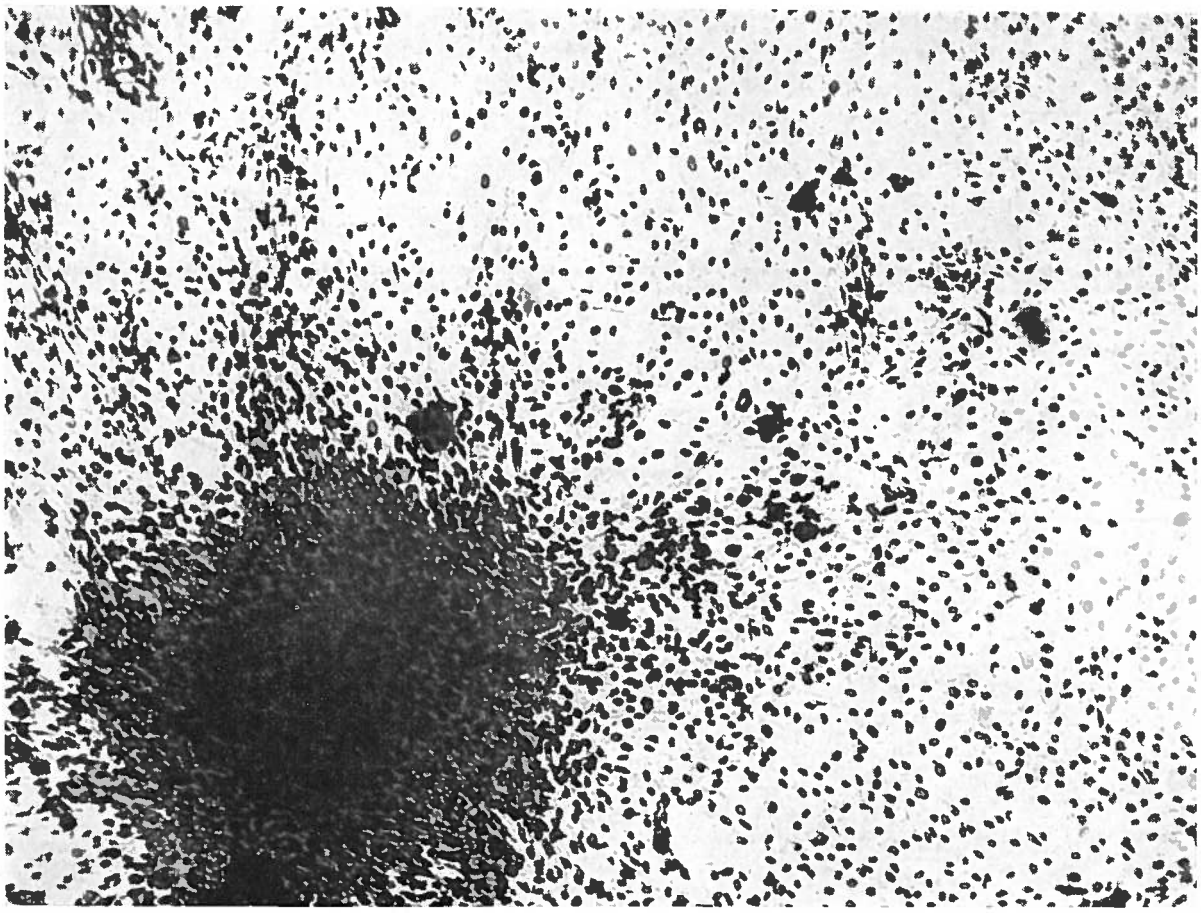


Fig. 8. Focus of rat cells transformed by a sarcoma virus isolated from chemically transformed cells infected with a leukaemia virus.

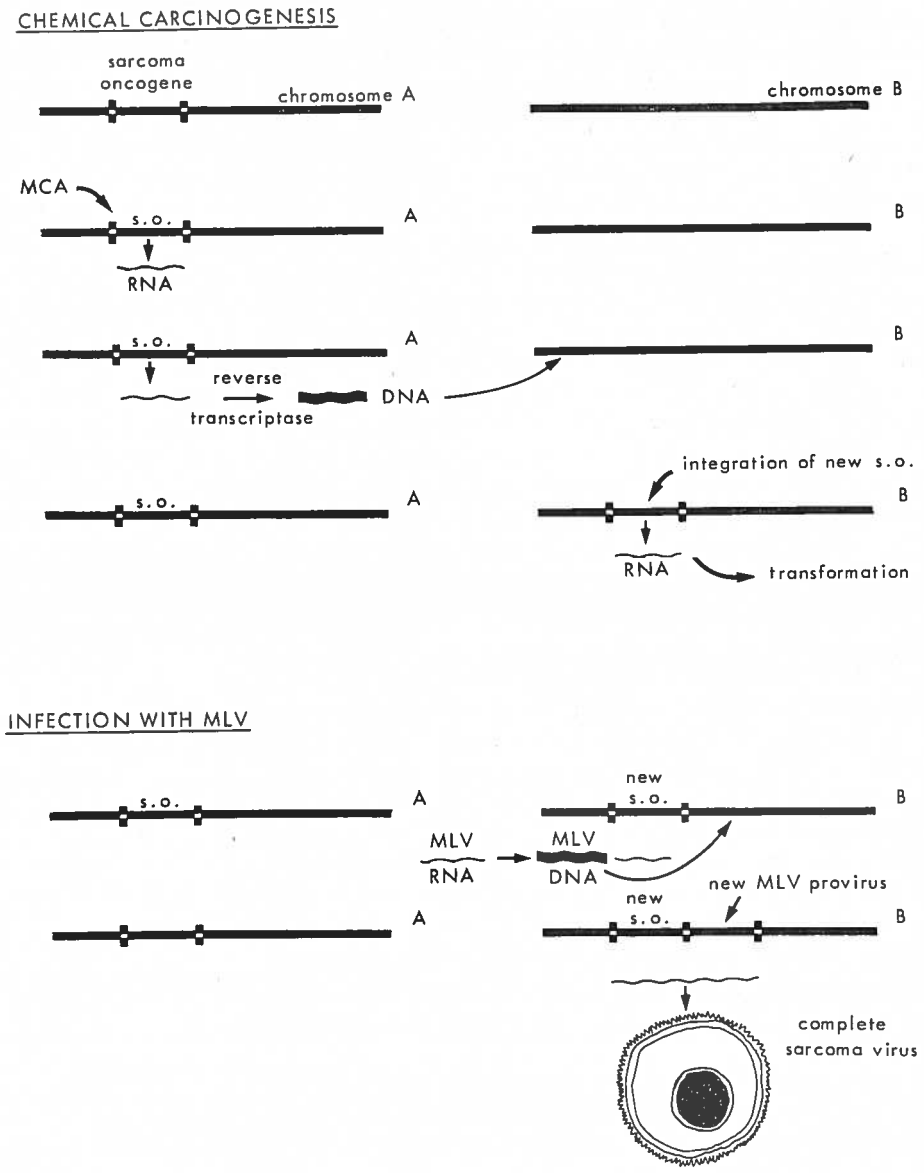


Fig. 9. Schematic representation of the hypothesis on retrieval of a sarcoma virus from chemically transformed cells following superinfection with a leukaemia virus.

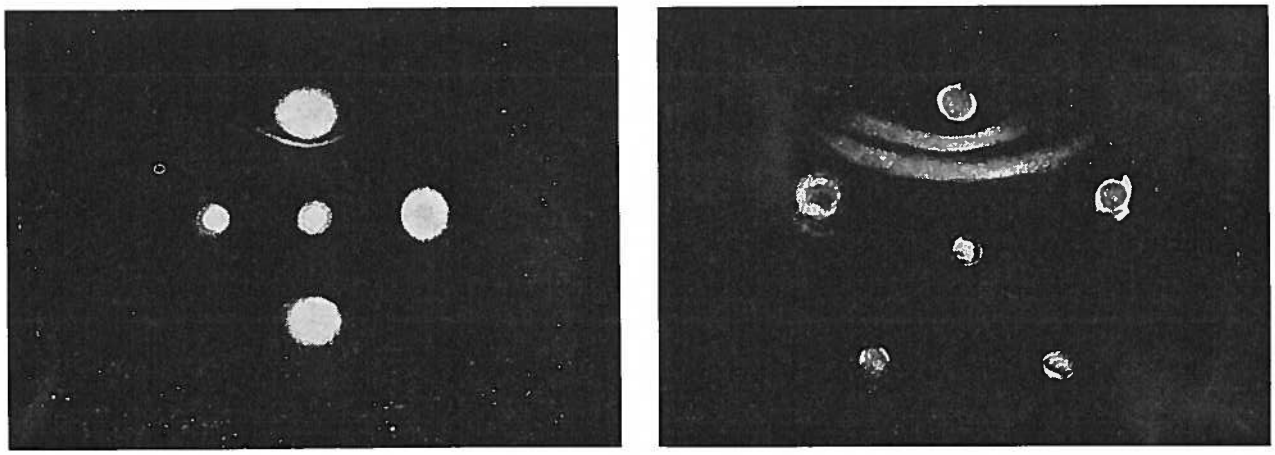


Fig. 10. Double immunodiffusion tests on sera of GR mice hyperimmunized with MTV. A: Precipitation of complete virus particles (upper well MTV, center well mouse serum); B: Precipitation of soluble antigens (upper well ether-disrupted MTV, center well mouse serum).

We assume that these antibodies have enhancing properties and that their production is the cause of the early emergence of tumours in these experiments.
Most likely, the induced anti-viral immunity does not simply interfere with the spread of virus from one cell to the other by means of antibodies. After infection has been established, immunity probably keeps the disease process in check by killing virus producing cells. It is likely that the latter phenomenon is cell-mediated, and it is possible that antibodies, which would be favourable in the initial stage of virus release, counteract the action of immunocytes in the second phase of viral carcinogenesis. It is obvious that considerably more fundamental research in this field is needed in order to develop vaccination methods, which provide a high degree of protection against the "spontaneous" development of some tumours in mice.

Perspectives for the human cancer problem

Animal tumour virology is conducted with the hope that several human tumours are also caused by a virus. Evidence is accumulating that man does indeed harbour *oncorna* viruses as assessed by some biochemical properties such as 70s RNA, reverse transcriptase, and homology of their nucleic acid to that of murine tumour viruses [8]. However, this is not sufficient evidence for the etiological role of these viruses in the tumours from which they have been isolated. From animal studies, it becomes clear that often an *oncorna* virus is merely present as a passenger in a given tumour line, for instance, leukaemia viruses in a mammary tumour.
Bioassays of human tumour material, which can only be done in heterologous hosts, have yielded only negative results. This is not so surprising, since *oncorna* viruses are often considerably less oncogenic (or not at all) to mammalian species other than those from which they have been isolated. But as became obvious in our own studies, even in the homologous situation, *oncorna* virus-induced tumours need not yield an infectious entity. Techniques are being developed in order to induce the virus to produce complete particles.
In some cases, oncogenic properties can also be deduced from the in vitro transformation of cells. With regard to the *oncorna* viruses, this only works for the sarcoma viruses at the moment. Models for the induction of leukaemia *in vitro* must be developed for animals. These will enable us to demonstrate, in the future, the leukaemogenic activity of a human virus. There are indications as haemopoietic cells can nowadays be cultured with great success [15], viral leukaemogenesis can soon be studied in tissue culture.
From the studies *in vivo* reported in this paper, it appears that some virus-induced leukaemias may be regarded as hormone-dependent tumours. With an increasing insight into the hormonal regulation of haemopoiesis, it can be envisaged that some forms of leukaemia can be treated more specifically than with the relatively crude chemotherapeutic agents.
The most important aspect of tumour virology is, however, that it could lead to cancer prevention. Animal experimentation, especially in the mouse mammary tumour system, provides the prospect that vaccination may prove to be a good prophylaxis against tumours such as breast cancer, or certain forms of leukaemia.

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The Occurrence of Polynuclear Aromatic Hydrocarbons (PAH) in outdoor air [1)]

Het voorkomen van polynucleaire aromaten (PNA) in de buitenlucht

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Summary

PAH are produced by the inefficient combustion of coal, oil, gasoline, and other organic matter.
Some of these hydrocarbons have carcinogenic properties; so their presence in air may be of importance in relation to the development of lung cancer.
Since December 1964, the PAH content of the atmosphere of the western part of the Netherlands has been determined on a regular basis by the atmospheric pollution division of the Research Institute for Public Health Engineering TNO. From the results of the measurements, it is found that the atmospheric PAH levels have continuously decreased, and that, in cities, the most important source of these compounds is not automobile traffic but, probably, the burning of domestic fuels.

Samenvatting

PNA zijn produkten van onvolledige verbranding van kool, olie, benzine en ander organisch materiaal.
Sommige van deze koolwaterstoffen hebben carcinogene eigenschappen zodat het van belang is hun aanwezigheid in de lucht in verband met het ontstaan van longkanker te onderkennen.
Sinds december 1964 wordt door de afdeling Buitenlucht van het Instituut voor Gezondheidstechniek TNO regelmatig het gehalte aan PNA in de buitenlucht in de Randstad Holland bepaald.
Uit de meetresultaten volgt dat de PNA-niveaus in de buitenlucht voortdurend zijn afgenomen en dat de voornaamste bron van de PNA in de steden niet het autoverkeer, maar waarschijnlijk „huisbrand" is.

Introduction

Polynuclear aromatic hydrocarbons (PAH) are produced by the inefficient combustion of coal, oil, gasoline, and other organic matter. Research on the occurrence of PAH in outdoor air is of importance because of the fact that several possess carcinogenic properties. Mortality, particularly due to lung cancer, has according to Hueper [1] increased since 1900 by more than 3000%. This is especially true with respect to large cities. The occurrence of PAH in outdoor air is often related to the development of lung cancer.
It should be pointed out, however, that only the potential carcinogenic properties for man of some of the PAH, occurring in outdoor air, have been demonstrated. Epidemiologic research has failed up to now to prove this relationship. A very disturbing factor in this epidemiologic research is the smoking of cigarettes. It has been demonstrated that substances with pronounced carcinogenic effects also occur in cigarette smoke. A list of 34 PAH and their carcinogenic properties is given in Table 1. This table is far from up to date. Many more PAH occur in outdoor air, while regularly new ones are discovered. There is also an indication in this table which of the hydrocarbons have been determined on a regular basis by the atmospheric pollution division of the Research Institute for Public Health Engineering TNO since 1964. It can be seen that 9 of these compounds are tested for on a regular basis.
Although only 4 or 5 of these (viz. 1.2-benzanthracene, chrysene, 1.2-benzopyrene, 3.4-benzopyrene and coronene?) have carcinogenic properties, knowledge of their occurrence is useful in attempting to identify the source of pollution.

The results of research have shown that the ratio of some of these PAH in outdoor air depends upon the source.
If the source is automobile exhaust gas, a ratio of
3.4-benzopyrene/coronene of < 0.4-1;
3.4-benzopyrene/1.12-benzoperylene of 0.2-0.6;
and pyrene/3.4-benzopyrene of 2-6 is found.

In domestic fuels these ratios are
3.4-benzopyrene/coronene > 1.7
3.4-benzopyrene/1.12-benzoperylene > 0.8
pyrene/3.4-benzopyrene 0.3-0.8.

From these ratios it follows that higher percentages of pyrene 1.12-benzoperylene and coronene are indicative of traffic as the source. This is especially true for the pyrene content.

* Publication no. 440 of the Research Institute for Public Health Engineering TNO, Delft.

TABEL 1

NAME	OTHER NAMES IN USE	B.P IN °C	CARCINOGENIC PROPERTIES	FORMULA
(BENZENE)	(BENZOL), (PHENE)	80.1	–	
NAPHTHALENE		210.8	–	
NAPHTHACENE	2.3–BENZANTHRACENE, TETRACENE	SUBL.	?	
ACENAPHTHENE	NAPHTHYLENEETHYLENE	278	–	
ACENAPHTHYLENE		265–75 (PAR. D)	–	
FLUORENE	2.3–BENZINDENE, DIPHENYLENEMETHANE	295	–	
PHENANTHRENE		336	–	
ANTHRACENE		340 COR. 226,5	–	
PYRENE	BENZO [d,e,f] PHENANTHRENE	393	–	
FLUORANTHENE	INDRYL, 1.2–BENZACENAPHTHENE	383	–	
ALKYLPYRENE			?	
1.2–BENZOFLUORENE	CHRYSOFLUORENE	398	–	
2.3–BENZOFLUORENE			?	
9.10–BENZOPHENANTHRENE	TRYPHENYLENE, 1.2, 3.4–DIBENZONAPHTHALENE	425	–	
1.2–BENZANTHRACENE	BENZO [a] ANTHRACENE, TETRAPHENE, NAPHTHANTHRACENE, 2.3–BENZOPHENANTHRENE	437	+	
CHRYSENE	1.2–BENZOPHENANTHRENE, 1.2, 5.6–DIBENZONAPHTHALENE	448	+	
PERYLENE	PERI–DINAPHTHYLENE	350–400 SUBL	–	
3.4–BENZOPYRENE	BENZO [a] PYRENE	495	+	
1.2–BENZOPYRENE	BENZO [e] PYRENE	492	+	
2.3–BENZOFLUORANTHENE			?	
3.4–BENZOFLUORANTHENE	BENZO [b] FLUORANTHENE		+	
10.11–BENZOFLUORANTHENE	BENZO [j] FLUORANTHENE		+	
11.12–BENZOFLUORANTHENE	BENZO [k] FLUORANTHENE		?	
1.12–BENZOPERYLENE	BENZO [g,h,i] PERYLENE	503	+	
ANTHANTHRENE			–	
CORONENE	1.12, 6.7–DIBENZOPERYLENE, HEXABENZOBENZENE	525	?	
1.2, 5.6–DIBENZANTHRACENE	DIBENZO [a,h] ANTHRACENE		+	
INDENO [1.2.3, c.d] PYRENE			+	
1.2, 3.4–DIBENZOPYRENE			+	
1.2, 4.5–DIBENZOPYRENE			+	
3.4, 8.9–DIBENZOPYRENE			+	
NAPHTO [2'.3' : 1.2] PYRENE			?	
INDENO [1.2.3–c.d] FLUORANTHENE	4.5–[PERI–PHENYLENE] FLUORANTHENE		?	
PICENE	DIBENZO [a,i] PHENANTHRENE	518–520	?	

LIST OF 34 POLYCYCLIC HYDROCARBONS AND THEIR CARCINOGENIC PROPERTIES

——— HYDROCARBONS OF WHICH THE CONCENTRATION IN AIR IS MEASURED BY TNO'S ATMOSPHERIC POLLUTION DIVISION

According to Sawicky [2] the ratio of pyrene/3.4-benzopyrene in exhaust gas from automobiles varies between 40-60. The explanation for the fact that in the outdoor air ratios of only 2-6 are usually found is that 3.4-benzopyrene is relatively stable in the atmosphere while pyrene is less so.

In addition to the compounds discussed up to now, other types of organic carcinogens have been demonstrated to occur in outdoor air. These are

polynuclear aza-heterocyclic compounds, such as:
dibenzo 2 (a, h) acridine and
dibenzo 2 (a, j) acridine,
polynuclear imino-heterocyclic compounds,
polynuclear carbonyl compounds, such as:
7H-benzo (d, e) anthracene-7-one,
alifatic and olefinic epoxides,
organic peroxides and lactones.

Even though it has been proved that these substances are present in the atmosphere, little systematic research has been done on their concentrations and trends in outdoor air. For this reason, these will be ignored in this publication.

There is little information regarding synergistic and antagonistic effects among these organic carcinogens, and these organic carcinogens and other compounds.
It is known, for example, that phenols potentiate and enhance the carcinogenic properties of organic carcinogens. It has also been shown that the carcinogenicity of 3.4-benzopyrene is enhanced if this substance is adsorbed onto certain inert particles — especially iron oxide.
Anti-carcinogenic effects have also been demonstrated amongst PAH. Kotin and Falk [3] have shown that dibenzo (a, h) anthracene, a compound which is carcinogenic, looses its carcinogenicity in the presence of hydro-and hexahydro derivatives of dibenzo (a, h) anthracene as well as benzo (a) antracene and phenanthrene.
Other authors have also pointed out that certain compounds, with little or no carcinogenicity, may diminish the activity of very potent chemically related carcinogenic compounds.
In the light of this information, it follows that the carcinogenicity of complex chemical mixtures (containing carcinogens) cannot be predicted solely on the basis of chemical analyses of the composition of these mixtures.
Of all the PAH occurring in outdoor air, 3.4-benzopyrene possesses the strongest carcinogenic properties.
The yearly emission of 3.4-benzopyrene in the USA from several sources is given in Table 2.
On a country wide basis, the contribution from traffic is low about 5%.

Table 2.

Yearly 3.4-benzopyrene emission in the USA
Source: Air Poll. Aspects of Organic Carcinogens (1969)
Litton Syst. Inc. Prep. for the APCA

Heating and generation of electricity	421.6 ton
Burning of wastes	20.2 ton
Industry	18.8 ton
Traffic	~ 20.6 ton
total	481.2 ton

For residential areas and street crossings with a high traffic density, the contribution from this source is higher in the summer.
No correlation, however, has been found between atmosperic levels of 3.4-benzopyrene and the death rate from lung cancer.

In this connection Şawicky [4] states:

"The number and type of carcinogens found in the atmosphere indicate that attempts to correlate carcinogenicity of the mixtures in our chemical environment with concentrations of 3.4-benzopyrene are probably naive in most cases and spring from our lack of knowledge of the composition of the mixtures with which we are dealing".

No standards exist for the concentration of PAH in outdoor air. Because of their potential carcinogenicity, the emission into the atmosphere should be kept to an absolute minimum.

Detection and analysis of PAH

Sampling occurs by passing large amounts of air through filter material.
Depending on the concentration in the atmosphere, from 30 to over 300 m^3 of air are needed for a valid analysis.
To prevent losses of PAH by oxidation, and decomposition by light, especially UV light, the filters should be stored in cyclohexane in the dark; preferably in a refrigerator.
The analytical techniques most commonly used are chromatographic and spectrophotometric.
The analytical technique which is used in our laboratory may be summarized as follows.
Following collection of the samples, the filters are placed for 16 hours in a soxhlet containing cyclohexane for purposes of extraction.
If necessary, the extract obtained can be purified by shaking it with a mixture consisting of 4 parts methanol and 1 part water.
Following this, most of the cyclohexane is removed by evaporation. The extract can now be separated into fractions by the use of column chromatography.

Table 3. *PAH content in µg/1000 m³; 24 Oct. 1956 - 10 Apr. 1957*

	Conway Valley (Caernarion)	Liverpool (Princess Road)
Pyrene	2	50
Fluoranthene	5	67
3.4-Benzopyrene	1	166
1.12-Benzoperylene	1	68

The PAH content of the fractions is then determined by spectrophotometric measurement at wave lengths between 270-400 nm. The concentrations of the different components are expressed as µg/1000 m³ of air.

The occurrence of PAH

Extensive research in America has shown that PAH are present in outdoor air almost everywhere. The concentrations in the big cities are much higher than in suburban areas. The average concentration of 3.4-benzopyrene in the cities during the year 1966 was 3 µg/1000 m³, while in the areas outside the cities this value was only 0.3 µg/1000 m³. Commins [5] also points to the great difference in concentration in these areas (see Table 3).

In addition to these differences, seasonal variations in concentrations in outdoor air have been shown.
During the summer months much lower concentrations are found than in the winter months.
Because of these seasonal variations, our laboratory has decided to determine the PAH concentration as averages over the months of
January + February
March + April
May — October and
November + December.
The highest concentrations are found over the months of November + December, and the lowest in the months May — October.
The results of some investigations which were conducted by our Institute in the western part of the Netherlands are presented in Table 4.

Because the highest concentrations are found during fog periods in winter we have included in the table data obtained from measurements during one of the notorious smog episodes in London.
From the data presented for the Dutch cities, it is quite apparent that much higher concentrations are found during the winter season than in the summer. This is an indication that the seasonal

Table 4. *PAH concentrations measured in outdoor air (µg/1000 m³)*

		Delft (TNO area)				The Hague				Vlaardingen				Amsterdam				Rotterdam			
		'68	'69	'70	'71	'68	'69	'70	'71	'68	'69	'70	'71	'68	'69	'70	'71	'68	'69	'70	'71
Pyrene	S	1	3	2	5		3	2	2	4	3	2	2		4	1	2	2	1	3	2
	W	15	16	5	1	14	6	2	4	22	24	11	8	22	19	8	4	10	10	3	5
Fluoranthene	S	n.d.	5	4	7		3	1	2	3	4	3	1		3	2	2	1	1	2	2
	W	13	17	6	1	11	6	4	3	27	25	10	4	25	18	9	1	8	8	3	4
1.2-Benzanthracene	S	2	1	1	2		<1	<1	1	3	1	3	3		n.d.	1	1	1	1	2	1
	W	16	10	8	4	6	7	4	2	29	25	19	14	20	11	7	4	17	12	6	5
Chrysene	S	•	•	2	4		•	1	2	•	•	5	4		•	2	2	•	•	3	5
	W	15	20	10	6	21	14	7	4	28	38	16	13	26	17	4	8	21	18	11	18
1.2-Benzopyrene	S	n.d.	2	2	5		2	1	2	3	4	5	6		•	2	1	4	2	3	3
	W	28	23	13	5	14	23	•	9	31	31	22	20	21	17	10	6	25	24	14	14
3.4-Benzopyrene	S	n.d.	3	1	3		4	4	n.d.	3	4	5	9		2	2	n.d.	3	1	3	2
	W	20	18	12	6	23	12	•	13	35	32	13	16	22	18	5	8	15	23	19	23
1.12-Benzoperylene	S	n.d.	1	2	2		2	2	2	1	3	4	n.d.		n.d.	2	1	2	3	3	2
	W	20	17	7	6	12	8	•	2	20	15	11	14	17	12	•	7	23	18	13	6
Anthanthrene	S	n.d.	<1	•	1		<1	<1	<1	n.d.	<1	<1	n.d.		n.d.	<1	n.d.	n.d.	<1	<1	n.d.
	W	n.d.	1	<1	n.d.	n.d.	1	•	n.d.	4	3	2	1	2	1	•	<1	2	3	2	1
Coronene	S	1	1	•	2		<1	<1	1	1	1	1	n.d.		n.d.	<1	n.d.	n.d.	1	<1	1
	W	n.d.	2	1	n.d.	n.d.	1	•	n.d.	2	3	2	2	2	2	2	1	4	3	2	2

S = Summer (May-October)
W = Winter (November-December)
n.d. = not detectable

trend is due mainly to fuel consumption for home heating rather than automobile exhausts.
This is further substantiated by the data presented in Figure 1. From this figure, it can be seen that data from cities in and outside the Netherlands (except for Los Angeles) give ratios of 3.4-benzopyrene/coronene, pyrene/3.4-benzopyrene and 3.4-benzopyrene/1.12-benzoperylene which are clearly in accord with those obtained when domestic fuels rather than automobile exhausts are the source.
For tunnels, border-crossings, and a city such as Los Angeles, the opposite is, understandably, the case.
It is remarkable that, during the smog episode of September 1971 in Rotterdam, ratios were found which indicated that automobile traffic was the primary source of PAH contamination. At that time, however, the maximum temperature ranged from 21°C to 25°C. Under these conditions, it can be assumed that there was very little need for home heating.
In Table 5, the 3.4-benzopyrene content for several localities in Europe and in America is given.

Traffic as a source of PAH

In recent years, opposition has arisen to the addition of lead compounds to gasoline in order to obtain the desired octane rating. Reduction in the lead content of gasoline is desirable for the following reasons:

(a) to minimize environmental contamination by the added lead — which is known to be a cumulative poison;

(b) to make feasible the use of catalytic after-burners for other toxic components in automobile exhaust gas.

Such after-burners are readily „poisoned" in the presence of lead. Although raising the aromatic content of gasoline (instead of the use of lead additives) is not the only possibility for obtaining the required octane rating, it is the only method which could be used on short notice. Experiments have shown that raising the concentration of aromatics in gasoline leads to an increase in the concentration of PAH in exhaust gas. In view of the carcinogenic potentialities of these compounds, this would not be a desirable procedure. It should again be pointed out, however, that not traffic, but other combustion processes, such as the burning of bituminous coal for heating purposes, are the most important sources of PAH in outdoor air. Diesel-

Delft fog 7-12-'66	Delft fog 11-2-'69	Delft fog 12-12-'69	R'dam fog[a] 31-12-'64	London[b] 1956	London fog 3-12-'57	Denekamp June '65	Beek Nov. '69	Beek July '69	
29	33	312	420	10 25	3170	48	61	20	Pyrene
28	37	377	725	10 25	4070	23	43	8	Fluoranthene
32	11	104	240			<1	17	7	1.2-Benzanthracene
72	23	209	325			•	•	3	Chrysene
64	14	175	200	8 20	740	13	15	7	1.2-Benzopyrene
55	11	138	190	12 50	2220	13	14	<1	3.4-Benzopyrene
23	13	126	145	8 25	1060	49	11	27	1.12-Benzoperylene
3	n.d.	11	14	2 5	380	10	2	2	Anthanthrene
6	3	19	35	4 10	280	28	5	16	Coronene

a) Report Keuringsdienst van Waren, Rotterdam
b) Royal Soc. Health Journal, 76 (1957) p. 776

Table 5. *3.4-benzopyrene content (µg/1000 m³)*

Locality	Date	Concentration	Date	Concentration
Delft	winter '68	20	summer '68	n.d.
	winter '69	18	summer '69	3
	winter '70	12	summer '70	1
	winter '71	6	summer '71	3
Vlaardingen	winter '68	35	summer '68	3
	winter '69	32	summer '69	4
	winter '70	13	summer '70	5
	winter '71	16	summer '71	9
Rotterdam	winter '68	15	summer '68	3
	winter '69	23	summer '69	1
	winter '70	19	summer '70	3
	winter '71	23	summer '71	2
Oslo (a)	winter '56	15	summer '56	1
Copenhagen (b)	winter '56	17	summer '56	5.4
London (c)	winter '56	50	summer '56	12
Liege (d)	winteraverage '58/62	113	summeraverage '58/62	14.5
Liverpool (e)	24.10.'56-10.4.'57	166	summer '56	33
Hamburg (f)	winteraverage '61/63	183	summeraverage '61/63	17
Milan (h)	winter '58	198	summer '59	1
Paris (i) (rue de Dantzig)	winter '58	300-500 (48 results of the measurements)	summer '58	
Düsseldorf	winter '63	75	summer '63	3.5
Detroit (Michigan) (g)	winter '59	31	summer '59	3.4
Chatanooga (Tennessee)	winter '59	31	average over the year '60	8.3
Hammond (Indiana)	winter '59	39	average over the year '60	3.9
Charlotte (N. Carolina)	winter '59	39	average over the year '60	5.7
Richwood (Virginia)	winter '59	45		
St. Louis (Missouri)	winter '59	54	average over the year '60	5.3
Nashville (Tennessee)	winter '59	55	summer '59	1.4
Altoona (Pennsylvania)	winter '59	61		
Birmingham (Alabama)	winter '59	74	summer '59	1.6

n.d. = not detectable

a) Brit. J. of Cancer. 10/4201 (Sept. 1956)
b) Danish Med. Bull. 3/205 (Nov. 1956)
c) Roy. Soc. Hlth. J., *76* (1956) p. 677
d) Arch. Belg. Med. Soc., *9/10* (1963) p. 578
e) Int. J. of Air Poll., *1* (1958) p. 14
f) Int. J. of Air and Wat. Poll., *8* (1964) p. 185
g) Air Poll. Aspects of Organic Carc. (1969) Litton Syst. Inc. Prep. for the APCA
h) Centro per Lo Studio Sugli Ing. Atm. (1958)
i) Revue Poll. Atmosph., *7* (1956) p. 316

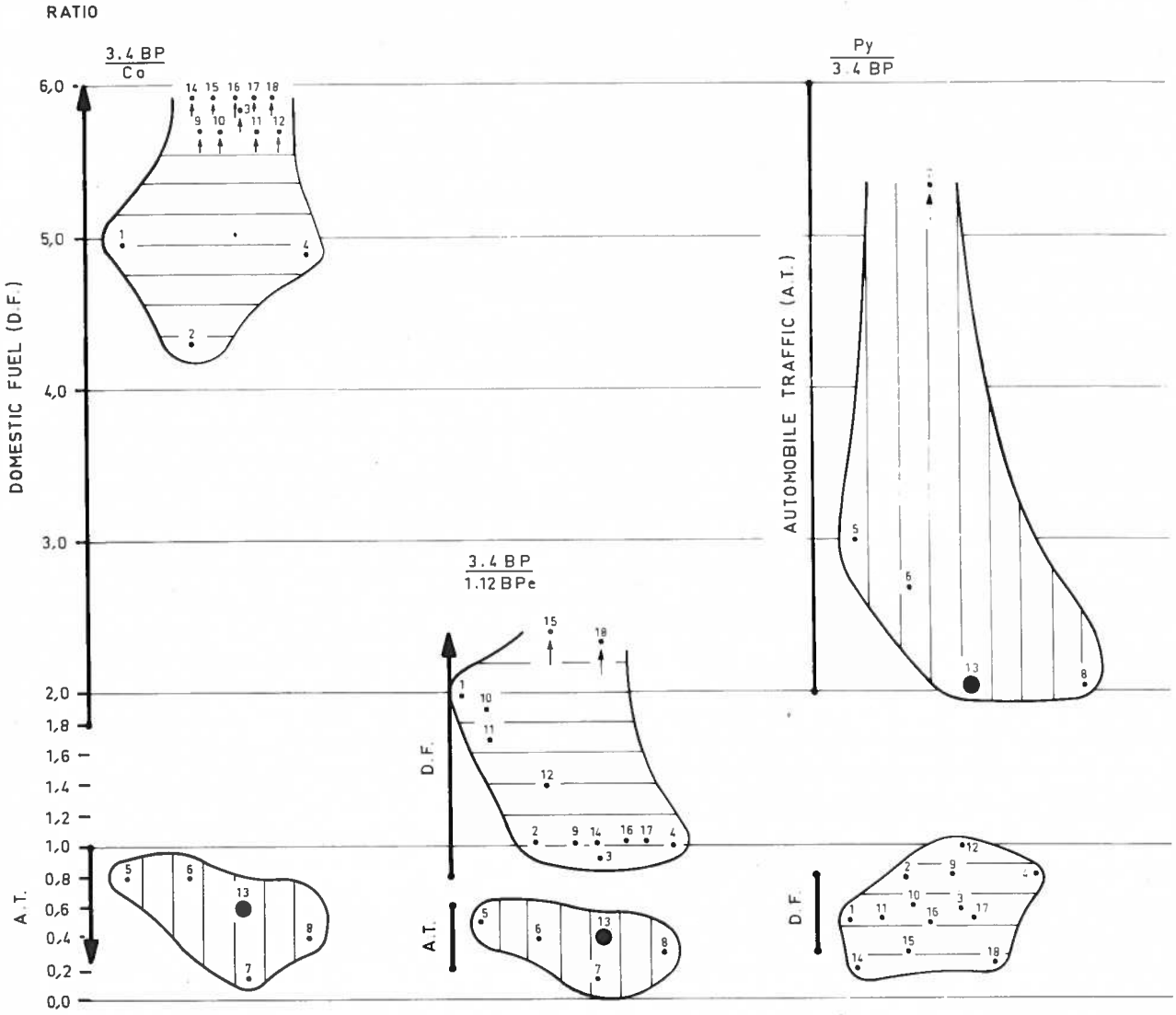

Fig. 1. PAH ratio for several localities

1.	London, 1956	*Source:* Roy. Soc. Hlth. J., *76* (1957) p. 776
2.	Liege, 1958/1962	Arch. Belg. Med. Soc. No. *960* (1963) p. 578
3.	Rotterdam-W, 1968/1969	IG-TNO data
4.	Detroit, 1959	Am. Ind. Hyg. Ass. J., *23* (1962) p. 137
5.	Blackwall tunnel 1960	Brit. J. of Ind. Med., *18* (1961) p. 250
6.	Denekamp, 1965	IG-TNO data
7.	Beek, 1969	IG-TNO data
8.	Los Angeles, 1959	Am. Ind. Hyg. Ass. J., *23* (1962) p. 137
9.	Delft-W, 1968/1969	IG-TNO data
10.	Vlaardingen-W, 1968/1969	IG-TNO data
11.	The Hague-W, 1968/1969	IG-TNO data
12.	Amsterdam-W, 1968/1969	IG-TNO data
13.	Rotterdam Smogepisode 1971	Rep. of the Keuringsdienst van Waren, Rotterdam, 1971
14.	Delft-W, 1971	IG-TNO data
15.	The Hague-W, 1971	IG-TNO data
16.	Vlaardingen-W, 1971	IG-TNO data
17.	Amsterdam-W, 1971	IG-TNO data
18.	Rotterdam-W, 1971	IG-TNO data

W = Winter season

Table 6.

Component	Diesel / Gasoline ratio for equal exhaust gas volume
Pyrene	0.12
Fluoranthene	1.7
1.2-Benzanthracene	0.79
Chrysene	0.07
3.4-Benzopyrene	4.2
1.2-Benzopyrene	3.9
1.12-Benzoperylene	3.8
Coronene	2.9

Data from J. L. Sullivan and G. J. Cleary, Brit. J. Ind. Med. *21* (1964) p. 117.

powered engines are also more important in this respect than are those using gasoline. This is illustrated by the data contained in Table 6. It gives the ratios obtained for PAH content when equal volumes of exhaust gas from the two sources are analyzed.
From Table 7 it can be seen that, since 1960, the 3.4-benzopyrene content of the air in Rotterdam has consistently decreased despite an increase in traffic density. The concentration of 1.12-benzoperylene, which is relatively abundant in automobile exhausts, has also decreased since 1964.

The emission of PAH by gasoline powered motors can be decreased by:

(a) better maintenance of the motor and provision for more complete combustion of the fuel,
(b) after-burning (catalytic) of the exhaust gas,
(c) a change in the composition of the fuel (aromatics are less favorable than highly branched aliphatic compounds; LPG is a favorable fuel).

The emission from diesel-powered engines may be reduced by:

(a) improved maintenance and better adjustment of the motor,

Table 7.

Year	3.4-benzopyrene 1 + 4 qu.	3.4-benzopyrene 2 + 3 qu.	1.12-benzoperylene 1 + 4 qu.
1960	56	•	
61	27	7	
62	46	4	
63	25	3	
64	26	6	32
65	19	2	34
66	6	0.5	
67	8	0.6	20
68	5	0.7	
69	3	0.4	5
70	2	n.d.	5
1971	2.5	0.3	7

Data from annual reports of the Keuringsdienst van Waren, Rotterdam.

(b) less strain on the motor, i.e. not overloading.

Furthermore, it has been demonstrated that the highest PAH emissions occur in the stationary, accelerating, and decelerating phases of the driving cycle. A more efficient traffic control system which would assure a free-flowing traffic pattern (synchronized traffic lights, for example) might improve the situation with respect to PAH emission.

Standards

Data from America show that the hydrocarbon content, indicated as C_xH_y, in exhaust gas from the 1970 model cars is 60% lower than was the case for models prior to 1966, when standards were established. The PAH content is 30% lower. The very stringent California standard for 1972 is about 1.5 g C_x H_y/km. Recent legislation provides that the whole of America will have standards which are even more severe by 1975 (for hydrocarbon emissions 0.29 g/km). Let us now examine the situation for Europe. A British survey has shown that, on the average, each automobile of the total British car population produces 2.6 g C_yH_x/km, based on the European testing cycle.
In the common market countries, standards for production models are 3.2 g C_xH_y/km for automobiles in the weight class of 1250-1470 kg; and, 2.7 g C_xH_y/km for those in the 750-850 kg range. It can be seen that these figures are, on the average, 2 times higher than those already established for California.
If one really wants to tackle the problem of pollution by automobiles, a first consideration should be the setting of standards for automobile exhaust gas (of which lead is only one of the toxic components) which are far more stringent than those now recommended by the European Economic Community. If we accept such standards for hydrocarbon emissions, a decrease in the lead content of gasoline with an accompanying increase in the aromatic content, will not necessarily affect the aromatic and PAH content of exhaust gas.
Older cars, which cannot meet the more severe standards, should be fitted with catalytic afterburners. If the proposed reduction in lead additives is effected, the danger of „poisoning" for these after-burners will be negligible. In addition to this, it is of interest to note that on a relative basis, the catalytic after-burners lower the PAH content more so than they do the total hydrocarbons.

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Detection and Measurement of the Concentration of Asbestos in Air*

Bepaling van de asbestconcentratie in lucht

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Summary

The attractive mechanical and chemical properties of asbestos have resulted in a steep rise in asbestos consumption since the 50's. Increasing evidence indicates that inhalation of even small quantities of asbestos can be detrimental to health.
Surveillance for the concentration of asbestos in air is therefore highly desirable.
As investigations of ambient air indicate that asbestos is mainly present as sub-microscopic fibers, it is necessary to determine these small particles.
The qualitative and quantitative aspects of the detection of asbestos fibers are treated and the determination of higher asbestos concentrations, as found during the manufacturing of asbestos containing materials, has been taken into consideration.

Samenvatting

Door de aantrekkelijke mechanische en chemische eigenschappen van asbest is het gebruik van dit materiaal de laatste decennia sterk toegenomen. Tevens nemen sedert de jaren zestig de aanwijzingen toe dat het inademen van zelfs zeer kleine hoeveelheden asbest, zoals die in omgevingslucht voorkomt, schadelijk kan zijn. Onderzoek naar de concentratie asbest in omgevingslucht is daarom gewenst.
Uit tot nu toe verricht werk is gebleken dat de in de omgevingslucht voorkomende asbestvezels overwegend submicroscopische afmetingen hebben.
Ingegaan wordt op de kwalitatieve en kwantitatieve detectie van sub-microscopische asbestvezels.
De bepaling van asbestconcentraties optredend tijdens het fabriceren en verwerken van asbest en asbesthoudende materialen, wordt eveneens behandeld.

Introduction

„Heatproof" and „shockproof" are words which are frequently used in advertising and, though advertising is not entirely objective, it indicates that both are highly valued properties of materials. Asbestos combines the properties of both heat and shock resistance.
Asbestos is the term applied to a group of natural occurring inorganic fibers belonging to two groups of silicate minerals known as amphiboles and serpentines.
Their discovery dates back to prehistoric times. They were first described by Plutarch who introduced the name „αοβεστα", meaning indestructible. Later, Charles V amazed and amused his guests by throwing tablecloths into a fire, from which they usually emerged whiter than when they went in [2]. Up to the end of the last century, asbestos remained a curiosity with very few applications. The discovery of large asbestos deposits in Canada gave a great impetus to its applications. Due to its unique mechanical and chemical properties, its use grew very rapidly as shown in figure 2 [3].
Table 1 [4] gives an estimate of the asbestos consumption in some European countries, while in table 2 [4], an estimate of its use on a world wide basis is shown.

Table 1. *Estimated asbestos consumption in a number of highly industrialised European countries in 1970.*

	tons per jaar
Belgium	59.000
France	167.000
Italy	109.000
Netherlands	24.000
U.K.	170.000
Western Germany	192.000

Table 2. *Estimate of major uses of asbestos. Breakdown in % of the total asbestos production.*

asbestos textiles	2
asbestos cement	66
friction materials and gaskets	4
asbestos paper	7
floor tiles	10
paints, roof coatings, caulks, etc.	3
plastics	1
miscellaneous	7
	100%

* Publication Nr. 442 of the Research Institute for Public Health Engineering TNO, Delft.

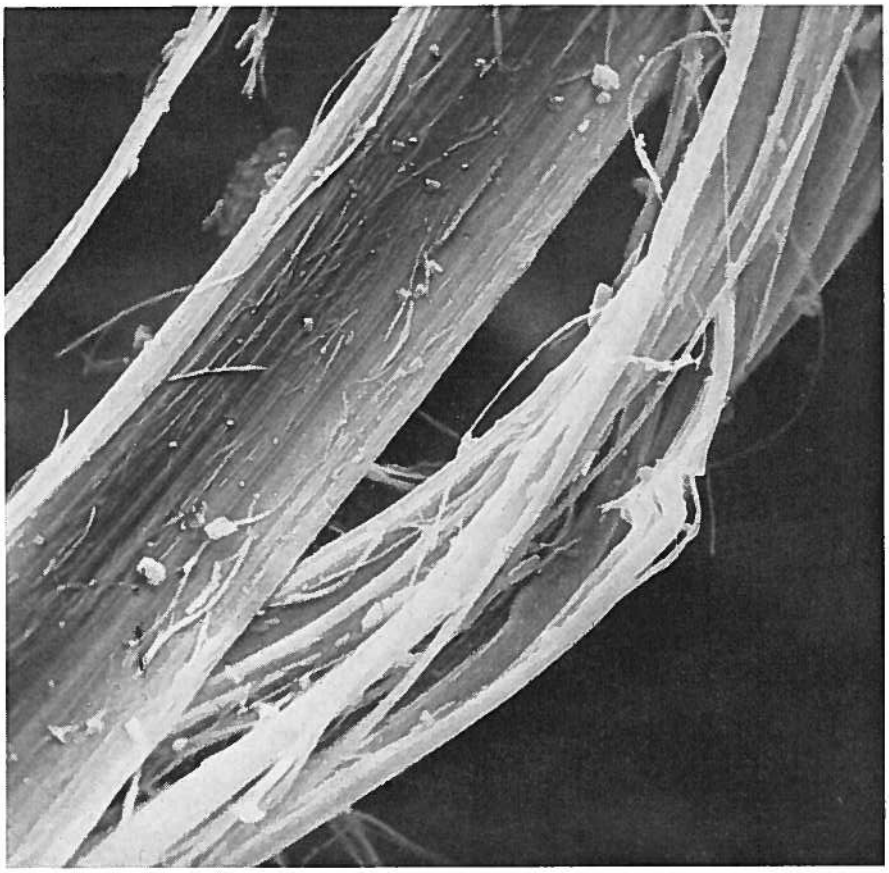


Fig. 1. Chrysotile asbestos fibers (2750 × C.E.M. Central Lab. TNO).

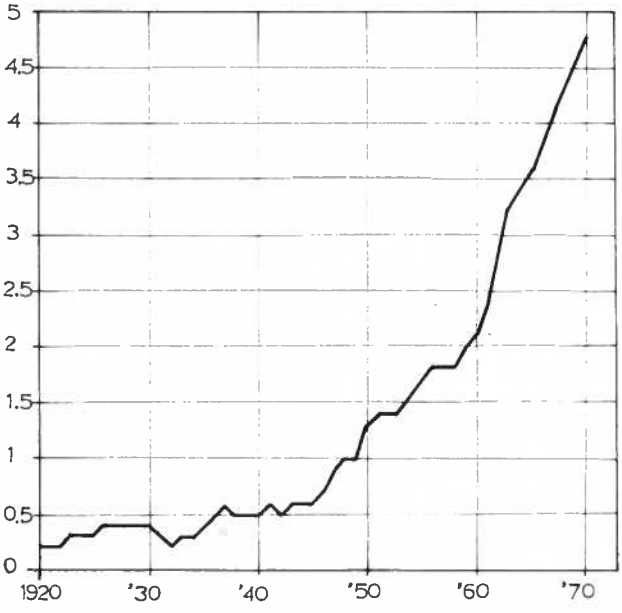


Fig. 2. Production of asbestos in 10^6 ton per year

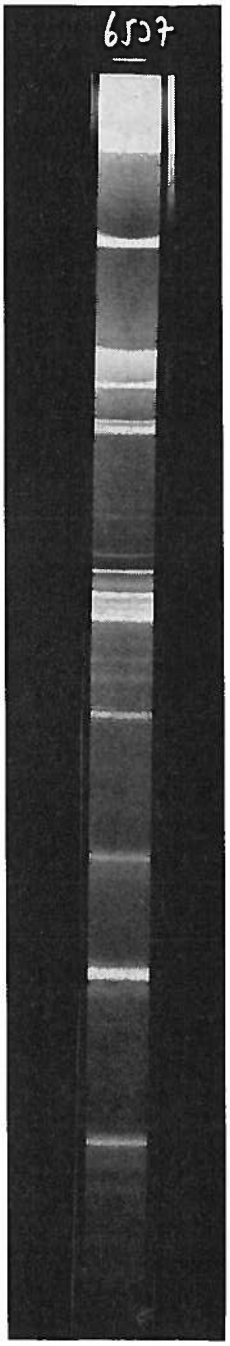


Fig. 3. X-ray diffraction of chrysotile asbestos (T.P.D. - T.H. - TNO).

Unfortunately, evidence becomes more and more convincing that asbestos, which from a technological point of view is an outstanding material, represents a serious health hazard — vide the article of Planteijdt in this same issue of „TNO-Nieuws". It is not surprising that attempts are being made to reduce its use: (a) by safety regulations which govern the precautions which have to be taken during the handling of asbestos [5, 6] and (b) by application of substitute products [7, 8].
Vigilance as to its concentrations in air is a necessity. The measurement problems arising from this need can be divided into two parts:

1. Determination of asbestos fibers in air under industrial conditions (e.g. work on asbestos containing materials);
2. Measurements of asbestos concentrations in ambient air (e.g. in buildings made of asbestos-containing construction materials, and in outdoor air).

In The Netherlands not much is known of the asbestos concentration of ambient air. Indications are that the concentrations are low (a few fibers per 1000 cm^3) and consist of submicroscopic fibers (diameter smaller than 0.1 μm, length in the order of 0.1 μm). Quantitative determinations of the number of fibers are difficult to make. The evaluation of methods to obtain reliable data is one of the projects of the Project group on Asbestos Exposure which was established by the Organization for Health Research TNO.
Methods pertaining to both problems are discussed in the subsequent paragraphs.

Qualitative and quantitative detection of asbestos in air under industrial conditions [9]

When selecting methods of investigation for the determination of asbestos fibres in dust samples precipitated from the air, the concentration to be expected is an important criterion.
Outside the asbestos-processing industry, the dust samples will contain but few asbestos fibres among much material of a different nature. In that case, high demands are made on the methods of investigation as regards selectivity and sensitivity. This problem is treated in the next paragraph. If the concentration is higher, e.g. during manufacturing of asbestos containing materials, light microscopy is an attractive analytical tool.
The dust trapped on a membrane filter is examined under the microscope. For this purpose, the filter is made transparent by means of glycerol triacetate. Since in normal light-microscopy the refractive indices of the transparent filter and of the asbestos fibres are virtually the same (so that the asbestos fibres cannot be properly distinguished), use is made of phase contrast. By means of this technique, fibres having a diameter of ca. 0.5 μm can still be detected. By definition, one speaks of fibres when the length to diameter ratio is at least 3 : 1 [10].
The presence of fibres other than asbestos fibres is possible, but in a low concentration they do not interfere. In phase contrast microscopy, synthetic fibres are lighter and straighter, while cotton fibres can never be brought into sharp focus over their entire length. Moreover, when the focusing adjustment is shifted, the latter fibres seem to curl up.
The relative accuracy of the count is ± 20%.
If the concentration of asbestos in the dust is higher, it can be determined by means of physico-chemical methods, i.e. with the aid of x-ray diffraction or infra-red spectrophotometry.

X-ray diffraction is based on the phenomenon that the atoms that are vibrating around fixed points in the mineral act as centres of scatter. The diffraction pattern (Fig. 3) is characteristic, while the intensity is a measure of the quantity of the asbestos present [11]. The sensitivity of the determination is ca. 0.5 mg asbestos. The asbestos content of the dust sample should be at least 3%.

Infrared spectroscopy is based on the phenomenon that the vibrating atoms in the mineral can absorb energy. However, only certain wavelengths can be used to magnify the amplitude of the vibrations of the atoms (resonance). At these wavelengths increased absorption of electromagnetic radiation occurs (Fig. 4). The absorption spectrum is characteristic, and the extent of the absorption is a measure of the quantity of asbestos. The sen-

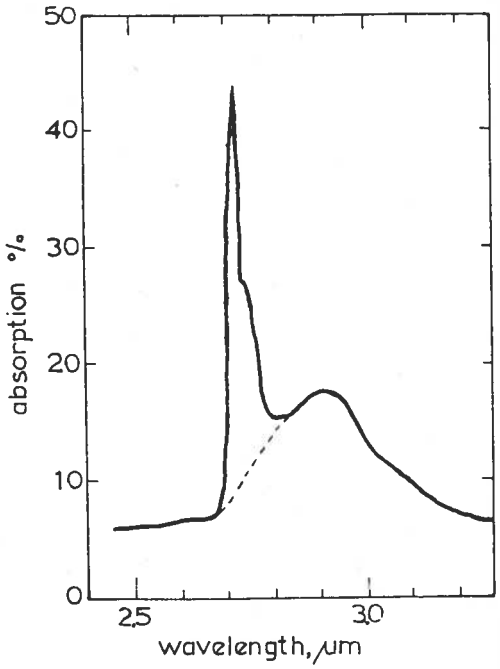


Fig. 4. Infrared spectrum of chrysotile asbestos

Fig. 5. Evaluation of several methods used for the determination of asbestos concentrations by creating a homogeneous dust concentration in the box shown on the picture

sitivity of determination is ca. 10 μg, while the asbestos content of the sample should be ca. 1% [12, 13].

Finally, it can be mentioned that when the airborne dust contains much asbestos, methods may be used that are not specific. Examples are atomic absorption and emission spectroscopy [9]. Within their limitations these are sensitive methods.
As asbestos is built up of elements which are very common such as magnesium, aluminium, and iron, these elements can only be used as indicators for the asbestos concentration if the concentration level of the asbestos is such that it stands out clearly against the always present background of these elements.

Detection of sub-microscopic asbestos fibers

The classic method of determining the concentration of sub-microscopic particles is by precipitation of the particles from a known volume of air on a substrate. For this purpose, among others, the following techniques can be used: filtration, electrostatic of thermal precipitation, and centrifugation [14, 15, 16, 17]. The particles are subsequently counted by use of electron microscopy. The problems which arise center around reproducebility and whether the numbers of particles counted are representative. The last two conditions are especially difficult to meet when filtration methods employing high polymer filters are used for the separation of solid particles from a known volume of air. The reason is that relatively elaborate manipulations have to be performed in order to prepare a suitable specimen for electron microscopy. Yet this method offers great advantages if long term averages of the concentration have to be determined. The instruments which are at our disposal are based on the alternative techniques mentioned earlier. They are all short time sampling instruments (sampling between 5-20 minutes). Using these instruments for the determina-

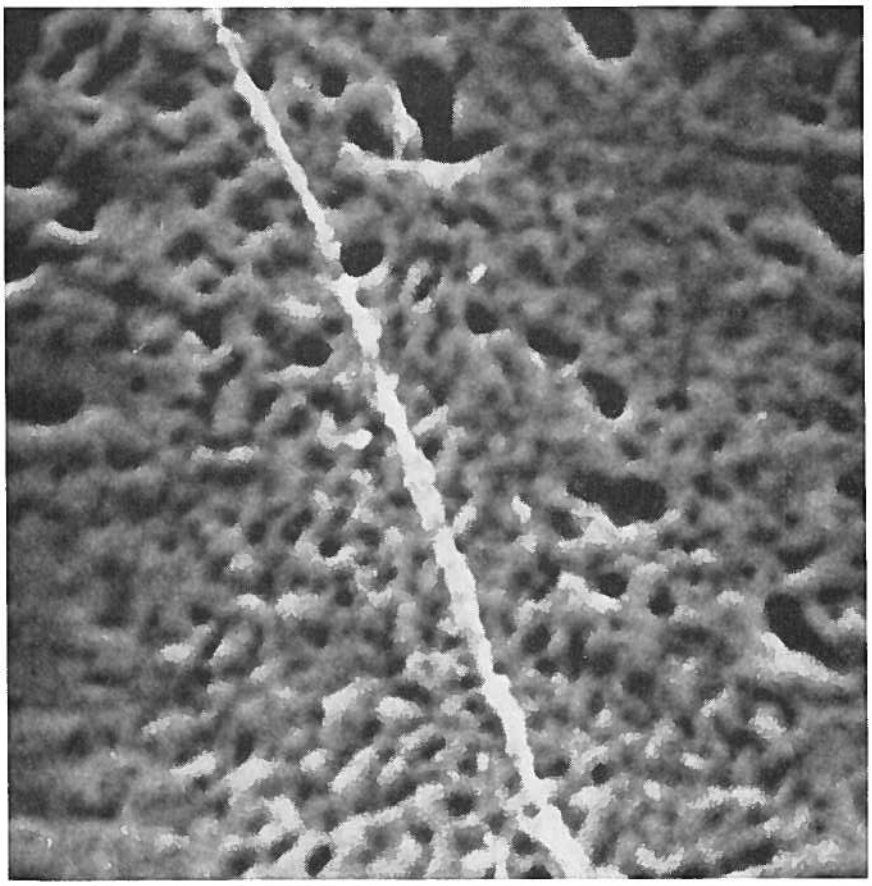


Fig. 6. Scanning electronmicrograph of a dust sample, as used for qualitative and quantitative evaluation of asbestos in air (11.550 × C.E.M. Central Lab. TNO).

tion of „long time averages" would be extremely costly. Finally it should be mentioned that asbestos, especially chrysotile, disintegrates easily into many fine fibers. The techniques used must prevent this as far as possible.
The latter property makes long time sampling e.g. with electroprecipitators, where the dust is continuously washed from the electrodes by a liquid, less attractive.
For evaluation purposes, the „filter method" was compared with direct short time sampling methods. In the latter case, the dust is collected on a specimen holder which can be directly used for electron microscopy.
The comparison was made by installing a number of filter samplers together with the short time samplers (thermal precipitator or electrostatic precipitator) in a box. Under the experimental condition the asbestos concentration, though fluctuating in time, is at a certain moment the same in the whole box (figure 5).
Using this method, it has not been possible to obtain comparable results when preparing specimens from filters for transmission electron microscopy. On the other hand, by taking samples from a filter, ashing them at 400° C in a golden specimen holder, and viewing the remaining deposit by a scanning electron microscope (stereoscan) [18] reproducible results are obtained (figure 6).
A disadvantage of using the scanning microscope (Cambridge Stereoscan) is the lower resolution (0.05 μm) as compared with the transmission electron microscope ($\sim$ 0.001 μm). Another drawback is that identification under the scanning microscope is only possible by X-ray fluorescence analysis of the elements which constitute the particle under observation; no information on crystal structure is given.
The latter information is provided by electron diffraction analysis which is accomplished by electron microscopy (figure 7a and b) [19, 20].
As it is believed that there is a connection between crystal structure and biological activity, information on crystal structure is important when structural transformations come into play under the influence of high temperatures. An example is braking. Use of asbestos containing brake linings results in the spreading of asbestos fibers into the surrounding air. According to some authorities, the asbestos decomposes at the temperatures which prevail during braking [21]. Therefore, work is still in progress which attempts to find a suitable sampling method for this situation.
Finally, it should be mentioned that the lowest practical level of detection with the stereoscan is 10^{-14} gr; with the electron microscope $\sim$ 10^{-15} gr. In principle, there is no limit to the amount of other material which may accompany the asbestos. The percentage of asbestos which can still be detected in the sample, is a function of the time

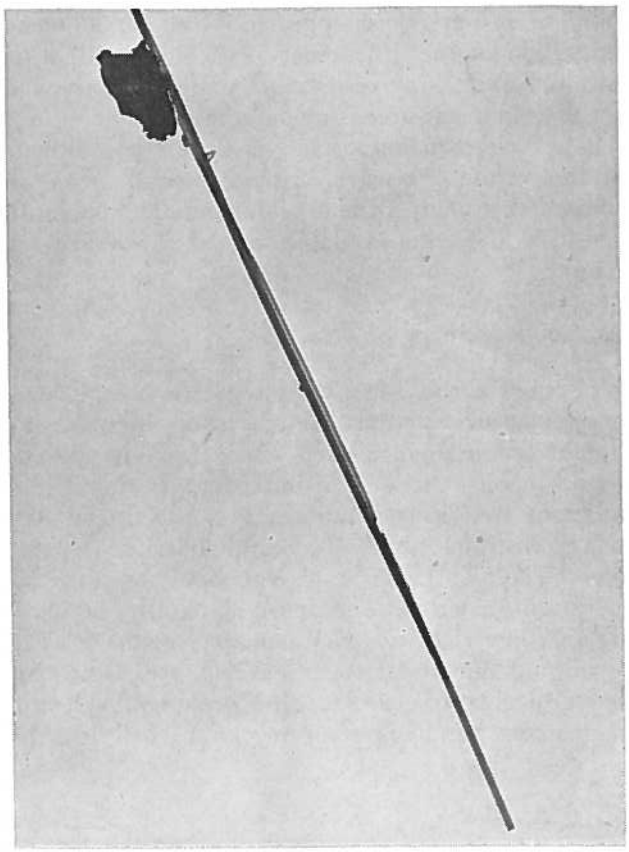
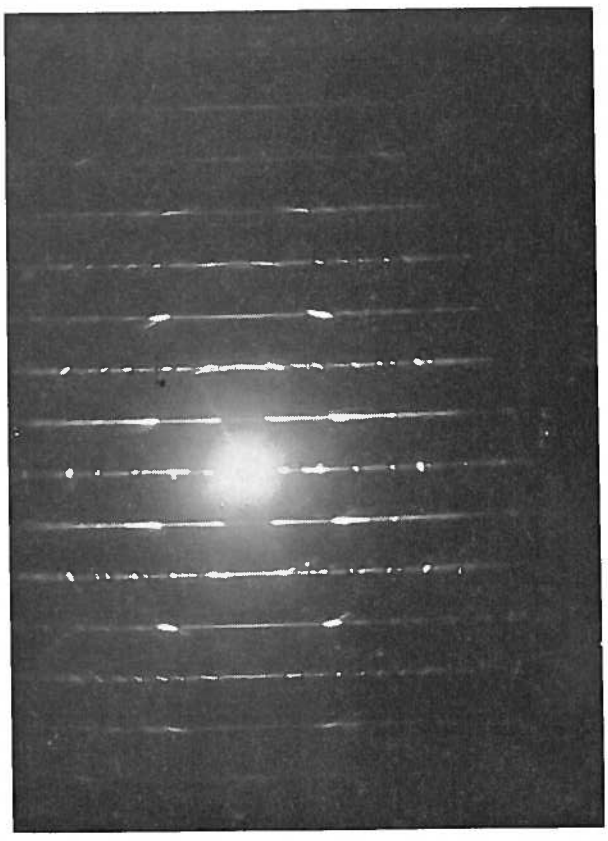


Fig. 7a. Electronmicrograph of chrysotile asbestos (14.500× Metal Institute TNO)

Fig. 7b. Electron diffraction pattern of the same fiber.



used to screen the samples. It is not an inherent limitation of the instrument as is the case with infrared and X-ray diffraction analysis which was mentioned in the preceding paragraph.
Though electron microscopy is a time consuming, and therefore expensive analysing tool, it is at present, by many orders of magnitude, the most sensitive detector available for the problem at hand.

Acknowledgement

Modern research sometimes requires complicated and expensive instruments and skills in order to obtain the desired results. These are not always found in one institute. In our case we should like to thank the Central Laboratory TNO, Metal Research Institute TNO, Technical Physics Department TNO-TH, Drs. J. F. van de Vate, Reactor Centrum Nederland and Ir. A. R. Kolff van Oosterwijk, Hertel & Co. N.V., member of the Project Group on Asbestos Exposure TNO, established by the Organization for Health Research TNO, for their interest and co-operation in the work described.

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Asbestos and Mesothelioma in the Netherlands

Asbest en mesothelioom in Nederland

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Projectgroup on Asbestos Exposure TNO

Summary

The relationship between asbestos and mesothelioma is still an object of intensive research in close cooperation between different disciplines in different countries. By the registration of all histologically verified cases of mesothelioma in the Netherlands and comparison with the mortality figures from the Netherlands Central Bureau of Statistics, some insight is gained into the frequency and geographical distribution of mesothelioma. An association with harbours and shipyards seems evident; asbestos is the most probable etiological agent. A detailed study of the professional history of mesothelioma cases is still in progress (J. P. J. Versteeg). The results of an electron microscopic search for asbestos in lung tissue in mesothelioma patients and in some control groups (F. D. Pooley, Cardiff) are given. Asbestos exposure is far more prevalent than is usually accepted. In nearly all cases of mesothelioma, both chrysotile and amphiboles could be demonstrated. Further studies are still in progress.

Samenvatting

Het causale verband tussen asbest en mesothelioom is nog steeds onderwerp van uitgebreide onderzoekingen waarbij nauwe samenwerking bestaat tussen speurwerkers uit verschillende disciplines in verschillende landen. Door de registratie van alle histologisch geverifieerde gevallen van mesothelioom in Nederland en vergelijking met de sterftecijfers van het Centraal Bureau voor de Statistiek wordt getracht inzicht te verkrijgen in de werkelijke frequentie en geografische verdeling van het mesothelioom. Het verband met havens en scheepswerven lijkt duidelijk; asbest is het meest waarschijnlijke aetiologische agens. Een gedetailleerde studie van de beroepsanamnese van mesothelioom-patiënten wordt thans verricht (J. P. J. Versteeg). De resultaten van een elektronenmicroscopische studie van asbest in longweefsel van mesothelioompatiënten en enkele controlegroepen (F. D. Pooley, Cardiff) worden gepresenteerd. Asbestexpositie is veel frequenter dan in het algemeen wordt aangenomen. In bijna alle mesothelioomgevallen kan zowel chrysotiel als amphibool aangetoond worden. Uitgebreider onderzoek wordt nog verricht.

As asbestos is not mined in the Netherlands but is predominantly imported as a half-product, the number of persons with a heavy and prolonged exposure to asbestos is only small in this country. This explains the low rate of asbestosis cases, and can also be considered as the reason that asbestos did not raise much medical interest until recently when the correlation between asbestos and malignant tumours of the pleura and peritoneum became evident (Wagner 1960). The first Dutch publications on this subject were those of Van der Schoot (1958), and Frenkel and De Jager (1961). Francke et al. (1968) reported a rising frequency of mesothelioma in Rotterdam. Stumphius (1969) published a series of 25 mesothelioma cases found on the island of Walcheren during the period 1962-1968. Most of the patients proved to be workers from a shipyard. In this same period, asbestos bodies were found in nearly 60% of 277 sputa from employees of this shipyard.

These, and similar investigations in other countries, raised many questions:

— what is the real frequency of mesothelioma?
— is there always an association with asbestos exposure?
— is there also an exposure of the population in general?
— is this population at risk and if so how can that be avoided?
— how can asbestos, aside from professional exposure, reach the people?
— is it present in the air, in the drinking water, etc.?

In order to study the medical problems concerning asbestos, and what could be done about the risk involved, the Organization for Health Research TNO installed a Study group on Asbestos Exposure in 1968. Different projects have been started by this group, some of which will be discussed in this article. Some others are discussed by Meyer et al in this same issue.
While many studies have been published on mes-

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Fig. 1. Chrysotile fibers in a slide of lung tissue from a mesothelioma patient. × 5000 (F. D. Pooley) (Negative).

Map 1. Number of mesothelioma cases (total 140 cases) reported to the Mesothelioma Register 1969-1971 from the different centres having one or more pathological laboratories.
○ = no cases reported
× = 1-4 cases reported
⊙ = more than 4 cases reported (5-37 cases).

othelioma and its causal relationship to asbestos, the real frequency of mesothelioma is unknown — even in small areas. The results of the investigations in Walcheren suggest that the real frequency is much higher than expected. All Dutch pathologists were asked to co-operate in the registration of all histologically verified cases of mesothelioma of the pleura and the peritoneum, starting from January 1969. In this Mesothelioma Register, covering the whole of the Netherlands' population of 13 million people, 140 cases were registered during 1969, 1970, and 1971. The division according to age and sex is given in table I.

The histological diagnosis was made on a biopsy only in 81 cases, and was made or confirmed at autopsy in 59 cases. In 6 patients, the tumour was reported to be present in the peritoneal cavity only, while in all other cases, the tumour was a pleural tumour, sometimes extending to the peritoneum or pericardium.

The geographical distribution could not be exactly determined due to the fact that the residence of the patients was not mentioned. More than half of the cases (78 out of 140) were reported from laboratories in two provinces (Zuid-Holland and Noord-Holland with a population of 5 million people). Most cases (37) were reported from the city of Rotterdam. The relative frequency was highest in the island of Walcheren (10 cases in a

Table 1. *Mesothelioma Register 1969-1971.*

age	10	20	30	40	50	60	70	80	90	T
male	1	—	3	13	28	37	31	7	1	121
female	1	—	1	2	5	5	4	1	—	19
total	2	—	4	15	33	42	35	8	1	140

population of 90.000 people). Other concentrations of cases were found in the area of Haarlem (10 cases) and Amsterdam (11 cases). Some university laboratories (Leiden and Utrecht, 10 and 16 cases respectively) also reported major numbers. The overall picture of this geographical distribution suggests a relationship with harbours and shipyards (Rotterdam, Haarlem and Amsterdam and Walcheren).

As this Mesothelioma Register contains a mixture of morbidity and mortality figures, the Netherlands Central Bureau of Statistics was asked to provide us with the number of patients that were reported to have died from a pleural tumour in 1969 and 1970. The year 1971 was not included because the data were not yet available, while the peritoneal tumours were left out because the reported high number in women might have been due to an admixture with ovarian tumours. In these 2 years, 166 persons were reported to have died from pleural tumours. The same areas as were reported above to have the highest incidence according to the Mesothelioma Register, now also proved to have the highest incidence: Rotterdam and neighbouring municipalities, 41 cases; Amsterdam, Haarlem, and neighbourhood, 18 cases. Only 2 cases, however, were reported from Walcheren. From the university towns Leiden and Utrecht, only 3 and 5 cases respectively were reported. This can be explained by the concentration of rare tumours in University Hospitals.

The hypothesis, concerning a relationship between the frequency of mesothelioma and harbours and shipyards, seems to be confirmed by these figures. The connection between mesothelioma on the one hand, and harbours and shipyards on the other hand, might well be asbestos. Further investigations seemed justified and were carried out.

A detailed professional history of about 30 cases of histologically verified mesothelioma in the Rotterdam area was compiled by J. P. J. Versteeg, M.D. In nearly all cases, a professional exposure to asbestos was certain, or at least probable. The detailed results of these investigations, and of further studies along with those of a control group, will be published elsewhere.

Another fascinating question was whether asbestos could be demonstrated in the lungs of patients with mesothelioma. To aid in the solution of this problem, Dr. F. D. Pooley, Department of Mineral Exploitation, University College, Cardiff offered to perform electron microscopic investigations on lung tissue. On our request, many Dutch pathologists sent us lung tissue from mesothelioma patients. In this way, tissue from the lungs of 28 mesothelioma patients from different parts of the country, together with material from 10 patients from an agrarian region in Zeeland, were investigated. In all cases, asbestos could be demonstrated in 6 μ slides as well as in KOH preparations from

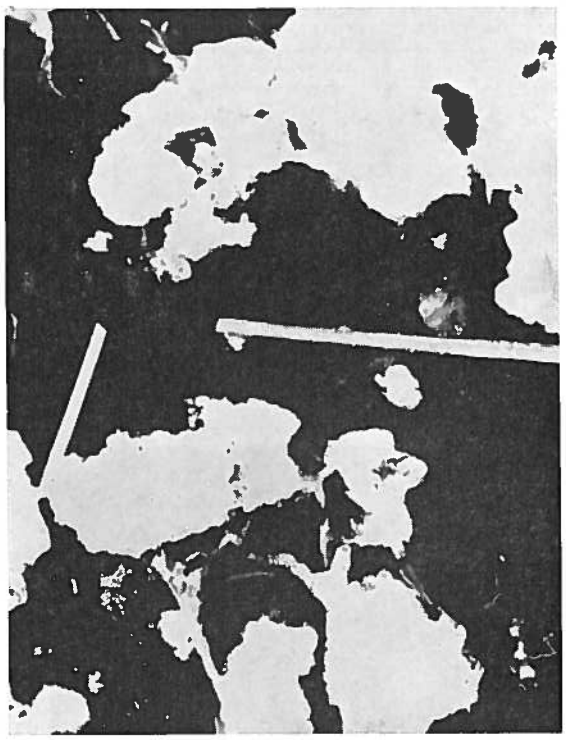
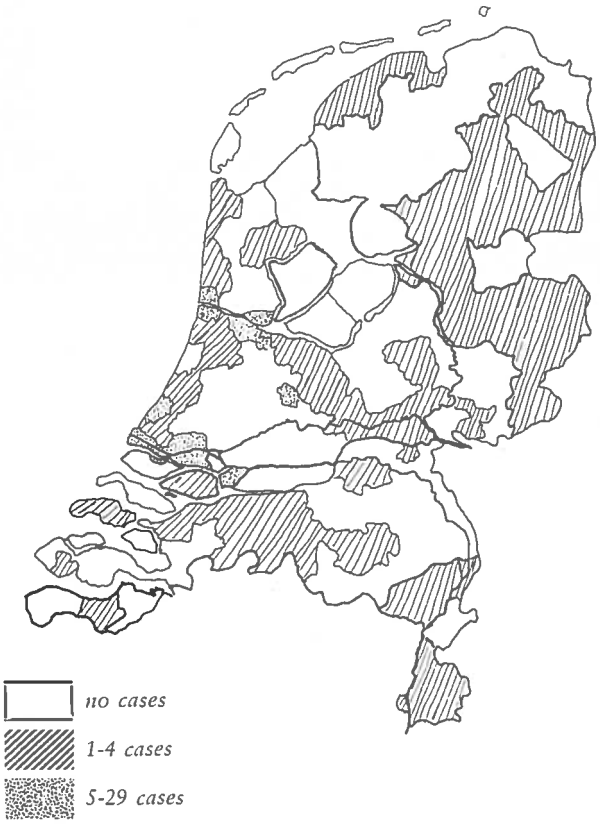


Fig. 2. Amphibole fibres in the extract from a KOH-preparation of lung tissue from the same patients as fig. 1. × 7200. (F. D. Pooley) (Negative).



Map 2. Pleural tumours as cause of death 1969-1970. Total 166 cases. Figures from the Netherlands Central Bureau of Statistics. Distribution according to economic-geographical regions.

Table II

	number of cases	6 μ slides				KOH-preparations			
		blank	C	C + A	A + C	blank	C	C + A	A + C
Mesothelioma	28	—	10	8	10	1	2	15	10
Controls	10	—	9	1	—	—	3	7	—
Special controls	3	—	3	—	—	3	—	—	—

Special controls: see text. Blank: no fibres detected. C: chrysotile only. C+A: chrysotile and amphiboles but mainly chrysotile. A+C: amphiboles and chrysotile but mainly amphiboles.

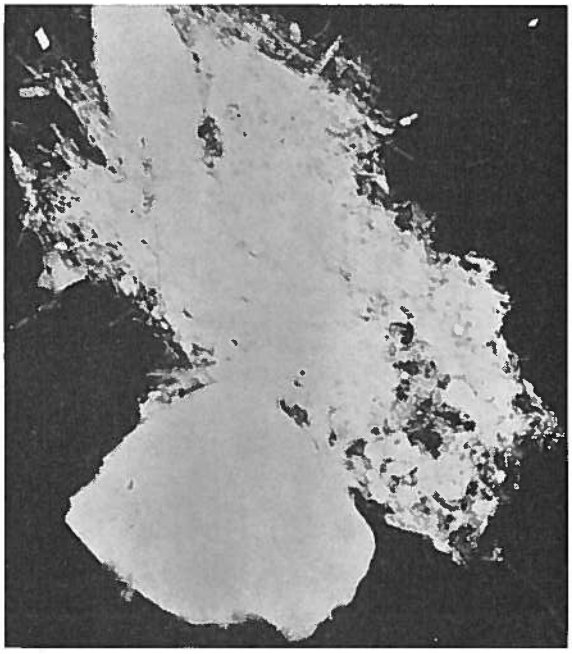


Fig. 3. Innumerable small chrysotile fibers in a slide of lung tissue from a patient of the control group. × 9000. (F. D. Pooley) (Negative).

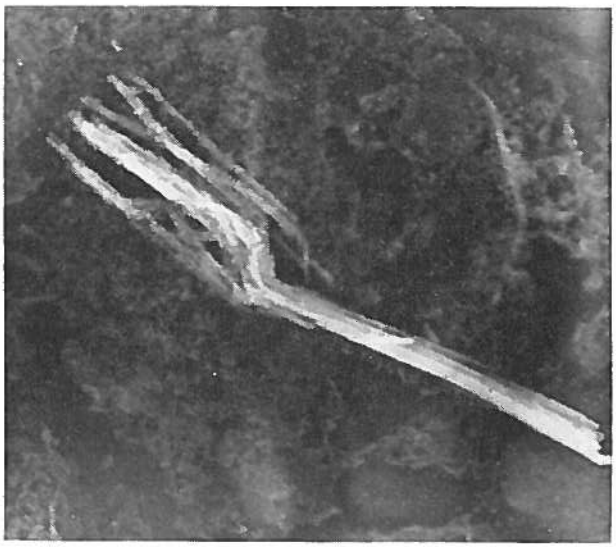


Fig. 4. Bundle of small chrysotile fibres in a slide of lung tissue from a mesothelioma patient. The whole length of this bundle is about 4 μ × 23.000 (F. D. Pooley) (Negative).

the lung tissue. On the basis of the electron microscopic morphology, together with the electron diffraction pattern, a distinction could be made between chrysotile and the amphiboles (Pooley 1972). The results are summarized in table II.

In the mesothelioma cases, chrysotile was always found in the slides and, in all but one case, in the KOH-preparations; in most cases (18 in the slides and 25 in the extracts from the KOH-preparations), amphiboles were found also. It is estimated that the KOH-preparations are about 300 times concentrated as compared to the slides. The findings, however, were essentially identical in 20 out of 28 mesothelioma cases. In 7 cases, amphiboles were present in the KOH-preparations, but not in the slides. In 1 case, which showed chrysotile in the slides, no fibres could be detected in the KOH-preparations.

In the control group, chrysotile was found in all cases, but in the slides only one case also showed some aphibole. In the more concentrated KOH-preparations, amphiboles were found in another 6 cases.

As there were no blanks in the control group, an additional „special" control group was investigated in order to make sure that no contamination (by formalin, paraffin etc.) could be responsible for these findings. This special control group consisted of pieces of lung tissue from a still-born baby, a premature neonate who died 14 hours after birth, and a young child who died from congenital malformations at the age of 14 months. In these three cases, no fibres could be detected in the extracts of the KOH-preparations, but a few fine chrysotile fibres were found in the slides. These fibres seemed to lie upon the slides, and not in the tissue. They were considered, therefore, to be contaminations that were probably introduced when chrysotile-containing paraffin blocks were cut with the same microtome knives as were the blocks from these babies. Some chrysotile from the lung sections of mesothelioma patients must have been still present on the edge of the knife.

The conclusions that can be drawn from these findings are;

— newborns and children have no asbestos in their lungs;
— older people (nearly?) always will have some chrysotile, and in many cases some amphibole, in their lungs; amphiboles, however, never predominate;
— in mesothelioma cases, asbestos could be demonstrated in all but one instance. In nearly all cases, chrysotile and amphiboles were both present, while in 10 out of 28 cases amphiboles predominate.

In the same pieces of lung tissue, we also searched for asbestos bodies in 30 μ sections which were stained for iron. In the mesothelioma cases, asbestos bodies could be found in 25 out of 28 cases — sometimes numerous, but usually scanty. In both control groups, no asbestos bodies were ever found.
Similar studies are being performed on another series of mesothelioma and control cases. An overall report of this whole study will be published later. Similar studies have also been performed by Pooley on material from other countries (Pooley 1972). All technical details can be found in that publication.
As usual, investigations seem to raise as many new questions as they give answers. Much more work will have to be done, both multidisciplinary as well as by international co-operation, before real insight into the relationship between asbestos and mesothelioma will be attained.

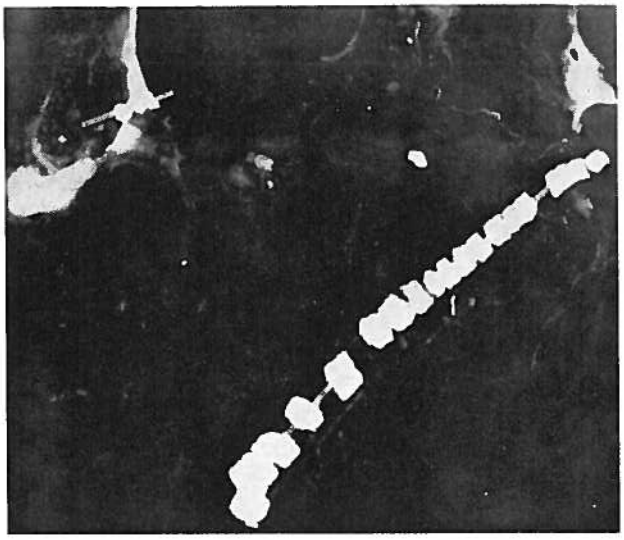


Fig. 5. Asbestos body and amphibole fibre in a slide of lung tissue from a mesothelioma patient. × 9000 (F. D. Pooley) (Negative).

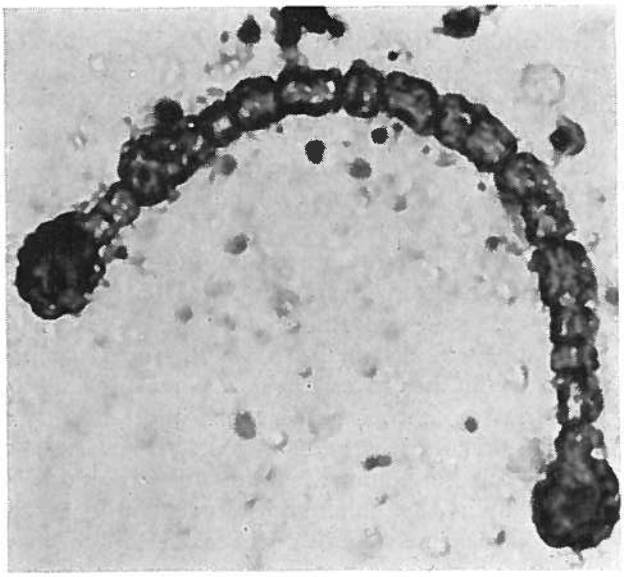


Fig. 6. Asbestos body in sediment of fluid from lung tissue of a mesothelioma patient. × 1500 (P. B. Meyer).

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International Studies on Cancer in Migrants

Internationale studies over kanker bij emigranten

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Summary

Risks of cancers at various body sites are found to change on migrating from one country to another. Studies of such migrants thus present a means of obtaining clues regarding cancer causality, and allow one to separate out, in part, environmental aspects from those of a genetic nature. The paper reviews the results of a number of such studies, while also relating the major suspect factors accounting for the findings noted.

Samenvatting

Het blijkt dat het optreden van kanker in verschillende organen samenhangt met het verhuizen van het ene werelddeel naar het andere. Een studie bij groepen emigranten kan iets meer licht werpen op de oorzaak van deze ziekte. Hierbij kan mogelijk gedifferentieerd worden tussen aangeboren en verworven oorzaken. Dit artikel biedt een overzicht van de resultaten van zulke studies, gebaseerd op literatuurrecherche.

I. Introduction

International studies are presently being conducted in order to investigate the effects of cultural mobility on cancer frequency in terms of the place of its occurrence; in other words, how a change in place of residence and life (the act of migrating) affects the subsequent development of the illness in those entering the new situation.

The migrant, in addition to adopting new ways of life, brings along with him both a genetic potential for disease and reflections of the environment of his country of origin. As a result, one can study not only the influences of new environments on variations in cancer rates, but also those influences one has retained from the past. The latter, of course, can be examined more precisely and with more validity by conducting studies in both the adopted and parent lands of the migrant.

Migration of human populations can be considered as a large-scale "natural" epidemiological experiment which is capable of generating useful hypotheses regarding cancer causality. The purpose of this paper is to review the significant findings of a number of these studies and to present certain ideas stemming from them with regard to the etiology of select cancer sites.

II. The studies

One of the earlier studies relating ethnic differences and migration to mortality from cancer at various body sites was that of Mancuso and Coulter [13] in 1958. The subjects of the study were male residents of Cuyahoga County, Ohio, 25-64 years of age. Mortality for each cancer site (i.e., lung, larynx, bladder and central nervous system) was found to be higher among the foreign-born white than among the native white. In addition, the rate for lung cancer did not show the same urban-rural gradient among the foreign-born as it did for the native-born American. Mortality for the native-born was found to be higher in urban than in rural areas, while that for the foreign-born remained at a higher level both in urban and rural locations. It is quite possible that early environmental exposures, i.e., smoking, air pollution, or other factors, in the parent country largely determine the migrant's risk for developing lung cancer, regardless of where he later settles. The pattern for the native-born can be explained, for the most part, by the fact that smoking is more prevalent in urban areas [8].

Specific-site mortality in the foreign-born white varied a good deal according to ethnic group. Higher rates for lung cancer were noted among those born in Poland, Yugoslavia, and the U.S.S.R. The Italian migrant's risk of lung cancer increased slightly, but still reflected, to a greater extent, the lower rate of his native land. Immigrants from England and Wales tended to show a similar mortality for this type of cancer as the native white, but less than that for their peers in England and Wales. Males from Russia were found to have the highest risk of developing cancer of the larynx.

Haenszel [9], in a later investigation, found significantly large differences between some migrant

groups and native-born United States whites in mortality from cancer of the esophagus, stomach, larynx and lung. Increases or decreases in risk were not uniformly distributed by body site across all ethnic groups but were specific for certain site and ethnic group combinations. Mortality from cancer of the esophagus was substantially higher in all of the foreign-born white males than in their native-born equals. The highest rates were found in Polish, Czech, and Irish migrants. High rates were also seen in women migrants from Czechoslovakia, Ireland, and Russia, being the highest in the latter. The Russian women were predominantly Jewish, and studies have indicated a higher susceptibility to the disease among Jewish females, suggesting a genetic factor [12]. Haenszel feels that the high incidence of esophageal cancer among the Irish may be explained by the high alcoholic consumption of Irish migrants. A study by Wynder and Bross [20] tends to support such an assumption. Reasons for the higher rates of this cancer in migrants from other countries, and for men and not women experiencing higher rates than those remaining in their particular countries, were not given.

All migrant groups in Haenszel's study showed higher mortality from cancer of the stomach than did the native-born American while tending to approximate the rates found in their home countries. The persistence of certain dietary habits might, in part, be responsible for the phenomenon. The study is in agreement with that of Lombard and Doering [11]. The investigators of this early migrant study (1920's) arrived at similar results and interpreted the differences as due to a general socio-economic status factor. Support for an environmental hypothesis is found in the U.S.-Japanese migrant study of Haenszel and Kurihara [10] who observed that American-born Japanese, or Nisei, have a reduced gastric cancer risk, compared with the Issei, or Japanese-born residents in America who have maintained the high rate characteristics of Japan. The findings are in general agreement with the earlier work of Buell and Dunn in California [1].

Male migrants from all countries, except England, exhibited higher mortality rates for buccal cancer than those remaining in their countries of origin. The higher oral cancer rate for the Irish migrants, and lower rate for those from the U.S.S.R. were found by Haenszel [9] to be consistent with the known relationship between cancer of this site and high alcohol consumption. Furthermore, the investigator pointed to the apparent similarity between the Swedish female's high rates for oral and pharyngeal cancer in the States and her high rates in Sweden, where an association between Plummer-Vinson disease and these conditions among females has been noted.

Compared with the native-born American, the English and German migrants showed a higher mortality from lung and bronchial cancer, but demonstrated lower rates than those in their countries of origin.

Although the migrants (many from parent countries with low breast cancer rates) showed a similar high breast cancer mortality as that of the United States, and thus, suggesting the effect of environmental influences, the work of Haenszel and Kurihara [10] has shown that both the Nisei and Issei retain the low breast cancer risk of Japan. This persistent low rate for second generation Japanese in America supports the notion of endogenous influences as well.

Cervical cancer rates were found to be low for migrants from the U.S.S.R., and high for Mexicans. The low rate for Russians apparently reflected the low cervical cancer incidence among Jews who comprised a large portion of the immigrants from the U.S.S.R. Beside studies [19] implicating exogenous factors, particularly those of a sexual nature, as important causes of cervical cancer, the short-term downward displacement of the Japanese rate upon migrating to the U.S. lends further support to an environmental etiology [10]. Apparently, the migrant Japanese female leaves behind conditions which influence her higher risk in Japan.

Prostate cancer mortality was found to be highest among those from Ireland, Norway, Canada, and Sweden, when compared with those from Italy, U.S.S.R. and Austria. Explanations for these findings were lacking, althrough the low U.S.S.R. rate may reflect the low prostate cancer rate among Jews.

Haenszel suggested further work, specifically with standardized methods, between investigators in the United States and in the countries of migrant origin. One such study was that of Staszewski and Haenszel [18] on Polish migrants to the United States. Age-specific cancer death rates for Polish migrants to the United States were compared with those for Poland and the United States native whites. Polish migrants continued to show the high stomach cancer rates typical of Poland. Although there is low mortality from cancer of the breast, colon, and rectum in Poland, the risk increased for these sites when Polish people migrated to the United States. It, in fact, reached the level existing among United States native whites. The death rates for cancer of the esophagus and larynx among male migrants exceeded those prevailing in Poland and the United States. The Polish migrants' lung cancer death rate varied in pattern from those of other ethnic groups, the risk for male migrants following esophagus and larynx in demonstrating rates higher than those for both country of adoption and country of origin. Other ethnic groups usually fell midway between those of home and host country. The Polish migrant also showed an increased risk above that of Poland for prostate cancer. It was, however, below that of the United

States. The authors mention the fact that Poles generally migrate from rural areas in Poland to urban areas in the United States, such as New York, Chicago, etc. Such urban areas are high-risk locations for cancers of the colon and rectum, among others, and apparently contribute somewhat to the higher risk of the Polish migrant for disease at such sites. The persistence of traditional diet and eating habits may have led to the Poles' high rate of stomach cancer.

Graham *et al.*, [7], studying patients admitted to the Roswell Park Memorial Cancer Institute in Buffalo, New York, found that foreign-born females showed a higher risk for cancer of the respiratory organs and stomach than did their native-born equals. The former, however, showed a lower risk for cancer of the corpus uteri. Furthermore, the female Polish migrant had a risk for gastric cancer three times that of other foreign-born women. Immigrant males exhibited higher risks for lung, stomach, and esophageal cancer than did native-born males. The foreign-born males' gastric cancer risk was two and one-half times greater than that for the native-born, while for cancer of the testes, the risk was considerably lower. Polish-American men had higher risks for lung, stomach and, particularly, esophageal cancer than did the other foreign-born, and native-born males. Italian migrants had high risks for cancer of the bladder, pharynx, and large intestine relative to other foreign migrant groups. The risk for buccal cancer among German-American men was only about two-thirds that of other foreign-groups.

The above findings held true after adjusting for factors such as age, urban residence, smoking, and type of occupation. The morbidity observations of Graham, *et al.* are consistent with those on mortality by Mancuso and Coulter [13] and Haenszel [9]. The Buffalo investigators suggest diet as an important etiological factor in the high rates for esophageal cancer among the Italian and Polish-American groups.

Quisenberry [16] studied the influence of ethnic difference and cultural factors on the occurrence of cancer in Hawaii, a multi-racial state of the U.S.A. The incidence of grastric cancer was highest for the Japanese migrant; the rate for Japanese males being greater than one and one-half times that for men of all races. Filipino men were found to have the highest rate for liver cancer, while Japanese males had the lowest incidence of lung cancer. The caucasian breast cancer rate was approximately five times that of the Japanese. Cancers of the colon and skin were more frequent in the white population. The rate of prostate cancer was nine times higher among whites than in Japanese. Nasopharyngeal cancer was found to be most frequent among the Chinese population, and the Hawaiian and part-Hawaiian appeared at greatest risk for cervical cancer.

Quisenberry [14, 15] conducted additional epidemiological investigations in order to find an explanation for the high incidence of stomach cancer among Japanese in Hawaii. It was shown that Japanese have no more gastric achlorhydria than caucasians, and that gastric cancer may lead to achlorhydria rather than the reverse. The observation that stomach cancer cases were approximately 12 years older, on the average, than ulcer cases is consistent with the hypothesis that ulcers serve as precursor lesions for a good number of gastric tumors. The finding takes on greater significance when one realizes that the Japanese in Hawaii not only have the highest incidence of gastric cancer, but of benign ulcers as well (in fact, one- and one half times higher than it is among caucasian men in Hawaii).

Furthermore, the Japanese, particularly the older ones, maintain a diet which is characteristic of Japan, that is, food high in carbohydrates (particularly rice) and low in animal protein (other than fish) and dairy products. White rice is low in vitamin B_1 and raw fish contains Thiaminase, an inactivator of vitamin B_1. The vitamin deficiency may be relevant as a causal factor. This population also consumes large amounts of hot beverages (sake and tea) and a variety of pickled vegetables, both of which might be involved in initiating or promoting the cancerous process.

In line with a genetic etiology is the fairly high percentage of gastric cancer and stomach ulcer cases found with blood group A typing.

It was felt by Quisenberry that, because changes in cultural habits were occurring in Hawaii along with an increase in integration and intermarriage of different races, a study of cancer rates over time might lend further support to specific causal hypotheses. He, along with his associates [17], followed the various rates in Hawaii for a period of approximately 10 years. It was noted that, although breast cancer consistently occurred less frequently in the Japanese female relative to other groups studied, the former's risk for this cancer apparently increased more rapidly. The discontinuance of certain nursing and mating habits due to migration was considered as a possible explanation for the change.

Stomach cancer trends showed a decrease for Caucasian males; a steady state for Japanese males; and an apparent increase for Hawaiians. The relative positions for the various ethnic groups remained the same (the Japanese having the highest rate, the Hawaiians an intermediate rate, and the Caucasians the lowest rate).

Although lung cancer was found to be least common among the Japanese (the Hawaiians and Caucasians being the same in rate and higher than the Japanese), all three groups showed an increase. The authors mentioned that Japanese men smoke more in Hawaii than when in Japan, and that this

may account for their increased cancer rate at this anatomical site. A similar explanation can be given for the rates in the other two racial groups.

It was mentioned earlier that English migrants to America have a high and low lung cancer risk relative to the native American and parent country, respectively [9, 13]. Because of the complicated nature of this mobility, difficulty arises in separating out possible endogenous from environmental factors associated with the disease. To partly remedy the situation, lung cancer investigators have utilized observations on migrants from England to formerly English-colonized parts of the world. Such immigrants act as natural controls, being essentially of the same British stock as the native-born and now living under similar conditions, while differing only in the fact that they have spent part of their lives in England before coming to their adopted country.

Both Eastcott [6] in New Zealand and Dean [2, 3, 4, 5] in South Africa and Australia have demonstrated a higher lung cancer mortality for the English migrant in their adopted lands, but a lower one relative to that in England. Differing smoking behaviours apparently could not account for the variation noted, as similar patterns of tabacco consumption were found in all countries involved. Furthermore, the length of time in England prior to migration was directly related to increased lung cancer risk. Rates for those migrating at 30 or more years of age were significantly higher than for those leaving before this age.

The results suggest that British immigrants, men and women, bring some of their greater liability to lung cancer with them, because of urban conditions experienced earlier in Britain. Yet, by terminating their residence in England, they lose much of still greater risk existing among people remaining at home. Whether the condition responsible is air pollution, climate, or some other factor(s) remains to be determined.

III. Discussion and recommendations

This paper has served to review the significant findings of some of the epidemiological studies relating cultural mobility to cancer. It was the intention of the author to not only emphasize the importance of such research as clues regarding cancer etiology, but also to present some of the factors involved in such migration, which are being considered as likely etiological components of specific-site cancers.

From the results presented, it appears that the migrant, for the most part, faces a greater risk of developing cancer. The tendency is for such people, upon entering and living in their new country, to increase their risks for cancer of various sites over those of persons remaining in their countries of origin. Gastric cancer appears to be an exception, for the migrant has, in most cases, a decreased risk for cancer of this site in his adopted location. Such findings suggest the significance of environmental factors in cancer etiology. Furthermore, cancer risk may be related to influences of the past as well as those of the present. The studies of Eastcott [6] and Dean [2, 3, 4, 5] suggest that early environmental exposure is important in increasing one's risk for lung cancer later in life. The role of genetic factors in determining the development, and course, of specific cancerous processes is also evident from the studies.

The following table summarizes, in brief, some of the major suspected causal factors stemming from migrant cancer studies.

Table

Cancer Site	*Factor*
Lung and Bronchus	Cigarette smoking Environmental exposures early in life in addition to smoking
Esophagus	Alcohol Diet Genetic factors
Stomach	Diet Genetic factors Precursor lesions
Oral cavity	Alcohol Plummer-Vinson disease
Breast	Genetic or other endogenous factors Nursing habits
Cervix	Environmental (?)

So, one can see the substantial benefit gained from studies of the culturally mobile population. The various ethnic groups tend to serve as their own controls in a „natural" epidemiological experiment where various factors related to environment may be separated out from those considered constitutional. While the hereditary characteristics of the migrant remain unchanged, cultural behaviour and environmental factors may be altered to a great extent in their adopted land. These changes apparently affect differences in the migrant cancer risk relative to both country of origin and new residence. Furthermore, exposures such as climate, pollution, and occupation change immediately; while others, such as diet and smoking habits, require more time for modification. As a result, a means of explaining certain time relationships exsist.

The investigator must be aware, however, of the following limitations associated with such investigations:

a. Possible self and forced selection on the part of the migrant;

b. A general lack in completeness and precision of birth entered on death certificates, morbidity reports, and population census records;

c. Difficulty in securing comparable statistics on causes of morbidity and mortality from country to country, due to discrepancies in standardization procedures, criteria, and diagnosis;

d. Problems in defining country of birth because of changes in national boundaries; and

e. International differences in medical care practice.

Regardless of the above, migrant studies have demonstrated the importance of identifying the place of birth and residence in cancer morbidity and mortality statistics. Apparently, age at migration should also be considered when attempting to evaluate a possible residual effect of past exposures. It is hoped that, in the future, further collaborative investigations between nations, such as that of Staszewski and Haenszel [18] regarding Polish migrants, will be carried out. Joint research, obviously, improves standardization of methods and analysis, and makes for greater reliability and valid comparisons. Studies on migrants in an adopted country and their siblings in the parent land will further help to clarify certain relationships by eliminating, in part, bias stemming from migratory selection.

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II. IMMUNOLOGY AND AGING

Immunologie en veroudering

1. Immunological surveillance against neoplasia
   Immunologische afweer tegen tumoren

2. The control of the immune response and its relevance in human oncology
   Genetica en immunologie. Betekenis voor de oncologie

3. Immunology of aging - Experimental approach and application
   Immunologie van de veroudering

4. Occurrence of spontaneous cancer with aging in an inbred strain of rats
   Spontane tumoren in oude ratten van een ingeteelde stam

5. Praomys (mastomys) natalensis in aging and cancer research
   De Praomys (mastomys) natalensis in ouderdoms- en kankerresearch

Immunological Surveillance against Neoplasia

Immunologische afweer tegen tumoren

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Summary

There is experimental evidence that induced and „spontaneous" tumours can be antigenic and that the host can react immunologically against tumour-specific antigens. On the basis of these and other relevant experimental results, „immunological surveillance" against neoplasia has been postulated. Although the evidence is circumstantial at best, there is reason to believe that immunological defences may also be operative in human cancer. A number of recent projects of the REPGO Institutes which bear on these problems are briefly reviewed.*

Samenvatting

Er zijn aanwijzingen dat experimenteel geïnduceerde en spontane tumoren antigeen kunnen zijn en dat de gastheer immunologisch kan reageren tegen deze tumor-specifieke antigenen. Op basis van dit soort experimentele gegevens heeft men vrij algemeen het bestaan van een immunologisch afweersysteem tegen tumoren aangenomen. Een dergelijke „surveillance" speelt waarschijnlijk ook bij klinische maligniteiten een belangrijke rol hoewel de bewijzen daarvoor meest indirect zijn.
In dit artikel worden een aantal relevante experimentele en klinische gegevens behandeld en sommige projecten van de REPGO-Instituten die direkt of indirekt met genoemde onderwerpen te maken hebben kort besproken.

In recent years the role of immunological defence mechanisms in oncology has been the subject of intensive investigations. The imaginative notion of such immunological „surveillance" against neoplasia was put forward by Burnet already in 1957 [1] and has been generally accepted as a useful working hypothesis. In the present communication we shall review some of the experimental and clinical evidence which supports this theory. Since the evidence for an involvement of the immune system in clinical cancer is tenuous, we shall deal primarily with experimental aspects of surveillance. Some implications of experimental results for current clinical immunotherapy of cancer will also be discussed.

Host reactivity against tumours

The starting point for any discussion of tumour immunology is the concept that many animal- and human tumours have antigenic characteristics distinct from those of the normal cells from which they originate. In the laboratory there is now solid evidence for the existence of tumour-specific antigens as well as for an immunological response of the host against antigenic tumours. Such responses are predominantly of the cellular type, i.e. host lymphocytes can become "killer cells" directed against the tumour. The process is thus similar to the rejection mechanism of transplanted normal tissue (the allograft reaction). In view of the experience obtained in the Radiobiological Institute in the field of transplantation immunology and because of current long-term programs on experimental radiotherapy of tumors and radiation carcinogenesis, it was opportune to apply some of the available immunological techniques and tumour models to investigate the role of immunology in carcinogenesis.
Humoral responses are also possible; the antibodies appearing in the host's serum can be toxic to the tumour cells and act synergistically with the killer lymphocytes. Unfortunately, antibodies of the „enhancing" or blocking type can also appear. These promote neoplasia by protecting tumours against the destructive activity of the killer cells. This and other complexities of tumour immunology still interfere with a more general application of classical immunological techniques in the prevention and/or treatment of clinical neoplasia. It is for this reason that knowledge about fundamental aspects of tumour immunology has to be extended and that some of the mentioned complexities have received priority in our research programs.

* Radiobiological Institute, Experimental Gerontology, and the Primate Center of the Organization for Health Research TNO

Evidence for surveillance

Let us now look at the actual observations which support the concept of immunological surveillance. The available clinical evidence is mostly circumstantial. For instance, a relatively high tumour incidence has been observed in individuals with a depressed cellular immune system, as found in old age and in genetically determined immune deficiencies. Another example is the increased tumour incidence in transplant patients on chronic immunosuppression; impaired surveillance is generally believed to be responsible for the increased tumour frequency in those patiens. Far less convincing are reports about so-called spontaneous regressions of clinical malignancies; the evidence that immune mechanisms play a major role in clinical tumour regression is very slender indeed.

More reliable and more readily interpretive data have been obtained from laboratory studies on immune surveillance; but interpretations can be rather difficult in experimental work, as well. For instance, in studies designed to prove increased susceptibility of immune deficient animals to chemical carcinogens, the drugs used to induce immune deficiency may have a carcinogenic effect of their own. On the other hand, the cytostatic effect of these same immunosuppressants may inhibit the growth of the malignancies which they are supposed to promote by their immunosuppressive effect. Table 1 shows some of the „shared characteristics" of immunosuppressive and tumour-inducing treatments which complicate the interpretation of many experiments in tumour immunology. The table also shows that thymectomy and treatment whith antilymphocyte serum (ALS) are not expected to be directly oncogenic. Since the Radiobiological Institute has studied the biological effects of thymectomy since 1960 and was directly involved with the development of ALS as an immunosuppresive agent [2, 3], we chose these methods to manipulate the immune defences of animals which did or did not receive oncogenic treatment. Some of the experimental results are presented in fig. 1 and table 3 (see below).

INFLUENCE OF IMMUNOSUPPRESSION WITH ALS ON THE INCIDENCE OF METHYMCHOLANTHRENE INDUCED TUMORS IN MICE

(Schematic representation ; from Balner and Dersjant, Nature 1969)

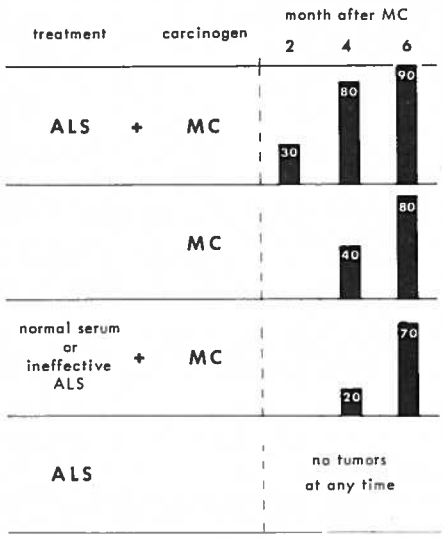

Fig. 1. The black vertical bars depict the percentage of animals with tumours at 2, 4 and 6 months after treatment with methylcholanthrene (MC). ALS was given for 2 months at weekly intervals, starting one week before an intracutaneous injection of 0.05 mg MC.

Experimental approaches

Table 2 presents common experimental approaches which are used to prove, or disprove immune surveillance against neoplasia. If immunological surveillance does indeed exist, it is difficult to understand how highly antigenic tumours can grow in the first place. One would expect immunologically intact animals to reject antigenic tumours as soon as they arise. To explain why such tumours can grow progressively in normal individuals, we must assume that the host's immune defences can somehow be circumvented. A widely accepted theory is the so-called "sneak-through" hypothesis, which implies that the growth potential of an antigenic tumour can sometimes override the host's immune defences [4]. This theory is supported by the finding that tumours arising

Table 1. *Immunosuppression and neoplasia*

	conventional immunosuppression		*neonatal thymectomy*	*anti-lymphocyte serum*	*chemical carcinogens*
Effect on cellular immune reactivity	+	++	++	+++	+
Oncogenic or leukemogenic effect	++	(+)	—	—	+++
Anti-tumour effect (anti-proliferative)	++	++	—	—	—

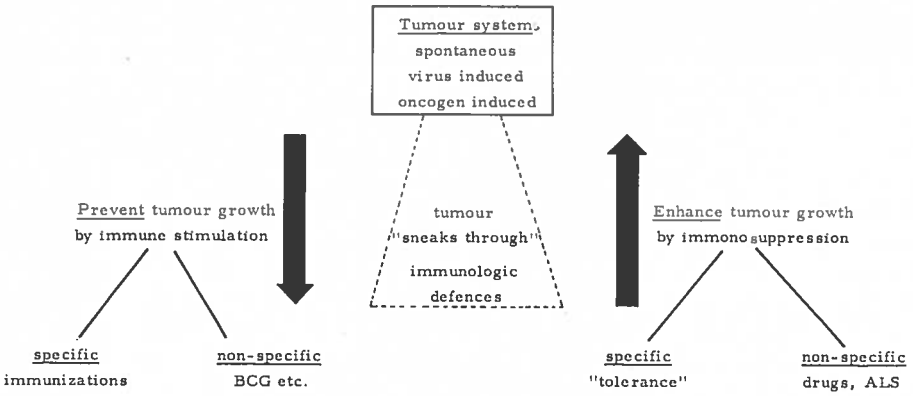

Table 2. *Approaches to study immunological surveillance in oncogenesis.*

early after application of the carcinogen are of the highest antigenicity; slowly growing tumours of high antigenicity would presumably be eradicated by the host's immune defences before becoming established.

As indicated in table 2, the concept of immune surveillance can thus be tested conveniently by modifying the host's immune reactivity. For instance, *non-specific* stimulation of the immune system (with BCG, various vaccines etc.) has been shown to lead to increased resistence to virus- and carcinogen induced tumours [4]. However, non-specific suppression of the immune system is a more productive approach and has been used more widely. For reasons indicated above, our own group has chosen thymectomy and ALS treatment to study the influence of depressed immune reactivity on tumour or leukemia induction [5, 6, 7, 8]. Typical for this kind of investigation is the experiment schematically depicted in fig. 1. It can be seen that chronic ALS treatment increased the susceptibility of mice to the oncogenic effect of methylcholanthrene, while ALS treatment alone (without a carcinogen) did not lead to tumour formation. Table 3 presents a survey of the principal investigations in this field; it can be seen that early thymectomy, as well an immunosuppression with drugs or with ALS has usually increased the susceptibility of experimental animals to oncogenic influences (viruses, chemicals, X-irradiation). Yet,

Table 3

depression of immune response by	cancer-promoting treatment	references and results obtained	
early thymectomy	virus	Allison e.a.'67, Nature 215, 185	+
		Vandeputte e.a.'63, Life Sci 7, 475	+
		Malmgren e.a.'64, JNCI, 33, 101	+
		Kirschstein e.a."64, Pr. Soc. EBM 117, 198	+
		Law, L.W. '65, Nature 205, 672	+
	chemicals	Miller e.a. '63, Nature 199, 920	+
		Grant e.a. '65, Nature 205, 1124	+
		Nishizuka e.a. '65, Nature 205, 1236	+
		Balner e.a. '66, JNCI 36, 513	0
		Johnson, S. '68, BJC 22, 93	+
	X-irradiation	Kaplan, H.S. '57, Can. 2d Canc. Conf. 2, 127	−
		v. Bekkum e.a. Proceedings Symp. Padova, 1971	0
cytostatic drugs and/or steroids	virus	Allison e.a. '66, JNCI 36, 859	+
		Haran Ghera, N. '67, Br. J. Canc. 21, 739	+
		Abelson e.a. '70, Cancer Res. 30, 2208	+
	chemicals	Teller e.a. '71, In: Host/Tum. Relationsh., 66 (Ac. Pr.)	+
anti-lymphocyte serum	virus	Allison e.a. '68, Proc. Soc. EBM 127, 207	+
		Hirsch e.a. '68, Lancet 2, 37	+
		Law e.a. '68, Nature 220, 611	+
		Many others	+
	chemicals	Balner e.a. '69, Nature 224, 376	+
		Cerrili e.a. '69, Transpl. 8, 774	+
		Rabbat e.a. '70, Transpl. 9, 164	+
		Trainin e.a. '70, JNCI, 44, 893	+
		Wagner e.a. '71, JNCI, 46, 1	0
	X-irradiation	Balner, H.'71, Eur. J. Cl. Biol. Res. 16, 981	−
		v. Bekkum e.a. Proceedings Symp. Padova, 1971	−

+ = increased susceptibility
− = decreased susceptibility
0 = no clearcut difference

when similar immunosuppression was applied to animals which were not given specific carcinogenic treatment, the incidence of „spontaneous" tumours was not increased [6, 9]. This seems to be in disagreement with the hypothesis of immune surveillance. If there is an immunological mechanism guarding against the occurrence of tumours, then immunosuppression should lead to an increased incidence of spontaneous tumours. Such controversial results can be due to inadequate experimental designs. For instance, the number of mice used in the above-mentioned experiments may have been too small to permit observation of a minor increase in the tumour incidence. Alternatively, the intricate „extra effects" of the various treatment (referred to in table 1) could also be responsible for results which are difficult to reconcile with the accepted working hypothesis of immune surveillance. Furthermore, non-immunological factors such as „organ-specific" resistance are known to keep tumour growth in check, and those factors will obviously not be influenced by immunosuppression.

An important recent finding is that resistance and susceptibility of mice to tumour viruses depends on their genetic make-up and that the genes determining such resistance are related to the animal's histocompatibility or tissue antigens [10]. Thus, genetic systems („the immune response loci" which are closely linked to the histocompatibility locus) are all- important in determining whether an individual will be susceptible or resistant to a particular tumour virus*. The implication is clear: only through an understanding of a species' histocompatibility system can we study and hopefully manipulate the immune response in a way which will lead to increased resistance to tumour viruses. Investigators at the Primate Center TNO have studied and unrevaled the histocompatibility system of rhesus monkeys, the RhL-A system [11]. In nearly every aspect, this system is similar to the HL-A system of man. Moreover, the monkey is phylogenetically close to man and serves thus as a better experimental model for humans than mice or rats. It is clear therefore, that the REPGO group is in an exceptionally favourable position to study the link between histocompatibility antigens and the immune response to tumour viruses in a primate species. A research program in this area has recently been started in collaboration with some of the world's most experienced investigators in the field.

Specific modifications of the immune response can also serve to test the hypothesis of surveillance. Specific non-reactivity is often referred to as „tolerance" and some investigators believe that antigenic tumours can establish themselves only because of existing tolerance. Experimentally, the tolerance approach is a rather difficult one. Therefore, most laboratory workers have preferred to perform experiments in which resistance to tumours is specifically increased rather than reduced. This is accomplished by immunizing animals against tumour viruses or against tumour-specific antigens. It has been shown that tumours or leukemia's induced by a particular virus share the same antigens. This means that effective prophylactic immunization of animals against those tumours is possible. However, antigenic tumours induced by a particular chemical carcinogen do not share antigens so that immunization against such tumours would seem to be impossible (except against an individual tumour of a particular animal). Yet, it has been reported recently [12] that the induction of sarcomas with methylcholanthrene can be prevented by vaccination with a leukemia virus, a finding which opens interesting avenues for tumour prophylaxis.

A complicating factor with regard to all types of active immunization is, as indicated earlier, the danger of inducing blocking or enhancing antibodies which could promote tumour growth. So far, no reliable methods are available which can predict or prevent the occurrence of such antibodies. However, once again the field of transplantation immunology may come to our rescue. Enhancing properties of antibodies can conveniently be studied also in the experimental allograft situation. Several teams within the REPGO Institutes are following this approach. As soon as the enhancing characteristics of antibodies can be reliably detected in those systems, the results will be applied to experimental oncology. The possibility of demonstrating enhancing antibodies in vitro may reduce the danger of inadvertently inducing them by active immunization.

Immunotherapy

Having reviewed some of the experimental studies on immune surveillance, one wonders how the results obtained relate to current trends in immunotherapy. Let us first consider the possibility of specific immunotherapy. There is reason to believe that at least some human leukemia's may be caused by viruses. If the causative viruses can be isolated, active immunization would seem to be an approach worth considering. Animal experiments have shown that the danger of inducing enhancing antibodies to leukemia cells is relatively small.

For non-leukemic tumours, the clinical situation may be more complicated, but active immunization is not impossible, a priori. Such tumours may also be caused by viruses. In that case, prophylactic immunization with the isolated virus can be con-

* Details regarding this subject can be found in Van Rood's article „The control of the immune response and its relevance to oncology" (this issue pg. 683).

sidered. But even if no viral or other causative agent can be identified, immunization should be considered as one of the possibilities. There is serological evidence that certain human tumours (osteosarcomas, melanomas, neuroblastomas, etc.) carry antigens specific for each tumour type. Therefore, active immunotherapy might again be kept in mind, preferably as adjunct following surgical excision of the tumour.

Another approach which is based on the existence of tumour-specific antigens, is the so-called adoptive therapy. Lymphocytes sensitized against tumour-specific antigens could be taken from another individual and injected into the patient; however, the applicability of this approach is very limited indeed and the few clinical attempts that have been made so far were disappointing.

In cases of leukemia, transplantation of bone marrow has also been attempted: after maximal erradication of a patient's leukemic lymphohematopoietic system, bone marrow from a normal individual is injected. The resulting graft-versus-host reaction can have some therapeutic effect on the host's leukemia but most of these patients eventually succumb to the consequences of the graft-versus-host reaction or to recurrent leukemia [13].

More closely related to the surveillance mechanism discussed above are clinical attempts to strengthen defences against neoplasia by non-specific stimulation of the immune system. BCG (Bacille Calmette Guerin) has been used most frequently but the results with BCG alone have been modest up to now [4, 14]. On the basis of current trends in experimental immunotherapy, the combination of non-specific stimulation with BCG plus specific immunization against tumour cells or tumour antigens [15] would seem to be an approach worth persuing also in the clinic.

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The control of the Immune Response and its Relevance in Oncology

Genetica en immunologie.
Betekenis voor de oncologie

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Summary

A systematic approach was made to recognise the histocompatibility systems in outbred species such as the dog and the monkey. It could be shown that, in man, next to the LA and Four loci, a locus exists which determines MLC reactivity.
The HL-A antigen W5 occurs in our group of Hodgkin-disease patients more frequently than in a control group. This has been the case in some, but not all, studies done by other investigators. These findings are discussed, and it is suggested that they might be due to variations in the linkage disequilibrium between the Four and MLC loci in the populations studied so far.

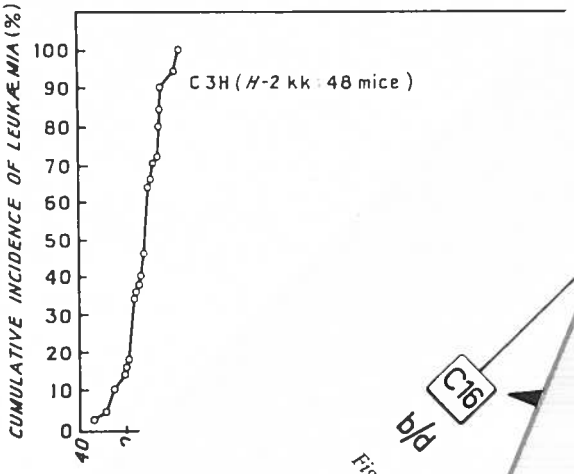
Samenvatting

Systematische pogingen om de histocompatibiliteits-systemen van de hond en de Rhesus-aap te herkennen zijn succesvol geweest en worden nog voortgezet. Naast de HL-A loci LA en Four werd op hetzelfde chromosoom een locus aangetoond dat MLC-reactiviteit bepaalt (het MLC locus).
Het HL-A antigeen W5 komt in onze groep van Hodgkin patiënten meer voor dan in controle-groepen. Sommige, maar niet alle onderzoekers, hebben hetzelfde gevonden. Overeenkomende resultaten en discrepanties worden besproken en er wordt verondersteld dat zij veroorzaakt zouden kunnen worden door een niet constant zijn van het linkage disequilibrium tussen het Four en MLC locus in de verschillende populaties, die zover zijn bestudeerd.

Introduction

All mammals which have been studied so far carry one histocompatibility system which is of overriding importance in determining graft prognosis. It is called the major histocompatibility system. The genetic information for this histocompatibility system is located on an autosomal chromosome. Recent evidence indicates that other loci which influence the immune response against well defined antigens, are situated in the same chromosomal region. It is also known that susceptibility to some oncogenic viruses is linked to this major histocompatibility system. It is the aim of this article to review briefly the pertinent experimental data, and to outline the protocols which are currently under study in our department to evaluate the relevance of these findings for human oncology.

As early as 1938, Gorer [1] noted that some inbred strains of mice were far more susceptible to infections with Gross leukaemia virus than others. Lilly [2] could show that this susceptibility was related to the presence, or absence, of a certain H-2 haplotype. A haplotype here defined as that part of the chromosome which codes for the major histocompatibility antigens, e.g. the H-2 system in the mouse. Figure 1 shows part of Lilly's data: mice carrying the H-2 k haplotype were highly susceptible to Gross leukaemia virus infection, while those carrying the H-2 b haplotype were resistant. The F 1's were also resistant, indicating that susceptibility was recessive. It is as yet not clear what the mechanism is by which H-2 haplotypes determine susceptibility to the Gross leukaemia virus infections. It is quite possible that this



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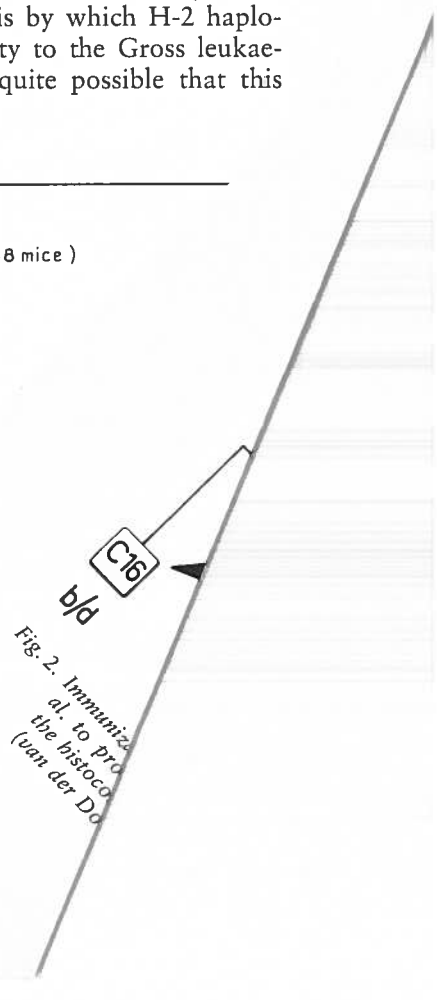
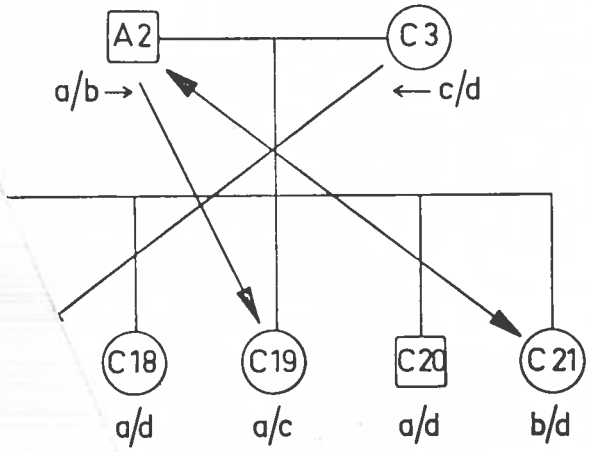


Fig. 2. Immuniz al. to pro the histoco (van der Do

susceptibility is under the control of the so-called immune response loci. These loci, which were recognised by McDevitt [3], Sela, Benacerraf and others (see for references 3), determine the ability (or inability) of the individual to develop immunity against a number of well defined artificial and biological antigens. Some of these loci lie in the direct neighbourhood of the loci coding for the determinants of the major histocompatibility antigens.
The recognition and study of these immune response loci have largely been done in inbred animals so far. Because recessive traits can be optimally recognised in inbred animals, it is to be expected that the situation in outbred animals (including man) will be much more complicated. For that reason, our group embarked upon a number of long term projects to investigate to what extent the findings in the mouse and guinea pig could be duplicated in outbred species. These projects consist of the following:

In the first place, our systematic attempt to determine the major histocompatibility systems in outbred species, and to evaluate the importance of these systems for organ transplantation, was intensified and extended. Together with Balner and McDevitt, an attempt was also made to determine whether the capacity to respond against artificial antigens is, in the Rhesus monkey, also governed by a locus situated near the loci coding for the major histocompatibility system. Finally, studies were done in man to better define that part of the chromosome which carried the information for the HL-A antigens, and to establish whether the susceptibility for certain diseases coincides with the presence of certain HL-A antigens or haplotypes.



ıtion protocol followed by Van der Does et duce, in a short time, reagents recognizing mpatibility antigens in a sib.
es, van Rood, Walker, Epstein 1972)

I. The study of histocompatibility systems in outbred species other than man

Balner et al. [4], and Vriesendorp et al. [5], have already reported on the progress which has been made in defining the antigens of the major histocompatibility systems in the Rhesus monkey and dog respectively. In general, the approach is as follows:
Animals are immunized by buffy coat cells and as a rule by skin grafts until leucocyte antibodies are detectable. These are then tested against a panel of lymphocytes and the results analyzed by computer, as was originally done for man [6]. This approach has been recently refined in the dog by Van der Does et al. [7] who performed the immunizations within families (Fig. 2).
In this way, the reaction pattern of the sera can be predicted. As a result, it is possible to obtain a full set of the reagents recognizing the haplotypes in a sibship of several generations in a fraction of the time that is necessary with the old approach.

With Balner and McDevitt, a study was initiated to determine the response of the Rhesus monkeys against (T,G)-A--L, an artificial antigen with a M.W. of 150,000. The animals studied so far (N = 49) did not make antibody. The study is continued with a slightly different immunisation procedure.

II. The study of the human histocompatibility system-HL-A.

The information for the HL-A antigens is located on an autosomal chromosome in at least two loci, LA and Four, which code for more than 10 and 20 allelomorphic antigens respectively. Eysvoogel from the Central Laboratory of the Red Cross in Amsterdam, together with our group, could confirm and extend the observations of others that the reactivity in the mixed lymphocyte culture test (MLC) is more influenced by differences in antigens of the Four locus than of the LA locus.
The fortunate occurrence of two crossing-overs in one family, one between LA and Four and (in another sib) one between the Four locus and the (at that time, still hypothetical) MLC locus made it possible to establish definitely that the MLC locus, that is the locus determining MLC reactivity, lies on the same chromosome as the LA and Four loci, but is separate from them. It is in all probability located nearer to Four than to LA (Fig. 3). Other loci which can influence MLC reactivity, but in a less consistent and strong way, are probably also present [8].

The question arises, of course, what is the biological significance of this MLC locus besides its capacity to influence MLC reactivity. One possibility is, that it is the human equivalent of the

immune response loci in the mouse, but no hard data on this point are available as yet.

Koch et al [9] could show, however, that matching for this MLC locus (in the presence of compatibility for LA and Four) improves skin graft survival (Fig. 4). This observation, which is of great practical value in organ transplantation, is also of crucial importance for studies which attempt to relate HL-A grouping with disease susceptibility. From the more than 50 unrelated HL-A identical pairs which were collected by Koch, and studied by Eysvoogel and Van den Tweel in the MLC test, only about 10 showed a low or negative MLC reactivity. This is in contrast with HL-A non-identical unrelated pairs, which almost always stimulate. It shows that a linkage disequilibrium, as was already known to exist for LA and Four, exists also for Four and MLC loci. More relevant for the study of susceptibility to disease is the fact that only about 20% of these pairs have a negative MLC reactivity. This indicates that they are not only identical for the LA and Four loci, but also for the determinants of the MLC locus. If the MLC locus is identical with (or lies in the direct proximity of) the locus that determines disease susceptibility, then it is to be expected that study of a group of unrelated patients versus an unrelated control group for a higher incidence of a certain HL-A antigen in the patient group will show, at best, a statistical, but not an absolute increase of a certain antigen. The findings in one population might also turn out to be non-reproducible in another population possessing a different linkage disequilibrium between that antigen and the gene determining disease susceptibility. This is indeed exactly what has been found. We have been, for instance, able to confirm the observations of Amiel [10] and Forbes and Morris [11] that patients with Hodgkin's disease have a higher incidence of the HL-A antigen W5 (Table 1). However, in a controled worldwide study, in which the same sera were used, this could not be confirmed. This might be due to different linkage equilibrium between W5 and the MLC locus determinant in the different populations studied.

It is clear that the study of diseases which have a familial preponderance might be much more revealing. A study of over 200 patients with mammary carcinoma, (in collaboration with Dr. F. Cleton from the Anthonie van Leeuwenhoekhuis in Amsterdam) is not yet completed to the point that we can indicate whether or not familial mammary carcinoma is linked to one HL-A haplotype. That this approach is, nevertheless, worth pursuing was recently demonstrated by Terasaki et al [12], who showed that (the rarely occurring) familial psoriasis was linked with the haplotype HL-A1 - HL-A17.

It is evident from the foregoing, that a major ef-

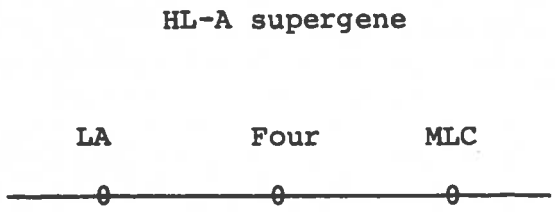


Fig. 3. Schematic presentation of the HL-A „super gene". An autosomal chromosome carries at least three loci, the LA and Four loci code for the HL-A antigens, the MLC locus for the MLC reactivity. (Eysvoogel, van Rood, du Toit, Schellekens 1972).

Table 1. *The incidence of the HL-A antigens R and W5 was significantly increased in 3 consecutive groups of Hodgkin's disease patients as compared to a healthy control group.*

	Total	R. pos.	W5 pos.
1st series	N = 98	43	33
2nd series	N = 50	23	13
3rd series	N = 25	13	6
Total patients = 123		79	52
Expected (contr. series 2,000)		46.6	32.5
X^2		22.5	11.7

(van der Does et al. 1972).

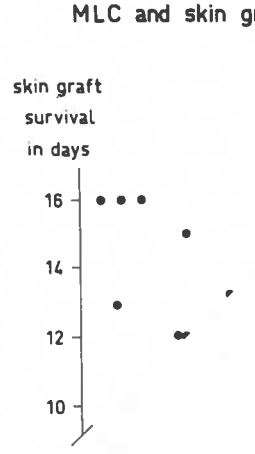


Fig. 4. MLC identic. MLC rea. 0.001). (Koch, Freder.

fort should be made to recognize the polymorphism of the MLC locus serologically. It is likely that when this has been achieved a major inroad will be opened to the understanding of individual disease susceptibility including cancer.

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Immunology of Aging - Experimental Approach and Application

Immunologie van de veroudering

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Summary

Heterogeneity is a prominent characteristic of the humoral immune response. An intriguing feature of aging in man is the increase in incidence of homogeneous immunoglobulins. This can be the result of a malignant process, but it can also be essentially benign. In view of the difference in prognosis and the possible necessity to administer potentially dangerous drugs in the malignant form a precise diagnosis is needed. Techniques are now available to reach this goal in the majority of the patients.
Recent observations in children may offer unexpected clues as to the nature of this feature of old age. Homogeneity could reflect an insufficient number of clones, which are available for antibody production, and old age may accordingly be associated with a loss of precursor stem cells.

Samenvatting

Een kenmerkende eigenschap van de normale humorale immunologische reactie is de heterogeniteit. Op hoge leeftijd ziet men echter frequent het verschijnen van homogene immuunglobulinepopulaties. Dit kan het gevolg zijn van een kwaadaardige groei, doch het kan ook een goedaardig symptoom zijn. Differentiëring is noodzakelijk gezien het verschil in prognose en ook gezien de wenselijkheid om bij de kwaadaardige vorm potentieel gevaarlijke geneesmiddelen toe te dienen. Dankzij verbeterde technieken is het thans meestal mogelijk een preciese diagnose te stellen.
Recente waarnemingen bij kinderen kunnen bijdragen tot het verkrijgen van inzicht in het wezen van de bevindingen bij ouderen. Homogeniteit kan een uiting zijn van een onvoldoende aantal cellijnen, die beschikbaar zijn voor de synthese van antilichamen. Dit kan een gevolg zijn van een nog onvoldoende groei en differentiatie van de stamcellen op jonge leeftijd en een verlies van de voorlopers op hoge leeftijd.

Immunology as a scientific discipline originally only concerned itself with the defense mechanisms against microbiological agents. At a later stage hypersensitivity reactions were included, followed by transfusion problems. Immunology now encompasses a much wider field and it can be defined at present as a science which deals with the different reaction mechanisms elicited by foreign substances, or more precisely by substances which are regarded as foreign by the organism. It thus covers immunity against infections and the allergic diseases, rejection of transplanted organs, immunohaematology, immunochemistry, immunogenetics, and tumor immunology. It encroaches upon inflammation and there are serious investigators who are willing to speculate on the role of immunological mechanisms for the regulation of normal growth. Classically one distinguishes humoral immunity from cellular immunity. The former is associated with the presence of circulating antibodies. These can be synthesized in any part of the lymphoid system, such as bone marrow, lymph node, the spleen, and they can exert their action in any part of the body and not necessarily at their site of production. In cellular immunity the immunological process is associated with cells, which have to be present at the site of action. The original term of delayed type of hypersensitivity is still widely used and it indicates that the time which is necessary for the development of the classical picture is considerable, for instance one to two days. The tuberculin test or the Mantoux reaction is the example, known to most of us. It will be clear that the analysis of humoral immunity can be expected to have been far more productive than that of cellular immunity. Blood constituents can be easily obtained in large quantities, whereas there are considerable limitations in the handling of cells. This statement receives support from the fact that the increase in knowledge in the field of humoral immunity has been little less than overwhelming. Protein chemistry has provided the major tools. It could be shown that all antibodies have a number of characteristic features in common and they are therefore regarded as members of one family, the immunoglobulins. The basic structure is now the totem figure of the immunologist (Fig. 1). The molecule is symmetrical and consists of two heavy and two light polypeptide chains. Per definition an antibody is an immunoglobulin with a specific affinity for a given structure, the antigen. It is therefore necessary that an immunoglobulin molecule contains a constant part, on which are localized the structures which are responsible for the

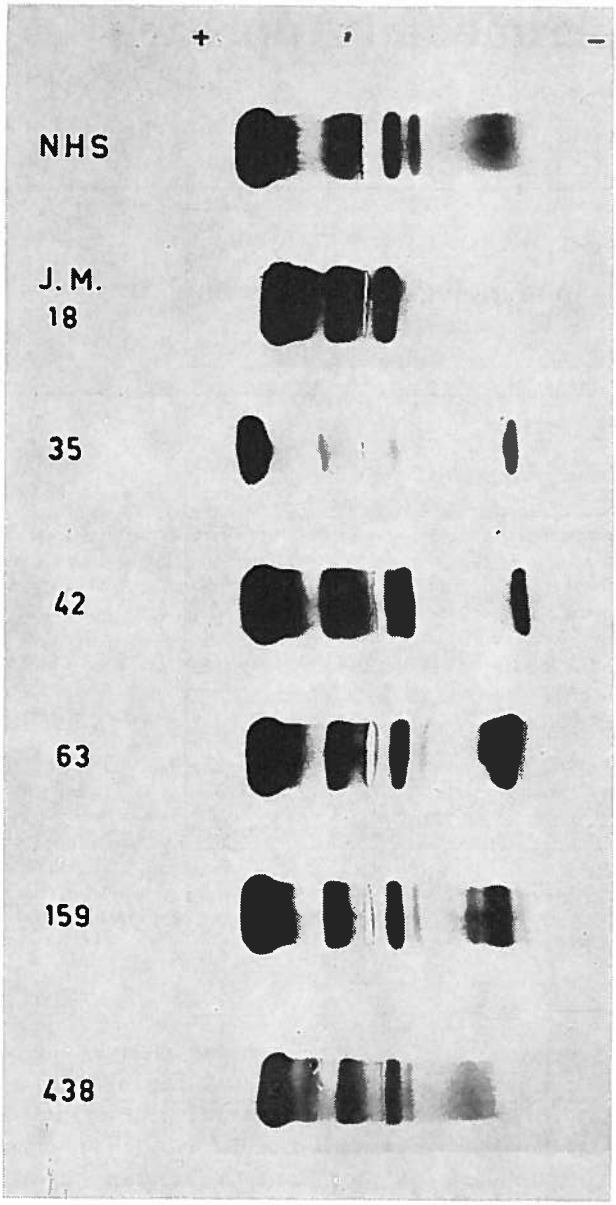

Table 1. *The family of immunoglobulins (Ig) can be subdivided as follows:*

Classes	*Subclasses*	*Types*
G	1, 2, 3, 4	kappa or lambda
M		kappa or lambda
A	1, 2	kappa or lambda
D	1, 2	kappa or lambda
E		kappa or lambda

Furthermore a large number of subgroups are now known, which are due to specific structures of the variable part of the light chain. In addition there are large numbers of variations on the basis of so-called allotypes.

Fig. 2. These electrophoretic patterns show the appearance of homogeneous immunoglobulin components in a patient (J. M.) suffering from severe combined immune deficiency and treated with bone marrow cells. NHS = normal human serum. The figures indicate the number of days after transplantation (from Rádl et al. 1972, with permission of the publishers).

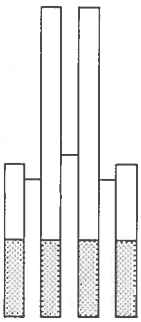

Fig. 1. Schematic representation of an immunoglobulin molecule. The structure is symmetrical with two light and two heavy polypeptide chains. The black areas are called variable parts, the open constant parts.

Table 2. *Frequency of paraproteinaemia in adults*

Authors	*year*	*source of specimens*	*number of specimens*	*age (yrs)*	*paraproteins (‰)*
Axelsson et al.	1966	mass screening	6995	> 25	0.9
				25 - 29	0
				80 - 89	5.7
Englišová et al.	1968	hospital admissions	369	65 - 79	1.6
		(excl. myeloma and macroglobulinaemia)	51	80 - 89	11.7
		home for the aged	26	90 - 99	19
Hällen	1963	homes for the aged	294	> 70	3
Hobbs	1967	new admissions	7200		0.9
Kohn and Srivastava	1972	blood donors	9420	20 - 62	0.2
		geriatric patients	1000	50 - 102	3
		same		90 99	6.2
Rádl and Rihová	1967	Spa attendants	7400	30 - 72	0.3

common characteristics of the immunoglobulin molecule, and also a variable part which has the binding capacity for the relevant antigenic structure. The actual situation is a bit more complicated: the variable part is not variable at random, nor is the constant part absolutely constant. Antibodies have in fact two major characteristics: specificity and diversity. Specificity has already been mentioned. With regards to diversity a number of subdivisions are recognized: three major and two minor classes are distinguished on the basis of the chemical composition of the heavy chain, each of which has either one of the two types of the light chain. Furthermore there are within the major classes a number of subclasses and within the variable part of the light chain a number of subgroups. There is no reason why they should not occur on the heavy chain. In addition there are large numbers of variations on the basis of so-called allotypes (Table 1). Administration of an antigen with single specificity can accordingly elicit an immune response of antibodies, each of which does carry affinity for the specific antigen and all of which belong to the family of immunoglobulins. The present information can account for a family size of several thousand members. From the latter point of view the pass-word is heterogeneity.

In view of the bearing of immunology on the well-being of aged persons and possibly also on the process of aging itself considerable attention is paid to this approach in the present programme. An intriguing feature of aging in man, at least with regard to the humoral immune response, is the increased incidence of homogeneous immunoglobulins in the serum, the so-called paraproteins. In these cases there is an increase of the level of one of the large numbers of immunoglobulins, often in combination with a decrease of the rest of the family. Their frequency is reported by several groups of investigators as about 1% in the adult population. The results of some of these studies are present in Table 2. They have been selected because of the large numbers of serum samples in each study, and also because some of them show the increase in frequency with age. The maximum figure of 19% was found by Englishova and collaborators who collected 26 specimens of inhabitants of a home for the aged in their 9th decade. Most of these groups have been selected, either on the basis of hospital admission, their willingness to act as blood donor, etc. Furthermore the distribution over the various age groups was not always equal, and finally different techniques have been applied. These factors can easily explain the differences in the results. It is unlikely however that a strong bias has been introduced, because the numbers were often very large. This is corroborated by the fact that Kalff (1970), who studied a sample of 252 specimens which had been collected on a purely random basis, found an incidence of 0.8% of paraproteins in his population. This figure is comparable to the figures found by the other investigators. Only a small minority of persons, in whom these homogeneous components have been found, suffer from a malignancy of the lymphoreticular system. The majority can therefore be classified as benign and idiopathice. Several estimates have lead to the conclusion that one malignant paraproteinaemia can be found for about every 200 of its benign counterparts.

A major question in the field of paraproteinaemia is whether the benign or idiopathic forms are related to the malignant diseases such as myelomatosis or Waldenström's macroglobulinaemia. Two sets of data are in support of a relationship. The first is that transitions of the benign form into the malignant disease have been reported. These cases have been reviewed by Waldentröm (1970). The frequency of course depends on the time of observation. In this respect the observations by Nörgaard (1971) are of special interest. He recently presented three cases of multiple myeloma in which the preclinical asymptomatic phase had persisted from 15 to 24 years. Although definite examples of this kind of transition have been presented it remains an exceptional phenomenon. The second set of data are derived from family studies. Nineteen such families have been reported (Festen et al. 1972) in which two or more members had a paraproteinaemia. In addition there is a report of a family in which in two generations three persons were found with an idiopathic form and three with myelomatosis (Meijers, de Leeuw and Voormolen, 1972). It has also been shown (Seligmann et al. 1967, Kalff and Hijmans, 1969) that IgM paraproteinaemia occurs more frequently in the members of the family of a patient with Waldenström's macroglobulinaemia than in control groups.

Several questions concerning the etiology of these phenomena can now be formulated more precisely. Are these idiopathic paraproteins antibodies to a particular antigen? Do they represent a premyeloma stage? Is their appearance a result of a gradual loss of certain lymphoid cell precursors, thus in fact an expression of an immunodeficiency? Is this related to the tendency to develop autoimmune disease or malignancy?

Although definite answers can not be given sufficient information is now available to allow practical applications for diagnostic purposes. Quantitative determinations of the individual immunoglobulin classes can help to distinguish the benign from the malignant form. Sometimes immunofluorescence can be most helpful, because with this technique the distribution profile can be constructed of the various cells, which are responsible for the synthesis of the immunoglobulins, as they are determined in the peripheral blood (Hijmans et al. 1971). In some cases this technique has proved to

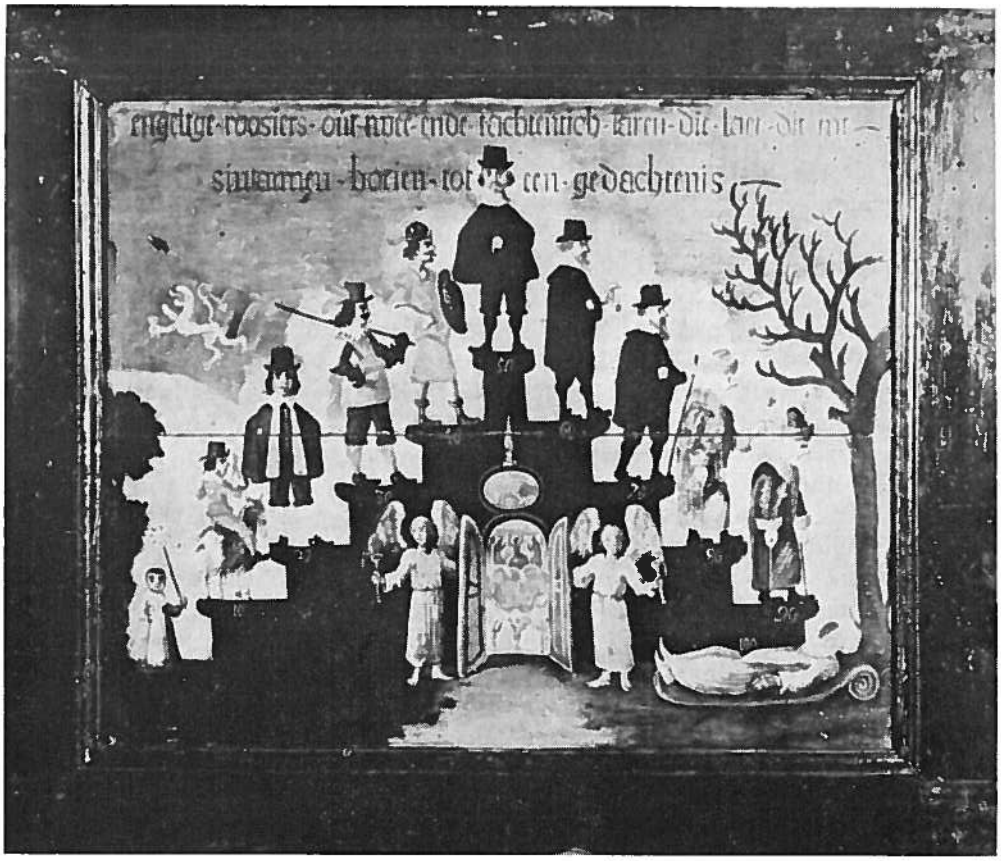

Fig. 3. Allegorical picture of the life-cycle, presented by a 82 year old lady at the Saint Anne Almshouse in Leiden in the 15th century.

Table 3. *Paraproteins in children*

Diagnosis		*No.*
Immune deficiency	— severe combined, no transplantation	2
	— severe combined, bone marrow transplantation	2
	— severe combined, thymus transplantation	1
	— severe combined, bone marrow and thymus transplantation	1
	— type Nezelof	1
	— no further classification	1
Leukemia	— acute myelomonocytic	1
	— stem cell	1
	— lymphoblastic	2
Wiskott-Aldrich syndrome		3
Familial lymphohistiocytosis		1
Sepsis in premature infant		1
Placental transfer		3
Not yet classified		1
		21

be indispensable for an acurate diagnosis. This is of more than academic interest because the so-called idiopathic paraproteinaemias usually do not require specific treatment, whereas the malignant form does. Furthermore the drugs, which should be administered to these patients are by no means harmless and a precise classification of the disease may therefore save the patient a potentially harmful medicine.

Unexpected clues may be offered by two recent observations, which may well lead to new ways to study this problem. One of them is that also in children such homogeneous populations of immunoglobulins can be found, and these occur almost exclusively in association with some kind of immunodeficiency (Table 3). The second observation concerns the appearance of transitory paraproteins in children with immunodeficiency diseases after successful transplantation treatment with bone marrow derived cells (Rádl et al. 1972). In these children the reconstitution of the immune system seems to follow the same way as in normal ontogenesis as e.g. the sequence of the appearance of the individual immunoglobulin classes is concerned. During the early period of reconstitution, the immunoglobulin spectrum is restricted in heterogeneity and during that time usually several homogeneous immunoglobulins could be observed. In the further course the serum immunoglobulin pattern became normal if compared with a standard of the recipient's age.

The appearance of paraproteins in these three different situations may have a common denominator — a certain kind of immunodeficiency. While in those babies the homogeneity of certain immunoglobulins might represent a situation of a response of limited clones of cells, because the other ones had not yet matured, the old-age paraproteinaemias can be a reflection of a situation, where only a limited number of clones are still available to respond to certain antigenic stimulations, because the other part of precursor cells is lost, or the mechanisms which generate a normal heterogeneous response are already exhausted. This hypothesis reminds one of old ideas showing the young — and old age as a mirror picture (Fig. 3). A promising experimental approach is the study of the serum proteins in irradiated monkeys after treatment with bone marrow derived cells. In preliminary experiments paraproteinaemia was indeed found (Fig. 4). These positive results will enable us to further analyse this problem in a situation which is easier to manipulate than those hitherto available.

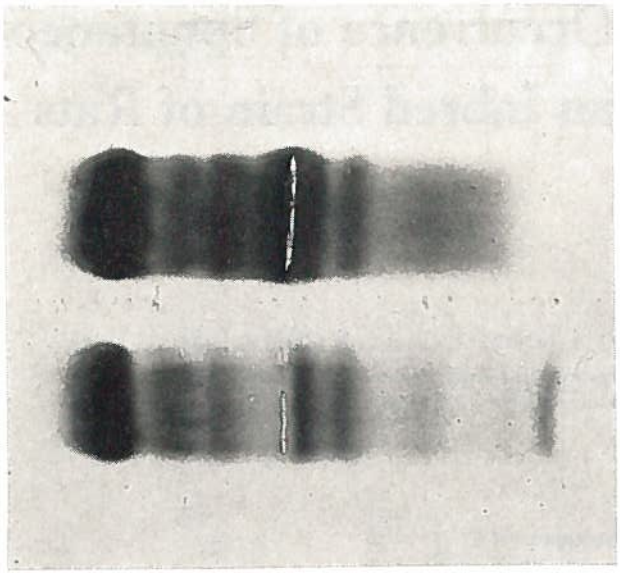

Fig. 4. The electrophoretic pattern of the serum of an irradiated monkey after bone marrow transplantation shows the appearance of a homogeneous component (bottom), which is absent in the control strip (top).

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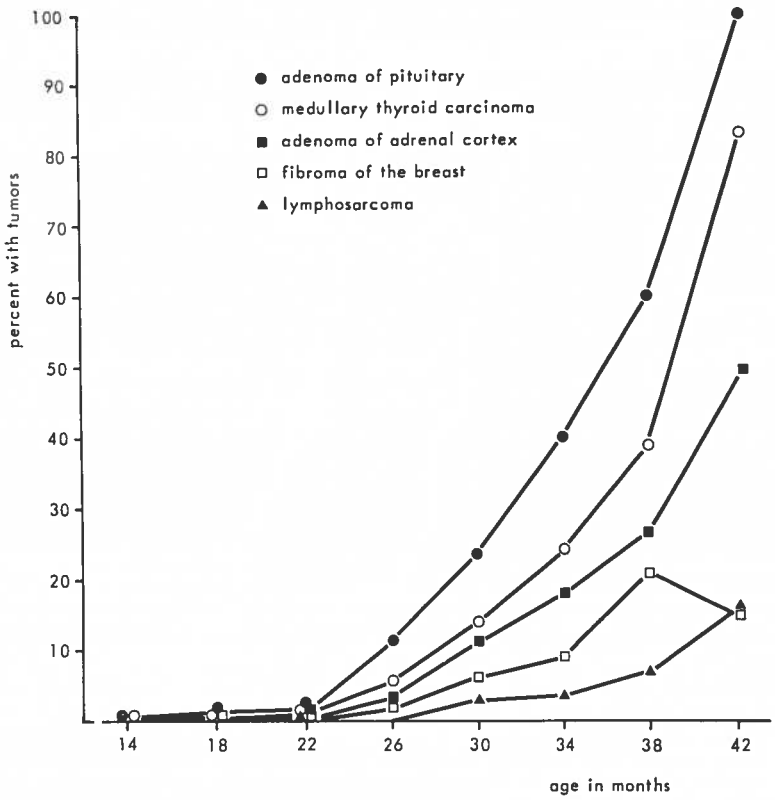

Fig. 2. Prevalence (per cent incidence) of five tumors in 290 female WAG/Rij rats. Plotted as 100 T/N where T = total rats having tumor in a given age group; N = total number of rats alive at that age.

and age for five of the more frequent tumors in the female WAG/Rij rat, is shown in figure 2. The tumors rarely occurred in rats of less than two years of age. The chromophobe adenoma of the pituitary, and medullary thyroid carcinoma, rapidly approached 100% prevalence in older age.

This suggests that perhaps all females may eventually acquire this neoplasma if allowed to live long enough. The adrenal cortical adenoma also increased significantly, reaching 50 per cent by 42 months of age.

Fibroma of the breast and lymphosarcoma tended to occur later in life, and the prevalence does not appear to increase markedly with age.

In another study on longevity and onset of lesions in male rats, it was found that the incidence of a certain tumor increased up to 2 years of age and then decreased. This meant that the probability of an older rat acquiring this tumor was less than for a younger animal (Simms et al., 1957). This led the authors to postulate that only a certain percentage of the population was susceptible to this tumor, and in the older population the susceptible animals had already acquired the disease and been eliminated by death. This phenomenon might explain our data on the fibroma of the breast.

A variety of tumors was frequently seen in a rat, and table 2 lists the total number of different tumors found per female rat according to age. The greatest number of tumors found in a rat was five, however, this number was found in six rats. In man, cancer in the same individual is more frequently multiple than would be predicted by statistical probability (Anderson, 1971). This is compatable with the theory that immune surveillance plays a role in preventing the occurrence of cancer. Table 2 is interesting in this regard, since it illustrates that rats dying with cancer ususally have several different kinds of tumors. The average number of tumors per rat dying with cancer does not appear to increase with age. This raises the interesting possibility that, regardless of age, once a rat becomes „susceptible" to cancer it is susceptible to a variety of tumors.

Some areas of study that may provide further insight into the relationship between multiple cancer and aging in the rat are necropsy of healthy rats of different age groups to determine the incidence of occult tumors; investigations into the possible stimulation of the formation of other tumors by an endocrine tumor, and a study of the immune status of rats having a number of different tumors.

Conclusion

This report provides data on the occurrence of spontaneous tumors in WAG/Rij rats. Not unexpectedly, tumor prevalence tends to increase with age and is a leading cause of death. Tumors were frequently multiple, but the number of tumors per rat did not appear to be age related.

Having acquired the baseline data, it will be possible to pursue the relationship of various factors such as environment, immunological status, and the appearance of spontaneous tumors. These data suggest that the rat can be a useful model in the field of carcinogenesis, just as it has been in so many other areas of biology.

Table 2. *Occurrence of multiple tumors in the female rat*

Age in months	total no. rats	total rats with tumors	No. of rats with given number of tumors					number of tumors/tumor bearing rats (mean ± SD)
			1	2	3	4	5	
9 - 11	1	0	0	0	0	0	0	—
12 15	4	3	1	2	0	0	0	1.7 ± 0.5
16 19	9	9	1	4	4	0	0	2.3 ± 0.7
20 - 23	7	7	2	3	0	2	0	2.3 ± 1.2
24 - 27	44	37	10	15	10	2	0	2.1 ± 0.9
28 - 31	84	78	23	23	27	4	1	2.2 ± 1.0
32 - 35	81	77	24	23	21	5	4	2.2 ± 1.1
36 - 39	54	51	12	24	11	4	0	2.1 ± 0.9
40 - 43	6	6	0	1	3	1	1	3.3 ± 1.0
Total	290	268	73	95	76	18	6	2.2 ± 1.0

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Praomys (Mastomys) natalensis in Aging and Cancer Research

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De Praomys (mastomys) natalensis in ouderdoms- en kankerresearch

Summary

The need to develop animal models that reflect realistic aspects of human aging and in which the hazard of the occurrence of cancer should be an integral part is stressed. The possible usefulness of the Praomys (Mastomys) natalensis for aging and cancer research is discussed.

Samenvatting

De noodzaak om proefdiermodellen te ontwikkelen waaraan de relatie ouderdom en kanker nader bestudeerd kan worden, wordt benadrukt. Nagegaan wordt of voor dit type onderzoek het gebruik van de Praomys (Mastomys) natalensis voordelen biedt boven de tot nu toe algemeen gebruikte inteeltstammen van muizen en ratten.

Cancer is one of the major causes of death in the aged. There are numerous reasons to believe that there exists a relationship between aging and cancer. In order to ensure that our animal models of aging reflect realistic aspects of human aging, the hazard of the occurrence of cancer should be an integral part of those models. For example, it must be taken into consideration that, according to presently fashionable views, a relationship exists between aging changes in the immunological system and the occurrence of malignant tumors in older age.

When searching for suitable animal models to study this relationship in more detail, it should be recognized that the widely used inbred strains of rodents have been developed and selected for cancer research, and not for aging research. Thus many strains exist where a certain type of cancer approaches 100% at a relatively young age. An example is leukemia in the AKR strain of mice (Russell, 1966; Murphy, 1966). For this reason, the introduction of other species may provide less artificial models.

The Praomys (Mastomys) natalensis (Davis, 1965), also called the multimammate mouse, is an animal intermediate in size between the mouse and the rat, (Fig. 1). It has an average weight of 80-100 gm, and a life span of approximately 3 years. At present, no inbred strains are available. Its potential value in cancer research is considerable, for it is a museum of spontaneous tumors (Oettle, 1967). In addition to the neoplastic lesions, it develops with age a wide range of degenerative and other non-neoplastic lesions, such as degenerative joint disease (Sokoloff et al., 1967), renal lesions (Snell et al., 1967) and thymic lesions associated with signs of autoimmune disease (Stewart et al., 1968; Strauss et al., 1968).

It seems therefore, warranted to explore whether or not the Mastomys is a suitable animal in which to study, in more detail, the relationship between aging and cancer.

In a previous report, the spectrum of spontaneous tumors in male and female Mastomys was described (Hollander et al., 1971). As in man, it was found that cancer is one of the major causes of death in aged Mastomys (unpublished data). The discovery by Snell et al. (1969 a, b) that gastric tumors are carcinoids has been most disappointing, because it is now evident that the Mastomys does not provide an animal model for the natural adenocarcinoma of the glandular stomach. However, the species provides us with non-malignant and malignant thymic lesions (Stewart et al., 1968; Strauss et al., 1968; Kurokawa et al., 1968). These differ from those observed in inbred mouse strains, and are similar to those found in man. Furthermore, tumors which are infrequent in other rodent species are found in a relative high frequency in Mastomys. Some of them are even infrequent in man, for example, carcinosarcoma of the seminal vesicle and adenocarcinoma of the rete testis (Snell et al.,

in press). The finding of proliferative hyperplasia of the prostate gland, and adenocarcinoma of this gland in female Mastomys (Snell et al., 1965; Holland, 1970) may give us a model for the study of the problem of prostate cancer in man. It is well known that cancer of the prostate gland is a very frequent finding in older males.

The observation of Sokoloff et al. (1967) that in Mastomys a severe degenerative joint disease of diarthroses and intervertebral disk develops regularly during the second year of life, has also been noted by us. We also observed a number of cases in which degenerative disk tissue protruded into the spinal canal. This gives rise to degenerative changes in the cauda equina, and occasionally lead to paraplegia. The nature of these lesions is still obscure.

In a few instances, in male and female animals over 2 years of age, a chronic thyroiditis was found. The infiltrate consisted of lymphocytes and plasma cells with accompanying destruction of follicles. The histologic picture bears some resemblance to Hashimoto thyroiditis in man.

In addition to the neoplastic and non-neoplastic lesions mentioned above, histopathologic investigations revealed an age dependent glomerulopathy (Van Noord et al., 1972). The renal lesions showed some resemblance to the lesions found in mice of the NZB/Bl strain and its outcrosses, and to the nephropathy of systemic lupus erythematosus in man. In the NZB mouse, an immunological disorder has been confirmed (Mellors et al., 1971; Lambert et al., 1968). In Mastomys kidney, deposits of immunoglobulins and complement have been demonstrated. These are suggestive of antigen-antibody complex mediated kidney disease (Van Noord et al., 1972). The nature of the antigen component of the immune complex in Mastomys is still unknown. In NZB mouse strains, DNA and Gross leukemia virus antigen have been found in the kidney (Mellors, 1971). Several differences exist between the disease in Mastomys and NZB mouse strains. Coombs' positive hemolytic anemia does not develop with age in the Mastomys and the percentage of animals with antinuclear antibodies is not abnormally high (Van Pelt et al., 1971). The kidney lesions are of a moderate nature, and do not result in impairment of renal function or severe proteinurea.

As has been stated, the nature of the antigen in the immune complex deposits in Mastomys kidneys is still unknown. Antigens that are especially suspect are: tumor antigens, viral antigens, and DNA. At this time, there is no proof that viral antigens and DNA are involved in the pathogenesis of the renal lesion. With regard to tumor antigens, it is worthwhile to mention that in human neoplastic disease, immune complex glomerulonephritis occurs — sometimes with demonstrable tumor antigen (Lewis et al., 1971). In a number of cases of Hodgkin's

Fig. 1: Adult male (to the right) and female Mastomys

disease, a nephrotic syndrome is found which is thought to be caused by an immunological reaction (Plager et al., 1971). Experimental studies in mice have shown, that the liability to develop immune complex mediated kidney disease may be related to a genetically determined deficient immune function (Soothill et al., 1971). Induced immunodeficiencies are also associated with immune complex nephritis (Teague et al., 1970). Furthermore, Makinodan et al. (1971) have shown a diminished immune response in aging mice.

The occurrence of an age related kidney disease in Mastomys seems to be of importance, not because of its severity, but because it may indicate an altered immune function. Furthermore, the pathology of aging Mastomys is dominated by the occurrence of multiple tumors, among which lymphoepithelial tumors of the thymus are of much interest. At present, lymphoepithelial tumors of the thymus in rodents have been described only in Mastomys. Stewart et al. (1968) have found no association between the occurrence of thymic hyperplasia, or thymoma, and the occurrence of other tumors in the same animal. With respect to the immune surveillance theory of cancer, it seems to be indicated to extend studies in Mastomys to the relationship between age changes in the immunological system and the occurrence of cancer.

Hellström et al. (1971) have emphasized the need to develop better animal models for immunologic prevention and cure of tumors. Mastomys might be the animal to be used in the future for these types of studies.

Therefore, it seems necessary to extend gerontological studies in this species with special attention being given to fundamental aspects of organ aging, and to the relationship between aging and disease — including cancer.

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III. DIAGNOSTICS

Diagnostiek

1. Thermography in cancer-detection

   Opsporing van borst-kanker met behulp van thermografie

2. The ultrasonic diagnosis of intracerebral tumours by means of Electronic Sector Scanning method: Electroscan

   Ultrageluidsdiagnostiek van hersengezwellen met de elektronische Sektor Scan methode: Electroscan

3. Aspects of regional cerebral circulation measurements in patients with intracranial tumors

   Aspecten van regionale circulatiemetingen bij patiënten met hersentumoren

Thermography in Cancer-Detection

Opsporing van borstkanker met behulp van thermografie

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Summary

Of 705 breast thermograms of a selected group of patients, 300 could be compared with the result of biopsy. In 631 cases there was also a radiographic examination available. A total of 106 cancers were demonstrated in this group of which 87 or 83% were predicted by thermography; with mammography only 66.6% was scored as suspect. In 17% the "diagnosis" was missed by thermography.
These data accord very well with literature findings.
Of the 148 scored as positive by thermograms — i.e. as pathologically changed heat-pattern — 20% showed no signs of malignancy of biopsy. However, in the course of 3 to 16 months after the original thermogram was taken, 7 showed a proved cancer.
The false-positive rate, as well as the fact that several of these cases developed a carcinoma in a rather short period thereafter, is sustained by literature.
The reliability of thermography justifies the use of this method for „screening procedures".

Samenvatting

Van de 705 in een niet aselecte groep verkregen mammathermogrammen konden er 300 worden vergeleken met het resultaat van het patholoog-anatomisch onderzoek. In 631 gevallen was ook een vergelijking met de resultaten van het mammografisch onderzoek mogelijk.
In totaal werden 106 carcinomen aangetoond, waarvan 87 (= 83%) op grond van het thermografisch onderzoek werden vermoed en 66⅔% op grond van het mammogram.
In 17% werd de „diagnose" dus door het thermografisch onderzoek gemist.
Deze getallen komen zeer goed overeen met die uit de literatuur.
Van de 148 positief gekwalificeerde thermogrammen — dus verdacht voor een actief proces — bleek in 20% deze verwachting niet door het patholoog-anatomisch onderzoek te worden bevestigd. In 7 gevallen echter bleek in de loop van 3 tot 16 maanden toch een carcinoom aanwezig. Ook dit „fout-positief" percentage blijkt overeen te stemmen met de gegevens in de literatuur, alwaar eveneens wordt vermeld, dat van deze aanvankelijk „fout-positieve" getallen een aantal binnen vrij korte termijn óók patholoog-anatomisch een carcinoom bleek te hebben.
De betrouwbaarheid van de thermografie is voldoende te achten om deze methode voor „screening" te gebruiken.

Introduction

All authors agree that only early diagnosis can add in improving survival rates in cancer patients. There is a striking unanimity in switching to detection and detection programs instead of experimenting with therapy.
For successful detection, the method to be used must be reliable; for a successful program, the method must be simple and not time-consuming. It must also be harmless to the patient.
For cervix carcinoma, the "Pap-smear" is an excellent example which meets each of these provisions.
One of the most resistant cancers with respect to treatment and survival rates is breast cancer. Most authors agree that only little has been gained in the 5-year survival rate during the last 50 years. On the other hand, it has been demonstrated that an "early" detected breast cancer has a better prognosis in spite of the diameter of the original tumour. Early diagnosis in this respect means before metastasis has taken place (Fisher et al., 1969). Programs with periodic mammography have proved their value (Gershon-Cohen, 1967). Technical problems, such as the costs of equipment, radiation hazards, and the number of radiologists needed, have held back the wide spread use of survey programs. So, a more simple method can be considered.

Thermography

This article will not attempt to give a detailed survey of thermography, as this information can be found in other publications (Aarts, 1965, 1967, 1969; Gershon-Cohen, 1967; Cade, 1969). The basic principle is to determine skin temperature by measuring its infrared emission. It has been known for more than 40 years that underlying processes, with increased metabolism and vascularisation, influence this temperature (Czerny, 1929). Lawson (1956) proved that this phenomenon can be used in the diagnosis of breast cancer.
During the last 15 years, much experience has

been gained in the field of thermography as expressed in numerous publications. Technical equipment has also been improved.
Systematisation of normal thermal patterns of the female breast has been achieved (Jones, 1969; Gros 1970; Almaric, 1972 and others). Standards for evaluation have been established; some simple (Aarts, 1971; Almaric, 1972) and some more complicated (Gros, 1971).
During the current year, the Social Security Administration of the Department of Health, Education, and Welfare of the U.S.A., has placed thermography on the list of reimbursable methods of investigations in the pathology of the female breast.
All of this underlines its value as a method of examination, but does not mean that it replaces other diagnostic aids. In Sweden (Melander), Scotland (Shawe), Canada (Ghys), and the United States (Strax), pilot-programs for mass-screening for breast cancer by means of thermography are in progress. In Italy, the United Kingdom, France, and the United States, thermography is used as a routine procedure in several Cancer Detection Centers.

Materials and method

As the aim of this study was to evaluate the efficacy of thermography in predicting abnormalities — a diagnosis of cancer can never be made on the basis of the thermogram — all women presenting themselves to the surgical department with breast complaints (in- and out-patients) were examined by thermography.
Because of the need for a reliable parameter, the histological diagnosis, we had to accept a selective rather than a non-selective group.
It is known that mammography, even in the hands of the most experienced investigators, fails to detect from 11 (Gershon-Cohen, 1967) to 25 percent (Wallace, 1968) of the cases of breast cancer.
Thermography was carried out in a cooled and air conditioned room at 18°C, following an appropriate time for "cooling down" of the patient as published elsewhere (Aarts 1965, 1969) and as generally accepted among thermographers.
A frontal, dorsal, and two oblique views were taken. The heat pattern was photographed on 35 mm film — in black and white in the beginning (fig. 1), later in colour (fig. 2) — for documentation. All isotherms were also recorded.
The evaluation was made according to criteria previously published (Aarts, 1971), and as shown in fig. 3.
Normally, no additional information was available, i.e. the evaluation of the thermograms was done without exact knowledge of complaints, clinical examination, or mammography.
In this selected group, we collected 705 thermograms. Only 300 of these could be checked by histology; 636 thermograms could be compared with radiography. These may be divided into three groups:

Group I. a total of 231 thermograms (Th.), compared with mammography (Rö.) as well as histology (P.A.) (table 1)

Group II. 69 thermograms (Th.), compared with histology (P.A.) only (table 2)

Group III. 405 thermograms (Th.), compared with mammography (Rö.) only (table 3).

Table 1.

TH.	Rö.	P.A.	N.
+	+	+	41
+	+	—	26
+	—	+	16
+	—	—	27
—	+	+	5
—	+	—	4
—	—	+	7
—	—	—	105
			231

Results

Although a simple judgment can never be made, and a certain number of equivocals is normal, in this comparative study all readings where "there is some activity but we have doubts about a malignant character" were scored as negative. This is of course not quite correct, but the data thus gained are more on the pessimistic than on the optimistic side. The results for group I can be seen in table 1.
In 231 cases, there were 69 carcinomas of which 57 were suspect by thermography. Fourty-six were diagnosed by mammography and 7 were missed by both methods. Of the 46 radiographically diagnosed cases, 5 were missed by thermography; on the other hand, of the 57 suspected by thermography, 16 were missed by mammography.
Of the 121 thermographically negative cases, 12 proved to have carcinoma; of the 110 positive scores according to the thermal pattern, 53 proved negative, although 26 of them were suspect for cancer on the basis of mammography.
In table 2, the results for the 69 patients in Group II are shown.

Table 2.

TH.	P.A.	N.
+	+	30
+	—	8
—	+	7
—	—	24

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The Ultrasonic Diagnosis of Intracerebral Tumours by means of Electronic Sector Scanning method: Electroscan

Ultrageluidsdiagnostiek van hersengezwellen met de Elektronische Sektor Scan methode: Electroscan

J. C. SOMER [1]), H. A. C. KAMPHUISEN [2]) and W. A. OOSTERBAAN [1])

Summary

A new form of ultrasound diagnostic possibilities is presented (The Electroscan).
Evaluation of some technical procedures in the field of neurology is presented and these are compared with the Electroscan. Without knowledge of the clinical findings a score of 64 per cent correct localizations of brain tumours could be made in a series of 17 patients.

Samenvatting

Een nieuwe vorm van ultrageluidsdiagnostiek (De Electroscan) wordt besproken en vergeleken met enkele andere neurologische onderzoekingsmethodieken.
Zonder voorkennis van de klinische bevindingen is met behulp van deze nieuwe methode een precieze lokalisatie van hersentumoren verkregen in 11 van de 17 gevallen (64%).

Introduction

The current practices of technical examination in the field of neurological disturbances would deeply shock Romberg, who wrote in the early 1800's that „The great aim which we must seek to achieve is the emancipation of medical science from the trammels of mere mechanical technicality".
At the moment, however, the technical procedures introduced in neurology during the last decades, such as electroencephalography, echoencephalography, and isotopic scanning, which have been claimed to establish diagnosis of neurological disease with 97 to 98 per cent accuracy (Walker) [6] cannot be abandonned. A new method almost never completely replaces older techniques and the still growing possibilities of technical investigations require an art of selecting the most appropriate technical aid.
Walker warns that there must be no misunderstanding: "a clinical neurological examination will suggest the correct diagnosis in 75 per cent of cases. No single unaided technical procedure can approach this accuracy. Even the much heralded isotopic scan fails to yield such information if the readers interpret the scan without knowledge of the clinical findings (about 60 per cent of 40 tumours were correctly diagnosed)".
The neurological history and examination, electroencephalogram, echoencephalogram, and X-rays of the skull are simple and safe means of quickly demonstrating a space-occupying process. An isotopic scan is without much danger for the patient, although it may require more time. For the hands of the investigator it can become a somewhat risky procedure (Hoencamp) [7]. Angiography carries a slight risk of aggravating a patient's condition*. It is a rather time consuming procedure for which anaesthesia is desirable, but it may yield invaluable information to the surgeon.
Any procedure, possibly modifying the blood brain barrier, such as angiography, may vitiate the results of an isotopic scan or an electroencephalogram. Consequently, the latter examinations are scheduled before angiography is performed.
Investigations with ultrasonic tools can be made and repeated at any time. The method is entirely harmless to the patient even in a comatose state.

Introducing a new method for clinical purposes is often a frustrating task.
The primary interest is, of course, to know what diagnoses can be made with it, which cannot be accomplished by the conventional methods. Starting experiments without having any experience with the method, and without knowledge as to which particular brain lesion it is best equipped for, one has, on the one hand, to work without any knowledge of the other neurological findings. On the other hand, one needs these findings for comparison and confirmation.

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* Krayenbühl and Yasargil [8] report in their literature study on cerebral angiography, an overall complication incidence (mortality and permanent neurological deficits) during carotic angiography of 0.2 to 5.0 per cent.

Electronic Sector Scanning principle

Electronic sector scanning is based on the use of a so-called array transducer, which consists of about 20 elements spaced half a wavelength apart (ca. 0.5 mm). According to Huygen's principle, one may appreciate that all the individual wavefronts of the elements will cause, by superposition, a resulting wavefront. This may form any angle with the array itself, depending on the time intervals between the excitations of the elements.
Direction of propagation is perpendicular to this flat wavefront. By rapidly varying the time intervals, direction of the beam can be swept over a sector of about 90°. In this way, scanning is possible at a rate of about 30 scans per second.
Receiving is directional as well, and direction of maximum sensitivity is similar to that of transmitting at every moment. The display on the oscilloscope-screen is such that a cross-section through the area under investigation is mapped into an echo-picture.
By this technique, the ultrasound probe is held stationary, just as with the conventional A-scan methods, and the screen displays instantaneous and continuous pictures.
A search can be made for the optimum pattern by moving the probe slightly and observing the screen simultaneously.
Moving structures can especially be observed directly. This method is particularly suited for brain diagnosis because the investigation is carried out in exactly the same way as the conventional echo-encephalography with A-scan. The only (but invaluable) difference is that here two-dimensional cross-sectional pictures are obtained.
For further information, consult the literature [1, 2, 3, 4 and 5].

Clinical Investigation

The two-dimensional electronic sector scan echo-encephalography (Electroscan) offers the possibility of obtaining clear information concerning spatial relations inside the skull. The pictures obtained by this method are usually complex and not easily interpreted. The midline structures are easily recognized and their location may be measured accurately, but for this determination conventional A-scan echoencephalography is more convenient. The Electroscan has proved to be particularly useful for the study of abnormal structures in a cerebral hemisphere. The evaluation of the results is carried out afterwards, on the basis of measurements on photographs made from the oscilloscope screen during the examination.
Usually 24 to 30 photographs are taken of each patient. These pictures are obtained from probe positions on homologous places on the right and left sides of the skull. The probe is placed so that scanning can be done in either a horizontal or a vertical plane.

At present, a series of more than 20 normals, and over 100 patients have been investigated. In the group of normal individuals the Electroscan pictures are not very interesting. The midline and end echoes can be found easily and the ventricle walls can be detected, but, in general, the area between the midline and the wall of the skull does not show clear reflections (empty area), although now and then arterial pulsations are present.
It should be mentioned that the prototype used here exhibited several shortcomings which undoubtedly can be overcome to a great extent in the future.

In the group of patients (aged between 14 months and 67 years), 17 were suspected of a space-occupying lesion, the localization of which was not mentioned to us. In some patients, clinical diagnosis by conventional means was difficult, and in others, the condition was very poor. For both reasons, the Electroscan investigation was requested before more dangerous methods of examination (angiography, pneumencephalography) were undertaken.
A complete evaluation of the observations on this material is not possible at present. This is because of the fact that a definitive diagnosis is not yet known in every case. (table I).

The nature of the space-occupying processes of the 11 correctly localized cases was as follows:

Meningoma	1
Arterio-venous aneurysm	1
Chron. subdural hematoma	1
Glioblastoma multiforme	4
Astrocytoma grade III	3
Cyste	1

Among the uncertain cases, the most likely clinical diagnosis was:

Not capsulated temporal abscess	1
Metastatic lesions	2
Jackson's epilepsy e.c.i.	1
Bundles of depth electrodes	1

In the light of Walker's statement that even the much heralded isotopic scan when used as an unaided technical procedure fails to come to a

Table 1. *Electroscan Localization*

Diagnosis	*Space-occupying lesion (17 cases)*
Correct	11 (64%)
Uncertain	5 (30%)
Incorrect	1 (6%)

correct diagnosis above 61% if the observers interpret the scan without knowledge of the remaining clinical findings, the Electroscan percentage is very encouraging.
The amount of material is, of course, too small to reach definite conclusions.

Material

Some patients will be described extensively, and the Electroscan pictures will be given, in order to show by which procedure a correct localization of the lesions was achieved.

Case I.

A woman, aged 55 years, had complained of headaches since her youth. These were localized particularly in the posterior part of the head. Lately, vision had deteriorated. The woman was obese (103 kg), loquacious, and somewhat confused.
The blood pressure was 220/110 mm Hg. The occular fundi were hypertensive and, on the right side, papilloedema was noted.
The left pupil was slightly wider than the right one. There was slight bilateral exophtalmos and a slight ptosis on the left side. No other neurological abnormalities were encountered. Elsewhere the diagnosis of pituitary tumour had been considered. The EEG was markedly abnormal and asymmetrical, with hypofunctional abnormalities dominating in the right frontal areas spreading over the entire hemisphere and to the left frontal area.
Conventional echo-encephalography showed a displacement of midline structures to the left side, amounting to 5% in the temporal-central areas.
The Electroscan investigation was carried out by an investigator who was not informed of the findings described above. When the probe was placed on the left temporal and left central areas, a massive "shadow" was observed in the right frontotemporal area, relatively close to the skull base and side (figure 1). It was concluded that these reflections originated from a space-occupying process. The conventional A-scan echo method had not shown echoes in the right frontal-temporal area. Subsequent neurological examination (angiography, scintigraphy figure 2) revealed this mass to be a large meningeoma in the right frontal-basal area. The meningeoma was neurosurgically removed.

Case II.

A man aged 34. In this patient, cerebral angiography had revealed an unusually large arteriovenous aneurysm. The location of this aneurysm was not known to the Electroscan investigator at the time of the examination. When the probe was

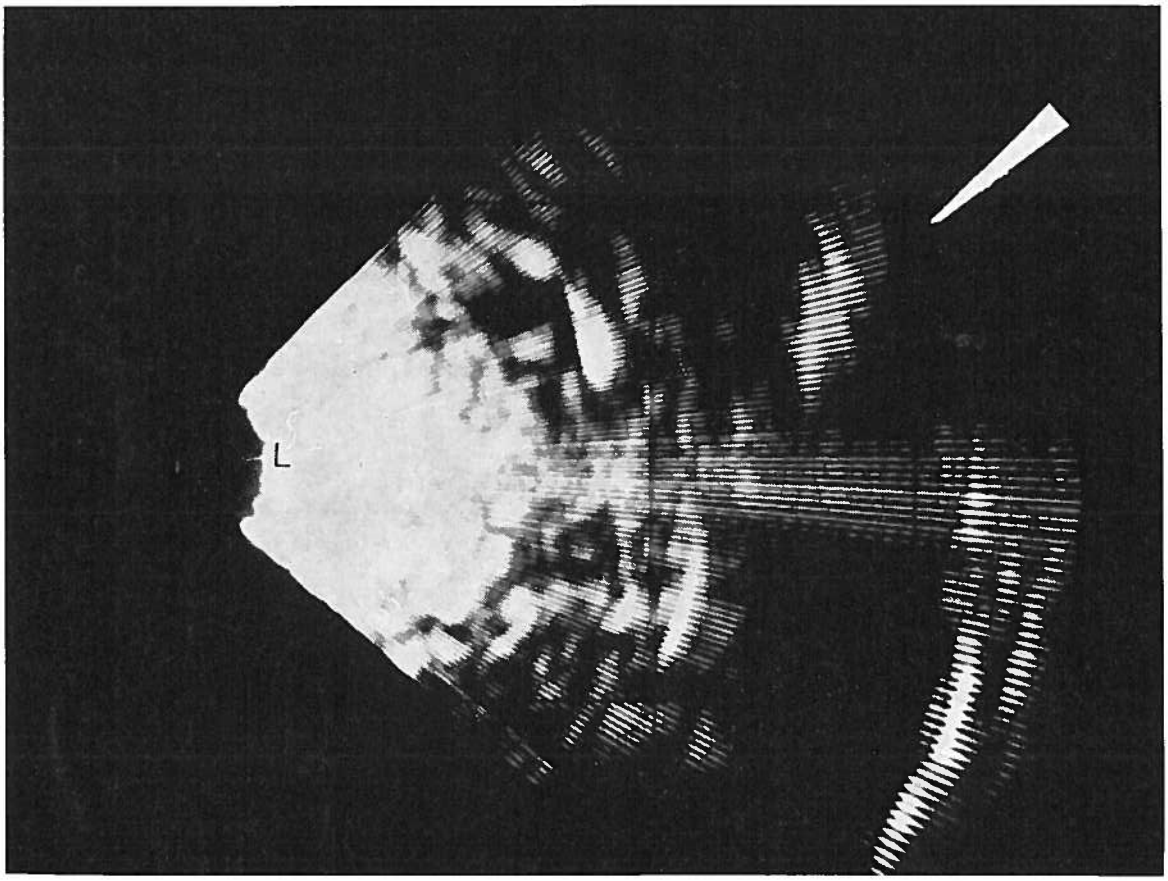

Fig. 1. Photograph of a horizontal Electroscan; probe left temporal (L). In the right hemisphere (arrow) there is a massive shadow which was interpreted as pathological. At operation, a large meningeoma was encountered in this area and subsequently removed. (Case I).

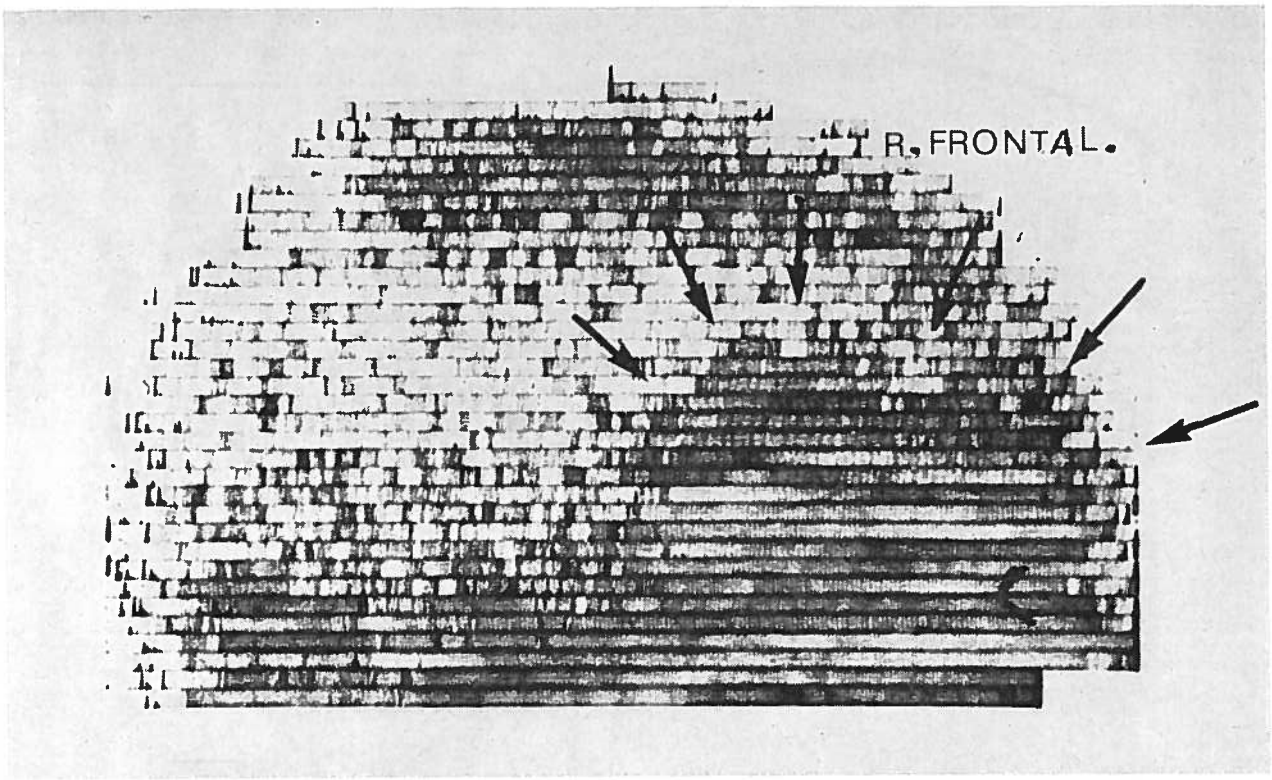

Fig. 2. Scintigraphy of case I.

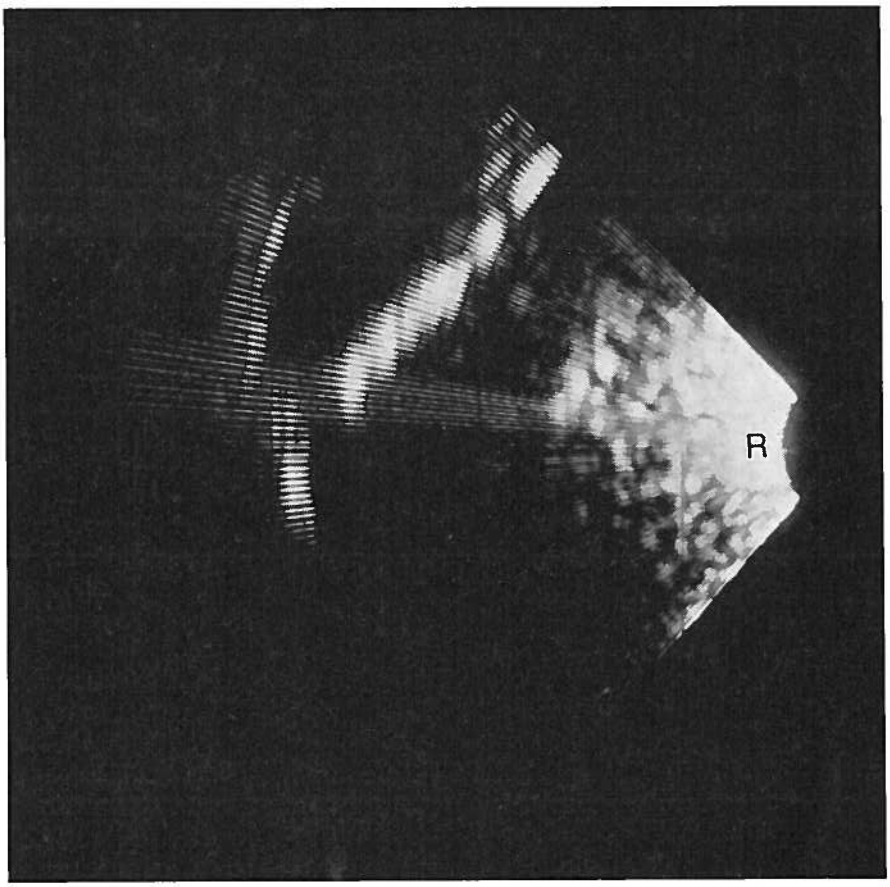

Fig. 3. The probe is placed horizontally in the right temporal area (R). The streak-shaped echoes are reflections in the left hemisphere from midfrontal to approximately centro-temporal.
The angiogram of this patient showed a very large arterio-venous aneurysm with voluminous varices in the area where these reflections occurred. (Case II).

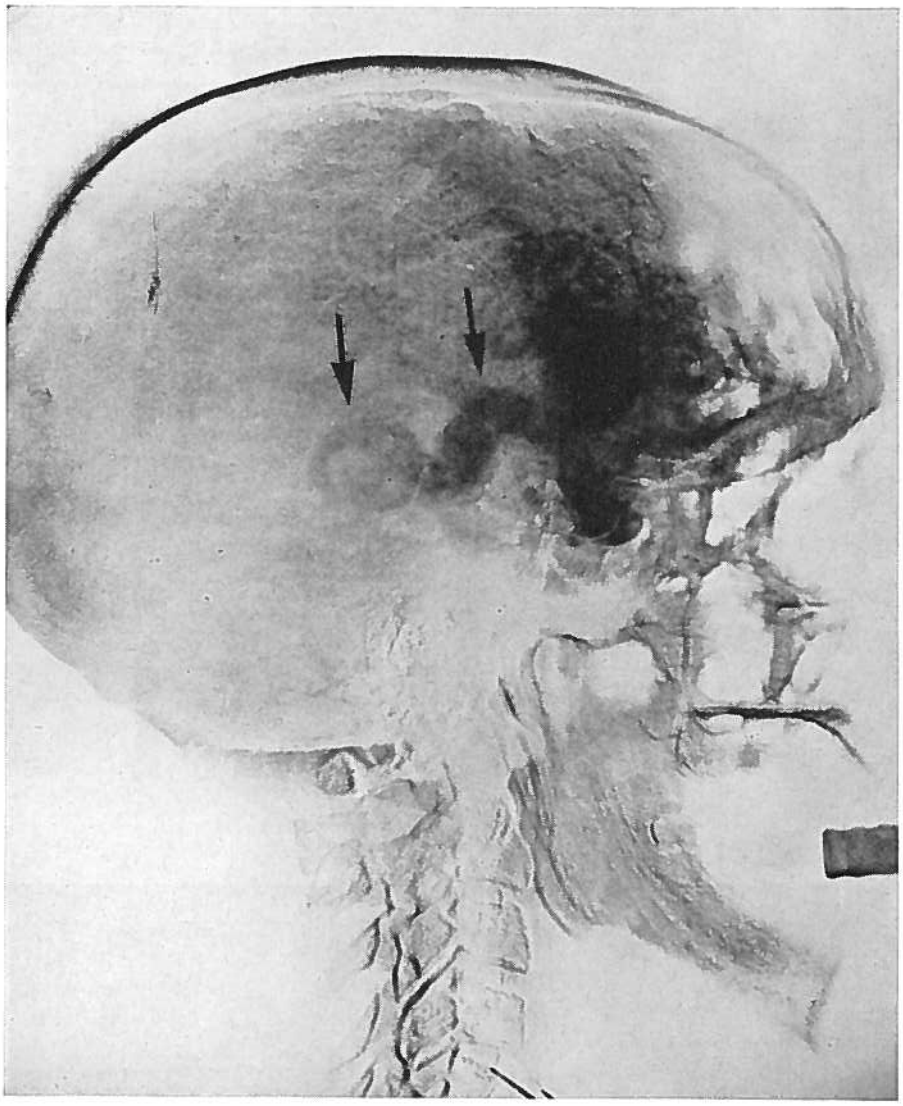

Fig. 4. Arteriogram (substraction technique) of Case II.

placed on the right frontal area in this patient, unequivocal echo-complexes were noted in the left frontal area. When the probe was placed on the right central area, conspicuous shadows were also seen in the left fronto-central areas. When the probe was moved gradually to posterior parts of the skull, echo complexes were visible in the left hemisphere to approximately 3 cm behind a vertical line through the tragus (figure 3).
Moreover, in the fronto-central area, the midline structures were displaced 7 to 8 mm to the right side. These pictures were obtained with the probe in a horizontal position. In this patient, conventional echoencephalography had not shown displacement of midline structures. It had been noted however, that, at probe location on the right temporal area, multiple echoes were obtained which made the determination of midline structures impossible. The EEG in this patient showed slightly diffuse, nonspecific abnormalities. No consistent asymmetry was noted. The left-sided carotid-angiogram (with proximal compression) showed a large angioma with voluminous branches in lateral direction distal of M3. (figure 4).

Case III.

A girl, aged 11 years, had complained of headaches for about 4 months. These occurred especially during the morning and were localized in the left frontal area. On one occasion, she collapsed at school. The ocular fundi showed papilloedema, but otherwise no neurological disturbances were found.
The EEG was markedly abnormal and asymmetrical, with severe hypofunctional abnormalities in the left temporal and posterior-temporal areas. The right hemispere showed no pathological wave forms. Conventional echoencephalography showed a displacement of midline structures to the right side, amounting to 8% in temporal-central areas and to 5% in the parietal area.
When the probe was placed in a horizontal plane at the right temporal side, the Electroscan investigation showed a large, irregular shaped shadow in

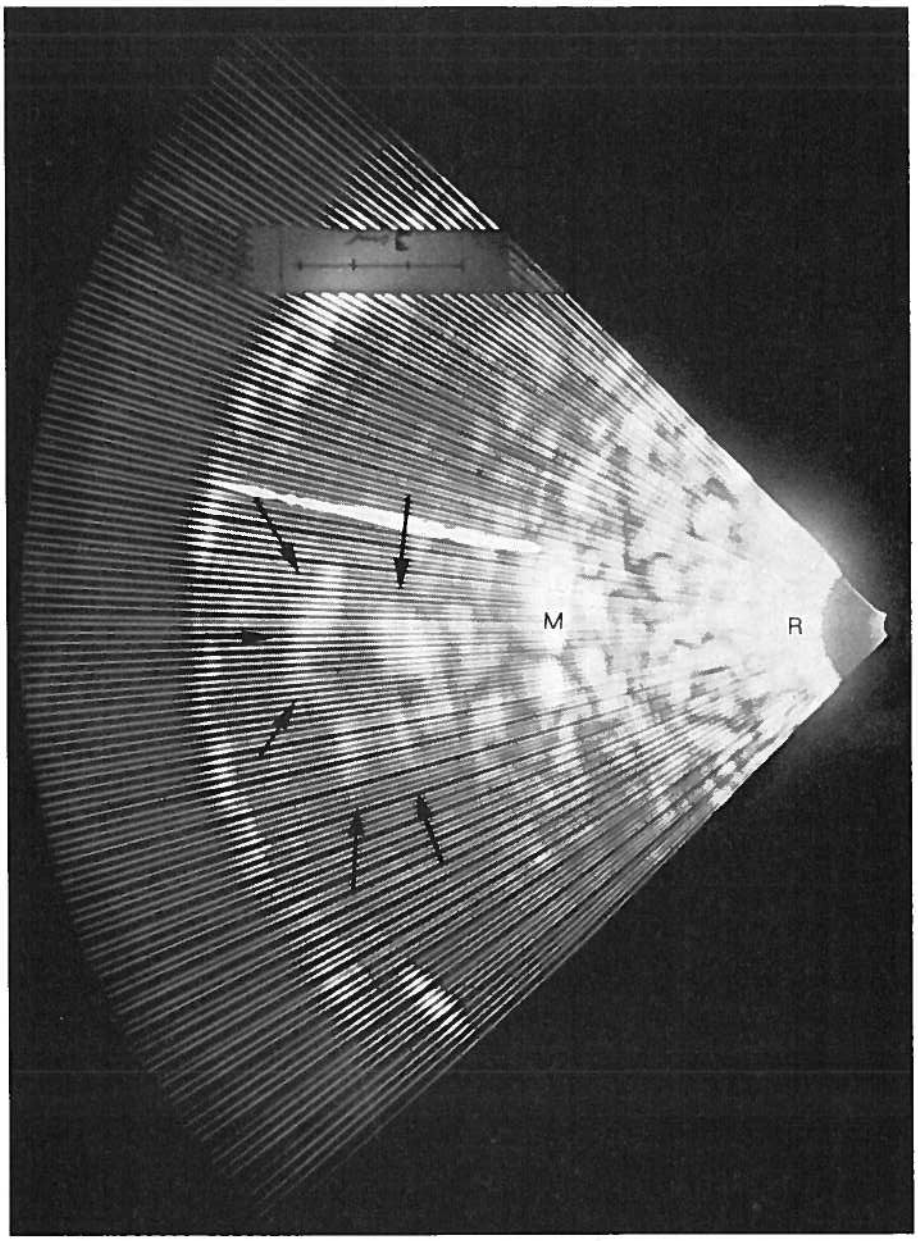

Fig. 5. The probe is placed horizontally in the right temporal area (R). M indicates the mid-echo reflections, which are displaced 5-6 mm to the right.
The arrows indicate reflections of an enormous cystic astrocytoma in the left temporal lobe. (Case III).

the left contro-temporal area (figure 5). It was concluded that these reflections originated from a space-occypying and possibly cystic process.
At operation, a malign cystic tumour (astrocytoma grade III?) was found in the left temporal lobe, infiltrating thalamic structures. After the sub-total operation, the condition of the patient was good and cobalt radiation was performed.

Conclusions

It is obvious that the Electroscan is useful as a supplementary diagnostic method for space-occupying processes in cerebro. In a small group of patients (17 cases), a correct localization could be made in about 64 per cent of the cases without prior knowledge of the clinical findings. The Electroscan in its present state does not replace the conventional methods of technical investigations, but recent developments have shown that the quality of the echo pictures can be enhanced considerably. The method has the advantage of being completely harmless. Furthermore, unlike angiography, pneumencephalography, and, to some degree isotopic scanning, the Electroscan can be applied repeatedly for screening very seriously ill and comatose patients.

Acknowledgement

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Aspects of Regional Cerebral Circulation Measurements in Patients with intracranial Tumours

Aspecten van de regionaal gemeten hersencirculatie bij patiënten met intracraniële tumoren

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Summary

The regional cerebral circulation was measured by means of the Xenon[133] clearance method in 16 patients with intracranial space-occupying lesions.
A summary of the results obtained is given as well as some illustrative examples.
Intracranial tumors may influence many aspects of the cerebral blood flow and its regulation.

Samenvatting

Bij 16 patiënten met een intra-cranieel ruimte innemend proces werd de regionale cerebrale bloeddoorstroming gemeten met de Xenon[133] uitwasmethode.
Na een kort overzicht van de literatuur wordt een samenvatting gegeven van de resultaten en deze worden geïllustreerd met enkele voorbeelden.
Intra-craniële tumoren kunnen invloed hebben op vele aspecten van de cerebrale bloeddoorstroming en op de regulatie hiervan.

Introduction

The neurological symptoms in patients with intracranial tumors are determined by many factors.
Apart from the volume of the tumor and its localisation, the following factors are of importance:

a) Increase in intracranical pressure and the rate of increase in this pressure.
b) The extent of the existing oedema of the brain in the tissue around the tumor — especially severe in metastasis.
c) The relationship between the tumor and the regional cerebral blood flow (rCBF).

The last three factors are interdependent, having a strong mutual relationship.
The degree of this relationship is variable and difficult to predict in the individual case.
Hence, it is understandable that, though diagnostic methods which are routinely used (arteriography, pneumoencephalography, positive contrast-encephalography, brainscanning) give fairly exact information with regard to localisation and anatomical extent of the process, they do not provide exact information concerning the patho-physiological processes in and around the tumor. Until a few years ago, the only technique available for the study of the cerebral patho-physiology in patients was electroencephalography.

The electroencephalogram (EEG) is an extremely sensitive device for the detection of cortical functional disturbances.
However, it gives little (or, at least, no specific) information about the functional state of subcortical structures.
Moreover, the electroencephalogram seldom gives explicit information about the cause of a functional disturbance and its localising value is limited (Cooper 1965).
It is for this reason that there is a widely growing interest in the measurement of intracranial pressure (Lundberg 1965, Jennett 1972) as well as in regional cerebral blood flow measurements.

Methods

The technique of regional cerebral blood flow measurements using radioactive gases has been described in detail elsewhere (see among others Lassen 1963, Høedt-Rasmussen 1965, Jonkman 1968). Broadly speaking, the investigations are conducted as follows: a physiologically inert radioactive gas (Xenon[133]) is injected through a teflon catheter into the internal carotid artery. (The gas is dissolved in a saline solution).
A few seconds after the injection the gas will have completely diffused throughout the whole brain tissue.
The injection is given very quickly and the clear-

ance of the xenon from the brain tissue begins immediately. The clearance curve can be observed with the aid of extracranially placed detectors. We used 16 detectors with crystals of 1.2 cm and Philips XP 1110 photomultipliers.
It is possible to measure in a fairly restricted local area if a narrow collimator is used.
From the clearance curves obtained, a number of parameters can be calculated (see also Høedt-Rasmussen 1965, Pálvölgyi 1968, Wilkinson 1968, Olesen 1971):

1. Regional cerebral blood flow through grey, as well as through white, matter (in cc/100 g. min.).
2. Mean regional blood flow through grey and white matter (in cc/100 g. min.).

This latter value can be obtained by several methods:

a. Weighted mean flow.
b. "Height over area" analysis.
c. Initital flow measurements.

For the undermentioned observations only the so-called rCBF (initial) (Paulson, 1969) was used.

3. The relative weight of grey and white matter in the region under the detector (in % of 100 g. tissue).

Our procedure differed only in detail from the techniques used by other investigators.
A special apparatus was developed to determine the exact localisation of the detectors in relation to the arteriograms previously made.

One must realize that, in patients with large tumors, it is possible that some detectors measure only the circulation through the tumor, and not through the brain tissue (see figure 1). An additional complication is that the diffusion-coefficient is known for grey and white matter, but unknown for tumor tissue. Because we were very interested in the relationship between the EEG and the results of the rCBF measurements, an EEG was registered continuously throughout the experiments, using standard methods (16 channels, 10-20 system; Jonkman 1970).
With this procedure, the regional cerebral circulation was studied in 16 patients with brain tumors. At the time of the investigation, all patients were hospitalized at the St. Ursula Clinic.
In all but 2 cases, the diagnosis was verified, not only by arteriography, but also by operation and/or autopsy.

Results

The mean normal value of the rCBF values is, according to other authors, 55 cc/100 g. min. (SD = 5.9).
In previous experiments performed on a group patients of different ages, results indicated that in old age there is a decrease in the mean rCBF.
This decrease is difficult to acertain since it is almost impossible to obtain normal values for older people.
But, even taking into account the physiological decrease in old age, we can state that we found *a very marked decrease in mean regional cerebral blood flow* in all our tumor patients. The lowest values were found in patients with extensive, dif-

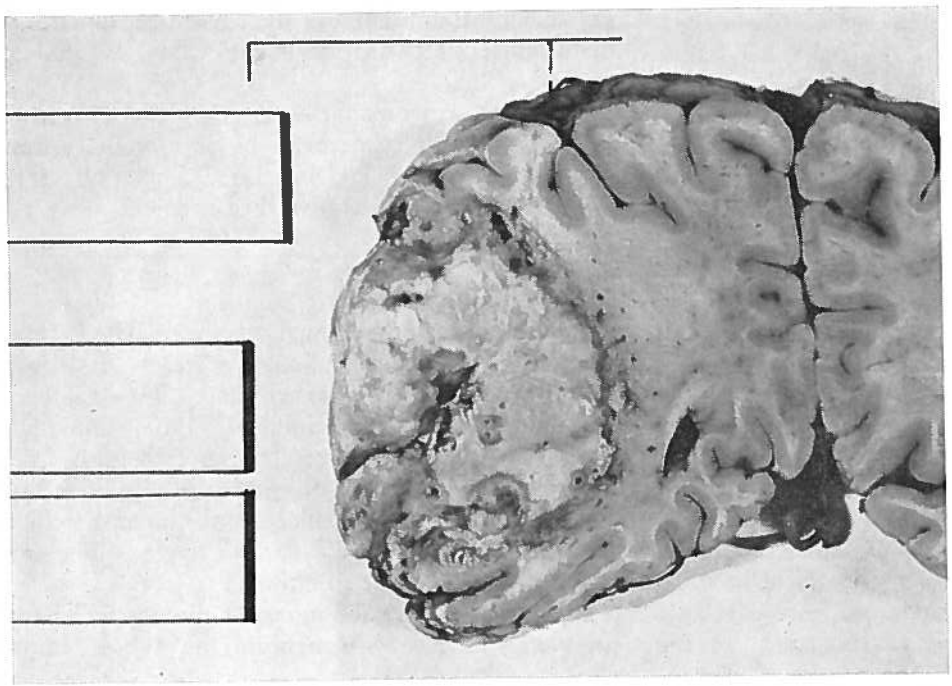

Fig. 1. Large cerebral tumor (astrocytoma). The contour of three detectors is outlined.

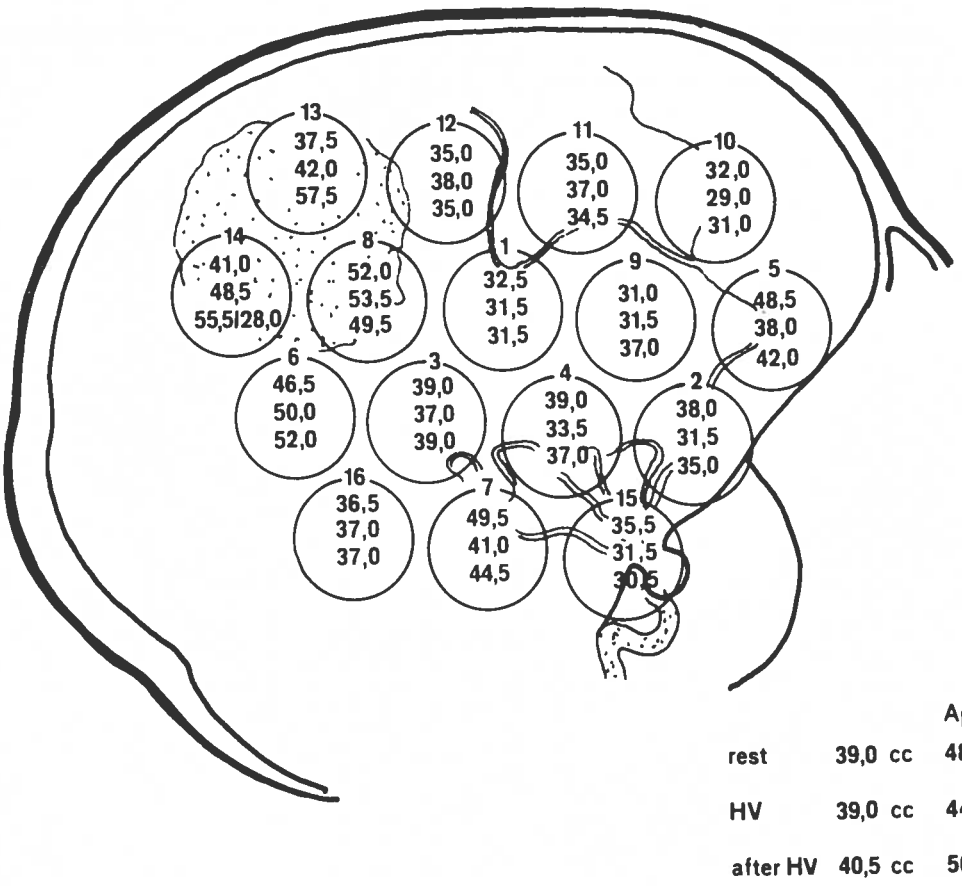

Fig. 2. Astrocytoma IV parietal. First measurement during resting state; second measurement after hyperventilation; third measurement again in the resting state (there was a spontaneous increase of A P_{CO_2}: 50 Torr!). Signs of inversed steal during the second injection (detector 5, 14, 13). In the resting state a relatively high rCBF is seen over a part of the tumor (8). (rCBF in cc/100 g. min.).

fuse, growing malignant tumors (almost all mean values were between 25 and 35 cc/100 g. min.).
The best circulation (relatively) was found in a patient with an epidermoid cyst in the middle cerebral fossa (42.5 cc/100 g. min.). Apparently, the cerebral circulation can adapt if the tumor is not destructive and is of slow growth.
In the literature, there are reports that the cerebral blood flow is diminished in the contralateral hemisphere also. We did not have the opportunity to verify this.
In all 16 patients *regional differences* were found. However, one must realize that there are tumors with a higher perfusion rate than normal brain tissue, and there are also poorly vascularised tumors. This means that it is often impossible to localise a tumor when only rCBF values are taken into account.
If the localisation of the tumor is known, one can conclude from the rCBF pattern wether the tumor is highly or poorly vascularised.
There was very good agreement between the values obtained by the cerebral blood flow measurements and the result of the angiography. That is, tumors which were highly vascularised angiographically also showed regions with high rCBF values.
When a detector is placed over a region with arterio-venous anastomosis, one will obtain a curve with an *abnormal configuration.*
The rapid increase in radio-activity after the injection is not then followed by the normal slow decrease, but by a very rapid decrease. These so-called "shuntpeaks" are found because a portion of the radio-active material does not take part in the normal diffusion/clearance process, but leaves the brain tissue immediately through the anastomosis.
According to the literature, such shuntpeaks can be found over many kinds of tumors (Pálölgyi 1969).
In our material, shuntpeaks were registered only in patients with meningeoma (2 cases).
The localisation of the shuntpeaks coincided very well with the localisation of the tumor.
These shuntpeaks were of much lower amplitude than those found in patients with congenital arteriovenous malformations. More important than the abnormalities in the resting state, or an abnormal configuration of the curve, is often an abnormal pattern during *reactivity testing procedures.*
The reactivity testing procedures can be devided into:

1. *Testing of the autoregulation*

Autoregulation is the capacity of the normal cere-

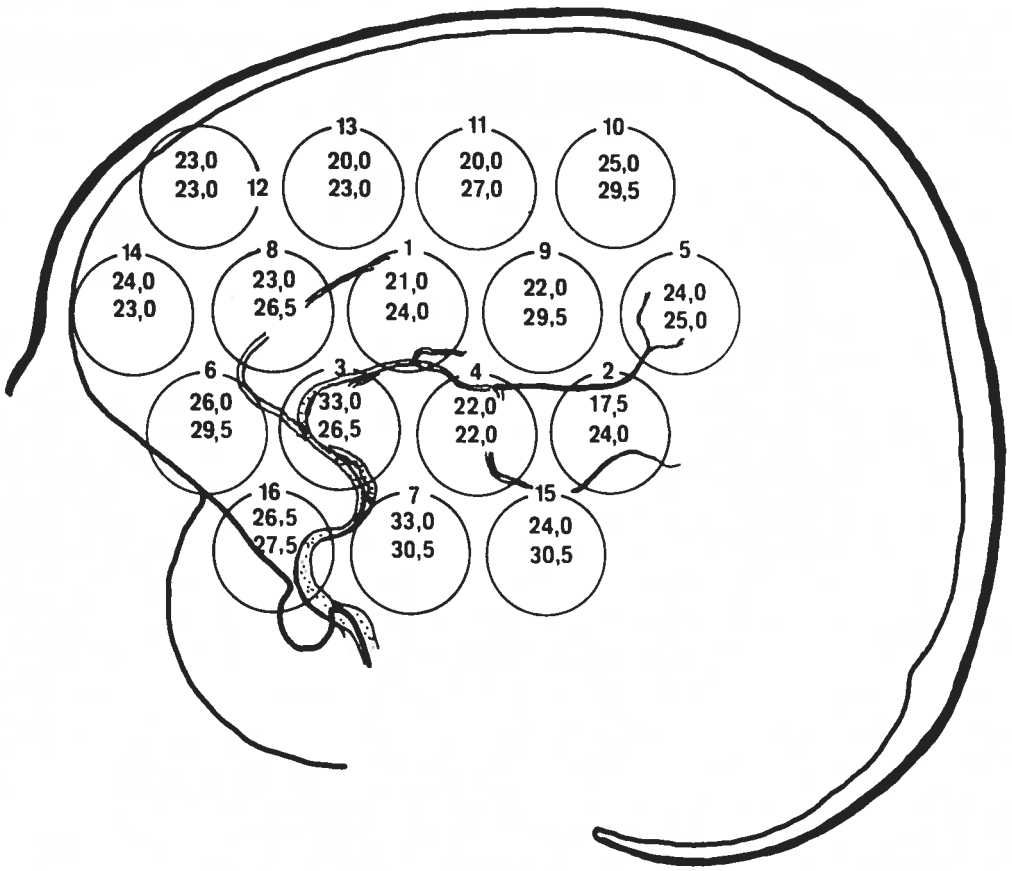

Fig. 3. Large astrocytoma IV in the left hemisphere. Diffuse marked decrease in rCBF. Minimal reaction to intra-arterial papaverine. Paradox reaction present (3). (rCBF in cc/100 g. min.)

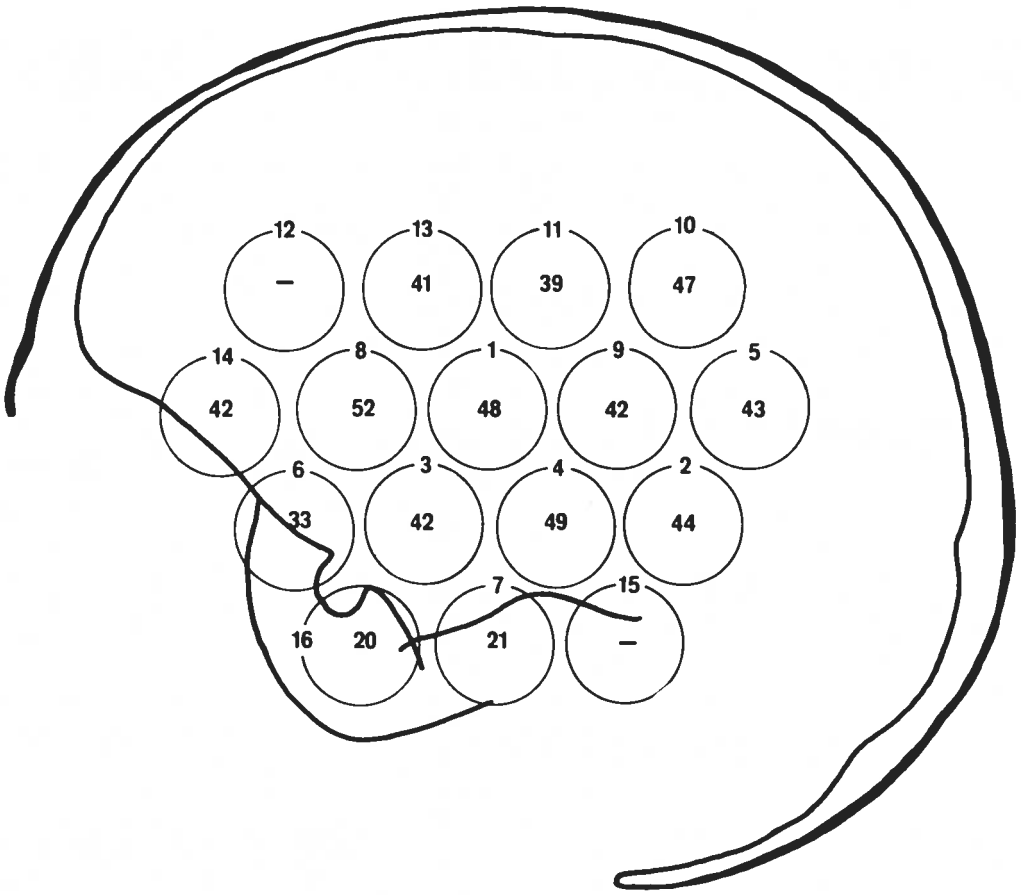

Fig. 4. Epidermoid cyst in the temporal region. Very low relative „weight grey" under detectors 6, 7, 16. (Weight grey in %/100 g. tissue.)

brovascular system to maintain (within certain limits) a constant rCBF with spontaneous or induced changes in perfusion pressure.
Most authors (Pálvölgyi, 1968) describe autoregulation disturbances in patients with cerebral tumors. These disturbances may be localized as well as diffused over the whole hemisphere. We did not repeat these investigations.

2. *Response to changes in arterial carbon-dioxide tension (A P_{CO_2}).*

In normal brain tissue there is a lineair- (Høedt-Rasmussen 1965) or exponential (Olesen 1971) relationship between A P_{CO_2} and regional cerebral blood flow (increase in A P_{CO_2} produces an increase in rCBF).
This reponse is frequently lost in a tumor region. The disapearance of this A P_{CO_2} dependency can induce a so-called intra-cerebral steal:
in this situation, an increase in A P_{CO_2} leads to a decrease in rCBF in the tumor region due to the fact that the cerebrovascular resistance is lowered in the region around the tumor, but not in the region of the tumor itself, while perfusion pressure remains constant. Under hypocapnia, an inversed steal may come into existence: the rCBF in the tumor region increases because the cerebrovascular resistance in this region does not increase as it does in the surrounding regions.
An example is given in figure 2.
A 60 year old female had an astrocytoma IV in the right parietal region. The first rCBF measurement was made in the resting state: the second measurement after three minutes of hyperventilation. The induced hypocapnia caused a decrease in rCBF in the fronto-temporal region, but an increase in more posterior located regions (detectors 6, 13, 14).
The tumor was highly vascularised according to arteriography. This was demonstrated by the relatively high rCBF in the region of detector 8.

3. *Pharmacological reactions*

More rapidly than by means of A P_{CO_2} changes, a rCBF increase can be induced through intra-arterial injection of papaverine (5-7 mg., diluted in 10 cc saline, rapid injection).
After such an injection under normal conditions the cerebral blood flow rises remarkably (often to 200% of the original value). The effect of papaverine disappears after only 1-2 minutes. The mechanism of this non-physiological increase in cerebral blood flow may be disturbed in the region of the tumor: the reaction may disappear, or even paradoxical reactions may occur.
An example is given in figure 3.
This is the case of a 70 year old man with a large astrocytoma IV in the left hemisphere. The cerebral blood flow in the resting state (values noted in the upper part of each detector field) was extremely low.
After 5 mg. papaverine was injected in the left carotid artery, we found under only a few detectors (6, 9, 11) a slight increase in perfusion. In other regions there was no change, while under detector 3 there was a region with a paradox reaction.
So, it becomes evident that the steal mechanism can also be activated by means of papaverine injection.

As mentioned above, we can calculate the relative weights of grey and white matter from the clearance curves. Although we are doubtful if the calculated values really correlate with the anatomical amounts of grey and white matter, one can say that there are apparently two compartments which have different clearance rates.
The relative weights of these compartments can be estimated. In normal situations, both compartments have about the same weight, and regional differences in normals are small (Wilkinson 1969).
In patients with focal lesions (tumors, vascular lesions), local abnormalitites in the grey/white weight ratio are found in most cases.
An example of this is given in figure 4.
It concerns the same patients who was mentioned in connection with the relatively best circulation: a man of 31 years with an epidermoid cyst in the middle cerebral fossa.
Apart from a (subjective) diplopia and a slight hypalgesia in the region of N. V, the neurological examination was completely normal.
According to the EEG, there were only minor irritative signs in the left temporal region.
Arteriography showed a poorly vascularised space-occupying lesion in the left temporal pole. The mean rCBF was only slightly diminished (42.5 cc/100 g. min.: A P_{CO_2} = 40.0 Torr).
The lowest values were found in the temporal region (in normal individuals the cerebral blood flow is also a little lower in the temporal region than in other locations).
More spectacular was the abnormal ratio of the weights of the two different compartments.
Under detectors 6, 7, and 16 there was an important decrease of the "weight grey". That means that in this region a relatively large compartment with a slow clearance rate has come into existence.
It must be pointed out that this two-compartmental analysis is only feasible when the clearance curves are computer analysed. In our laboratory this was done by a Digital PDP15 computer. The results of the correlation between EEG and cerebral blood flow in these patients will be published elsewhere.
Summarizing the EEG findings, it can be stated:

1. There is generally a good correlation between mean rCBF and the severity of EEG abnormalities.

2. Induced increase in cerebral blood flow (increase of A Pco_2, injection of papaverine) has in general amazingly little influence on the EEG, but pathological signs may become clearer.

Conclusion

Our findings have confirmed the data published by other authors: most intra-cerebral tumors led to disturbances of many aspects of the cerebral blood flow.
The cerebral blood flow measurements do not play an important role in tumor diagnosis, or the localisation of intracranial processes.
The combination of arteriography and rCBF studies can lead to a better understanding of the pathophysiological processes around the tumor, and thus may lead to a better correlation between the pathological process and the clinical signs. For optimal use of the method, we think the following points are of importance:

1. It is necessary to use automatic analysis of the clearance curves.

2. It is essential to calculate all parameters (including the relative weights).

3. The results are more interesting when the regional cerebral blood flow is determined not only in the resting state, but also during hypercapnia, after injections of papaverine, and, if possible, after blood pressure changes.

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IV. THERAPY

Therapie

1. Experimental chemotherapy of tumours
   Experimentele chemotherapie van tumoren

2. Evaluation of possible advantages of 15 MeV neutrons for tumour radiotherapy
   Onderzoek naar de mogelijke voordelen van 15MeV neutronen voor de radiotherapie van tumoren

3. Experimental treatment of leukemia: Attempts to improve the results of extracorporeal irradiation of the blood by cell mobilizing agents
   Behandeling van leukemie door middel van extracorporale bestraling van het bloed

4. The relationship between tumour vascularisation and response to radiotherapy
   Het verband tussen vascularisatie van de tumor en de gevoeligheid voor radiotherapie

Experimental Chemotherapy of Tumours

Experimentele chemotherapie van tumoren

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Summary

In the field of tumour chemotherapy four fields of activity are described in which experimental studies may lead to the development of new approaches to improve the success of clinical treatment. The fields are:

(1) Selection of drugs which may be active against slow growing tumours.

(2) Differential manipulation of cell proliferation rates of tumour cells and normal cells in order to increase the therapeutic index.

(3) Evaluation of relative effectiveness of regional versus systemic chemotherapy.

(4) Specific modification of the process of tumour metastasis with drugs.

The results are described briefly.

Samenvatting

In het gebied van de experimentele chemotherapie van tumoren zijn over vier onderwerpen onderzoekingen gaande, namelijk

1° over de selectie van cytostatica die mogelijk effectief zijn tegen langzaam groeiende menselijke tumoren

2° over de mogelijkheid een grotere therapeutische breedte te verkrijgen door het manipuleren van de celproliferatiesnelheid

3° over de relatieve effectiviteit van regionale infusie van cytostatica

4° over specifieke beïnvloeding van het metastaseringsproces.

De resultaten worden kort beschreven.

Introduction

The mechanism by which chemotherapeutic agents are effective in tumour therapy is based on a difference between the malignant and the normal cells which is responsible for a greater sensitivity of the malignant cell to cytotoxic agents. The difference varies from one tumour to another and possibly also from one type of cytostatic agent to another; it has been defined with certainty only for a few experimental models and there is certainly not one unique property which discerns malignant cells from normal cells. But there is one common property of those tumours which can be treated successfully with cytostatic agents in man; each of these tumours is growing rapidly and its cells are continuously dividing. This finding focuses our attention on the importance of rapid proliferation as an aspect of cell sensitivity to chemotherapeutic agents. Experimental studies by Bruce and his colleagues in Toronto (1968) have indicated that most cytostatic agents are more effective against rapidly proliferating cells which seems to give a logical explanation for the clinical results.
Unfortunately only a limited number of tumours have the property of rapid growth and many slow growing tumours are completely insensitive to chemotherapy. For this reason, studies are undertaken to identify methods which may lead to more effective chemotherapy of slow growing tumours.

Four fields of study have been approached by our institute in the last few years:

(1) Selection of drugs which may be active on slow growing tumours.

(2) Selective modification of the cell proliferation rate to obtain more pronounced differences in sensitivity between normal tissues and malignant tumours.

(3) Study of the experimental basis for local or regional chemotherapy.

(4) Exploration of tumour metastasis as a process which may be open to chemotherapeutic modification.

The present status of research in these fields will be reviewed briefly.

1. Selection of drugs which may be active against slow growing tumours.

This might be accomplished by the use of a screening system containing one or more slow growing tumours (Sandberg and Goldin, 1972). However, in the hope of getting insight into mechanisms of action another approach was sought (Van Putten, Lelieveld and Kram-Idsenga, 1972): For a large number of cytostatic agents the effectiveness in killing one cell type is studied under

two conditions of proliferation, namely in rapid proliferation and in rest.

From these studies the ratio of the slopes of the dose-survival curves may be obtained which gives a numerical expression of the degree to which the effectiveness of each particular drug is dependent upon the proliferation rate of the cell. Such studies might preferably be performed on tumour cells, but a technically simple system with malignant cells is not available. Instead the normal mouse haemopoietic stem cell in vivo was chosen for these studies, as this cell is normally not in mitotic cycle, but can easily be brought into rapid proliferation by inducing a deficiency of haemopoiesis with radiation. Examples of the dose-effect curves are given in fig. 1. The ratio of the resulting slopes is compared to the effectiveness of the drug, given at the maximal tolerated dose, in reducing the volume of a transplantable tumour in similar mice. The volume of the treated tumour expressed as a fraction of control tumour volume is presented in table 1 as a tumour volume reduction fraction together with the slope ratios for the different drugs.

If drug effectiveness were mainly related to the proliferation dependence of its action, drugs with high slope ratios should be the most effective agents. However, it is evident from the table that a number of drugs which have low slope ratios are highly effective anti-tumour agents. If this high effectiveness is not based on proliferation specificity, it must be based on other discrimating factors which cause tumour cells to be more sensitive than normal tissues. Such factors, independent of the rate of proliferation of the cells should be most important for effects on slow growing tumours and thus an expansion of this study may indicate which of our cytostatic agents are most promising for use in the majority of human tumours which grow much slower than the transplantable animal tumours.

Table 1

	Ratios of slopes of curves for killing resting and proliferating cells	*Tumour Volume Reduction Factor*	
Trilophosphamide	1.4	0.20	++
Bis Chloroethyl Nitroso Urea	1.5	0.20	++
Di Methyl Myleran	1.5	0.55	—
Di Anhydro Mannitol	1.6	0.14	++
Cyclohexyl Chloroethyl	1.6	0.17	++
Nitroso Urea Isophosphamide	1.6	0.14	++
Nitrogen Mustard	1.6	0.50	—
Myleran	1.7	0.83	—
Chlorambucil	1.8	0.59	—
Cyclophosphamide	2.3	0.14	++
Nor-mustine	2.3	0.63	—
Tri Ethylene Melamine	2.3	0.27	+
Melphalan	2.5	0.38	+
5-Fluoro Uracil	4.0	0.33	+

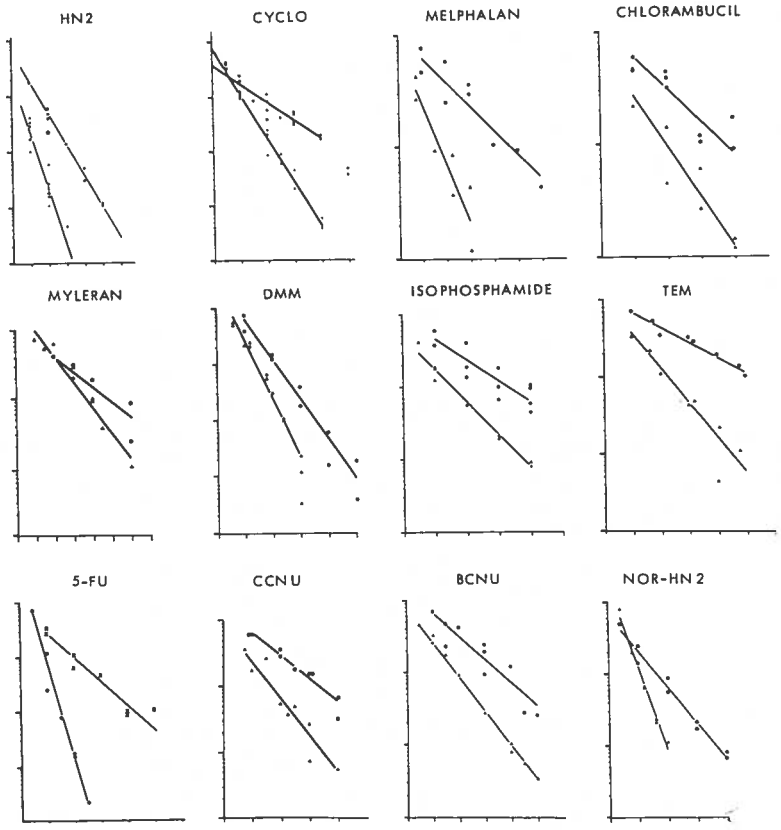

Fig. 1. Dose-effect curves for the killing of resting (upper curves) and rapidly proliferating (lower curves) haemopoietic spleen colony forming cells by different chemotherapeutic agents.

2. Selective modification of cell proliferation rate

One of the problems of the use of agents which are most active against rapidly proliferating cells lies in the observation that the cell proliferation rate changes in the course of chemotherapy (see for instance Skipper, 1971).
This change is unfortunately not restricted to an increase of the proliferation rate of the malignant cells in the tumour; it occurs also in normal tissues such as haemopoietic stem cells and intestinal epithelium. Methods which would temporarily block the increased proliferation of haemopoietic stem cells, or stimulate proliferation of malignant cells, would increase therapeutic effectiveness of many of the presently used drugs. A specific block of proliferation of normal cells without simultaneous influence on leukemia cells was caused by the prolonged application of anaesthetic gases (nitrous oxide, halothane) as described by Bruce, Lin and Bruce (1970). Analogous observations have been made on the cells of transplantable solid tumours (Evenwel and Van Putten, unpublished) and studies are continuing in order to elucidate the mechanisms involved and to evaluate the possibilities of application of the principle in clinical chemotherapy.

3. Experimental basis for regional chemotherapy

Regional intra-arterial infusion of chemotherapeutic drugs has found clinical application in a number of malignant conditions, but it has also encountered criticism. Part of the criticism is associated with the experience by some authors of a high rate of complications from intra-arterial infusion, such as embolism, hemorrhage and infection, but other criticism is aimed at the lack of proof of higher effectiveness of regional as compared to systemic chemotherapy. For this reason studies were started (Sindram, 1972) on a model system in the rat comparing the use of methotrexate (MTX) in optimal dose schedules by regional infusion and optimal dose schedules in systemic therapy in order to learn whether regional therapy may offer better potential anti-tumour effect than systemic treatment with the same agent.

Table 2

Treatment and no. mice	*Metastases observed 17 days after inoculation*	
	Lymph Nodes	*Lung*
Control (145)	77%	8%
400 rad total body X-rays (45) ..	60%*	33%*
CCNU (75 mg/kg) (18)	0%**	44%**
Triton WR 1339 (5 × 550 mg/kg) (30)	70%	7%
PolyMethAcrylic Acid (5 × 40 mg/kg) (27)	52%*	7%

* $p \leq 0.05$
** $p < 0.01$

Preliminary results indicate that regional therapy with MTX gives superior effects on the tumour in comparison with systemic therapy when tested after continuous infusion at maximal tolerated doses. Studies are continuing to test the use of a number of alternative dose schedules.*

4. Metastasis as a process open to chemotherapeutic modification

Another approach to find specific properties of malignant disease which might be open to treatment with drugs lies in the study of the process of metastasis. Many factors are involved in the development of hematogenous or lymph node metastasis and some of these seem open to manipulation (Garattini and Franchi, 1972). A model for the specific study of lymph node metastasis in the mouse was developed.
After injection of tumour cells into the testicle of the mouse, lymph node metastases are seen in high frequency and lung metastases in low frequency. Some results of treatment in this system are presented in table 2. (Van Putten and Kram-Idsenga, unpublished). It is clear that an effective chemotherapeutic agent such as CCNU (Cyclohexyl-Chloroethyl-Nitroso-Urea) will prevent the growth of metastasis in lymph nodes, partly by damage to the nodes, partly also by destruction of tumour cells. Nevertheless some tumour cells remain intact, pass through the nodes into the thoracic duct and the blood stream to give rise to lung metastases. Similar effects may be seen after total body irradiation. Triton WR 1339, a compound active in inhibiting metastasis formation in a number of other tests (Garattini), was without effect. Treatment with Poly-Meth-Acrylic Acid (PMAA) seems to reduce the number of lymph node metastases without increasing lung metastases.
Such studies are presently of limited interest for practical application, but it is assumed that the analysis of the types of modifying agents will give us an indication of the processes involved in the formation of metastases and permit us to develop better agents.
It is realized that a study of these four areas of experimental tumour chemotherapy does not cover the whole of the field. No research is devoted to development and synthesis of new drugs or to pharmacology and screening, to name only a few areas. It is felt that our studies aimed at optimal applicability of available agents form a relatively homogeneous program which can adequately be handled by a small group. Close contact with other centers in this country, and on a larger scale,

* This study which was initiated at the Radiobiological Institute TNO with support from the „Koningin Wilhelmina Fonds" of the National Cancer League, is now continuing at the Free University in Amsterdam.

(continued pg. 721

Evaluation of Possible Advantages of 15MeV neutrons for Tumour Radiotherapy

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Onderzoek naar de mogelijke voordelen van 15MeV neutronen voor de radiotherapie van tumoren

Summary

Radiobiological studies with different types of radiation, carried out during the past decade, have yielded experimental data indicating that fast neutrons might provide advantages for the radiotherapeutical treatment of some types of tumours. An outline is given of the sequence of investigations, which started with fundamental biological studies on survival of cultured cells and have finally led to irradiation of tumours in patients. Characteristics of the 15 MeV neutron beams employed for the clinical studies are described and a preliminary assessment of responses of lung metastases in patients is presented.

Samenvatting

Radiobiologische onderzoekingen welke de laatste 10 jaren met verschillende stralingssoorten zijn uitgevoerd, hebben aanwijzingen opgeleverd dat snelle neutronen voordelen zouden kunnen bieden voor de radiotherapie van bepaalde typen tumoren. Een overzicht wordt gegeven van de reeks van onderzoekingen, welke begonnen met fundamenteel biologisch onderzoek betreffende celoverleving en resulteerden in bestraling van tumoren in patiënten. Een beschrijving wordt gegeven van de eigenschappen van de 15 MeV neutronenbundels welke voor de klinische bestralingen worden gebruikt. Tenslotte wordt een voorlopige conclusie vermeld betreffende het effect op longmetastasen in patiënten.

1. Introduction

The treatment of cancer by radiotherapy can attain local and regional control of the disease in an appreciable proportion of patients, because all tumour cells are rendered incapable of unlimited proliferation, while the normal tissues in the treated region are capable of restoring their integrity and function. For the optimization of a treatment the radiotherapist must select the type of radiation, the dose and the dose distribution in the patients, and the dose fractionation schedule which are expected to produce this control with the greatest probability.

In the treatment of cancer by radiation considerable progress has been made during the past 25 years with respect to its technical aspects. The introduction of high energy X-rays, gamma-rays and fast electrons during the past decades, has provided the possibility to attain significant improvements in the distribution of the dose in the patient, especially important for deep-seated tumours. In addition, the precision and consistency with which a prescribed treatment can be delivered has increased. As a consequence of these developments, combined with better insights in the clinical behaviour, the probability of local control of a number of types of cancer has been improved, e.g. for certain stages of Hodgkin's disease, reticulo sarcoma and bladder carcinoma. Nevertheless for many

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cooperative programs in the European Organization for Research on the Treatment of Cancer make it possible to integrate the data obtained in a much wider field of knowledge and thus to get a maximal benefit of the research for clinical application.

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types of malignant diseases failure to attain local or regional control of tumours by radiotherapy is all too frequent.

Beams of energetic protons, deuterons or alpha-particles, as well as negative pi-mesons might provide still better focussing of the dose to the prescribed area, but in radiotherapy they have so far been applied to only a limited extent, because accelerators required to produce adequate beams are large and expensive, they cannot easily be installed in hospitals and beam flexibility is difficult to obtain. Beams of negative pi-mesons of sufficient intensity for radiotherapy will only become available in the future.

In addition to possible advantages from further improvements in the spatial distribution of the dose in patients, increases in the probability of local control of tumours might conceivably be obtained by modifications of the dose fractionation schedule. However, fundamental insights obtained in the responses of specific tumours and normal tissues from radiobiological research, have not yet provided an adequate scientific basis for optimization of treatment regimes to be suited individually to each type of tumour.

Radiobiological studies with different types of radiations, carried out during the past ten years, have yielded experimental data indicating that fast neutrons and negative pi-mesons might provide advantages which are not based on dose distribution patterns, but on biological factors such as anoxia of tumour cells and repair of sub-lethal damage. These factors can influence responses of tumours and normal tissues differently [1]. As a consequence of these studies, applications of fast neutrons to tumours in patients have been started in two centres. At Hammersmith Hospital in London fast neutrons produced by a cyclotron are employed, while we use 15 MeV neutrons from a relatively small generator in the Radiobiological Institute. In addition to this difference in neutron source, a difference exists between the types of approach taken in these two centres for the evaluation of the possibilities of fast neutron radiotherapy, as will be discussed later.

For an adequate evaluation of possible advantages of fast neutrons, it is necessary to assess not only the relative biological effectiveness (RBE) of fast neutrons for effects on various types of tumours, but also the RBE for effects on various types of normal tissues. In a radiotherapeutical treatment, the dose which can be administered to the tumour is always limited by the risk of causing severe damage to normal tissues which must retain the capacity to function adequately. The limitation of the total dose depends on many factors, e.g. the location and size of the tumour, the sensitivity of the various types of normal tissues in the irradiated area and the importance of the function of that tissue for the well-being of the patient. The dose-limiting tissues may be skin, intestinal tract, spinal cord, connective tissues, etc. If in a particular case measurements show that a given dose of neutrons produces as much damage to the tumour as twice as large a dose of X-rays, then the RBE of that neutron dose is equal to 2 for the effect considered. However, if that dose of neutrons would also cause damage to the dose-limiting normal tissue with an RBE of 2, then therapeutically nothing would be gained.

The essential gain factor is therefore defined as the ratio of the RBE values of a given dose of neutrons for damage to the tumour and for damage to the normal tissue respectively. If this factor is significantly larger than 1, the use of fast neutrons can be advantageous, provided that other factors, e.g. depth dose characteristics, do not offset the gain from radiobiological factors. The purpose of this communication is to outline briefly the sequence of investigations carried out to evaluate possible advantages of fast neutrons, starting with the acquisition of fundamental radiobiological data, leading finally to treatments of patients.

2. Radiobiological aspects of fast neutron irradiation

2.1. Dose-effect relations for cultured mammalian cells

Results of investigations carried out during the past fifteen years have shown that radiation-induced damage to normal tissues and tumours in animals is determined to a large extent by responses of those constituent cells which are capable of unlimited proliferation, i.e. the clonogenic cells or stem cells. Furthermore, responses of individual cells after irradiation in vivo, have been shown with a number of experimental systems to be similar to responses of the same type of cells grown and assayed in vitro, provided that various experimental conditions, e.g. oxygenation status, are equal. Consequently, data obtained with cultured mammalian cells, irradiated and assayed in well-controlled conditions, can provide relevant information for the interpretation of effects produced by ionizing radiations in tumours and normal tissues, although quantitatively, differences can exist between cells of different tissue or tumour origin [2].

In studies concerning impairment of the capacity for proliferation of mammalian cells, dose-effect relations are generally presented as survival curves. In these curves the fraction of cells which have retained the capacity for unlimited proliferation is plotted on a logarithmic scale as a function of the dose on a linear scale. Survival curves obtained for cultured cells of human kidney origin irradiated with heavy ions, fast neutrons and X-rays, have demonstrated three main differences, which are important for the application of fast neutrons in radiotherapy. These differences concern the relative

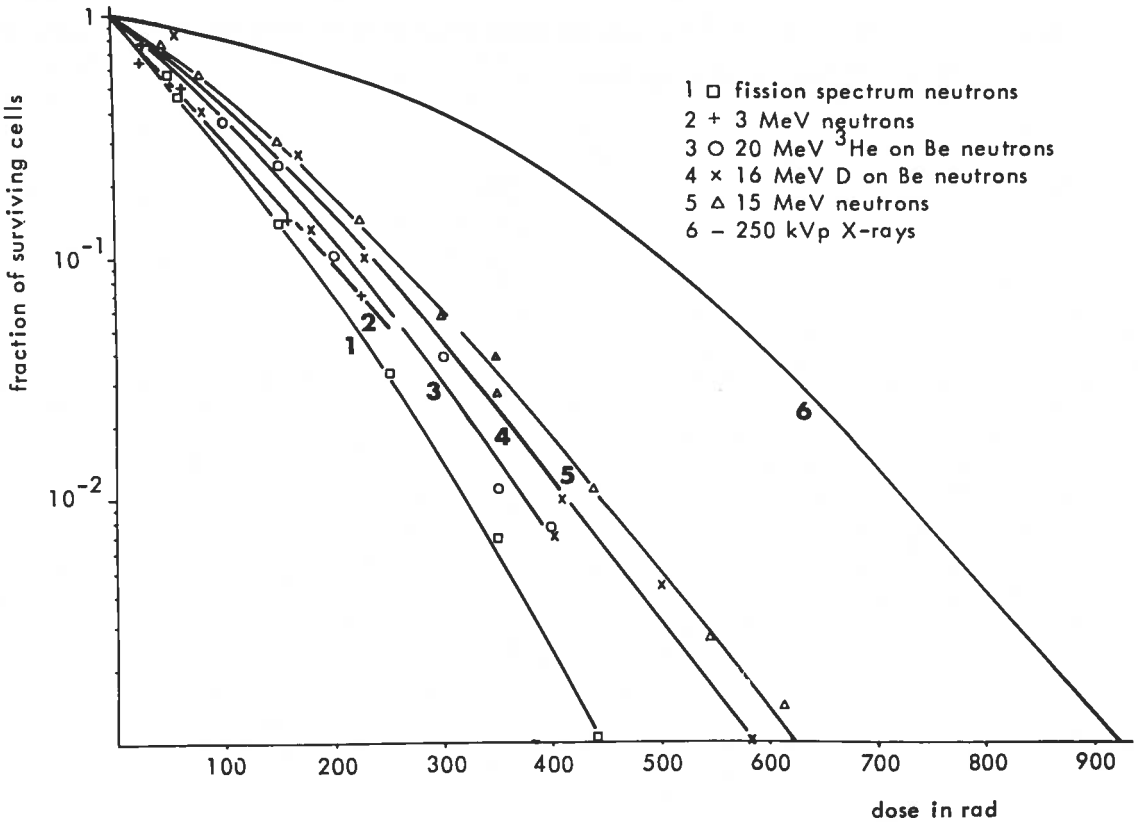


Fig. 1. Survival curves obtained for cultured cells of human kidney origin by irradiation with different beams of fast neutrons and with 250 kVp X-rays.

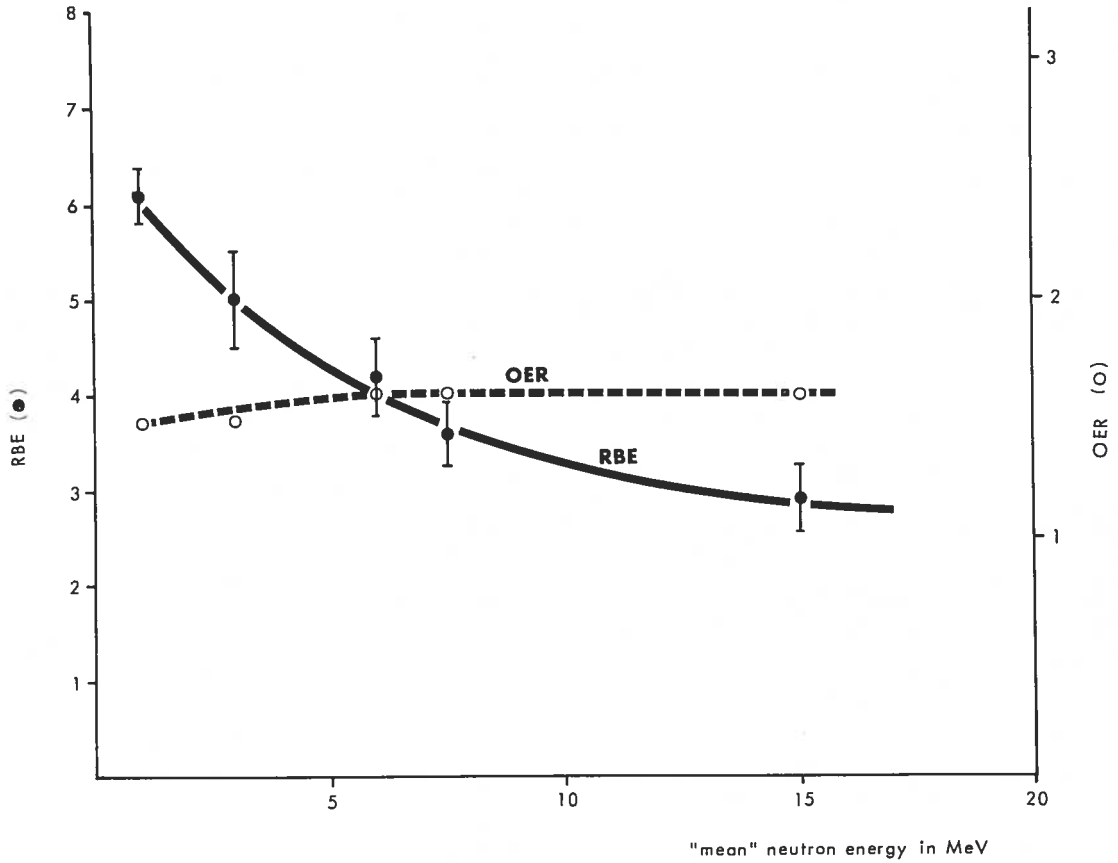


Fig. 2. RBE and OER of fast neutrons as a function of mean neutron energy for impairment of the proliferative capacity of cultured cells of human kidney origin. RBE values correspond to low doses, inducing 20 per cent cell reproductive death, as derived from curves 1 to 5 of figure 1, respectively.

biological effectiveness (RBE), the effect of dose fractionation and the influence of the oxygenation of cells [3-5].

As noted earlier, the RBE of a dose of neutron radiation of a specified energy is defined as the ratio of a dose of X-rays and a dose of neutron radiation required to produce quantitatively equal effects in the cells, tumours or tissues considered. In fig. 1 a number of survival curves are presented, obtained for cultured cells of human kidney origin irradiated with neutrons of different energies. These curves illustrate that for all neutron beams the survival curves exhibit less curvature in the low dose region than the curve obtained with 250 kVp X-rays. As a consequence of these differences in shape, the RBE of a specified neutron beam depends on the level of damage considered.

The differences in shoulder width of the low dose regions of the single-dose survival curves are related to differences in repair of sub-lethal damage which can occur in the time interval between subsequent doses applied in fractionated exposures. Studies of the effect of dose fractionation of 15 MeV neutrons and 250 kVp X-rays on mammalian cells in culture have shown that the influence of repair of sub-lethal damage is much larger after X-irradiation than after neutron irradiation [6].

Many investigations with a variety of biological systems have shown that severely hypoxic cells are more resistant to X-rays, gamma-rays and fast electrons than well-oxygenated cells. This difference is expressed as the oxygen enhancement ratio (OER), defined as the ratio of two doses required to produce quantitatively equal effects in severely hypoxic cells and in well-oxygenated cells, respectively. In a series of experiments with cultured cells of human origin, the oxygen effect has been studied for X-rays and for different neutron beams with mean energies ranging from about 1 MeV to 15 MeV.

The results demonstrated that the OER values vary only little with neutron energy; as shown in fig. 2, the values range from 1.5 to 1.6 in this energy interval. These factors are considerably smaller than the OER of 2.6 which has been obtained for 250 kVp X-irradiation.

2.2. *Significance of differences in dose-effect relations between fast neutrons and X-rays for radiotherapy*

As noted earlier the observation that the RBE of fast neutrons is generally larger than 1, i.e. that these radiations are more effective for producing cell reproductive death than X-rays, gamma-rays or fast electrons, does not indicate directly that they might provide an advantage in radiotherapeutical applications. The relevant criterium for such an advantage of fast neutrons is, that in comparison with X-rays more damage can be caused to tumour cells for equal damage to normal tissues in the treatment field, i.e. that the RBE for producing damage to the tumour is larger than the RBE for producing damage to those normal tissues that are dose-limiting in the treatment considered.

In radiotherapeutical treatments with X-rays, gamma-rays and electrons, the radiation is administered according to a schedule which, by the proper choice of various parameters, namely the spatial distribution of the dose in the patient, the sizes of the dose fractions, the intervals between fractions and the total dose, will just be tolerated by normal tissues, while causing maximum damage to the tumour. With respect to the effect of dose fractionation, two possibilities may be envisaged. Firstly, it is possible that the response of the dose-limiting tissue is characterized by a survival curve with a shoulder which has a greater width than the shoulder of the survival curve for the tumour cells. In such a case fractionation of a given total dose would have a greater sparing effect for the normal tissue as compared with the tumour. Consequently the use of a type of radiation which enhances this difference is indicated, i.e. X-rays or gamma-radiation should give optimal treatment results.

A second possibility which can be envisaged is that the shoulder width of the survival curve of the tumour cells is larger than that of cells of the dose-limiting normal tissue. In such a case fractionation of a given total dose of X- or gamma-rays in a large number of fractions would have a greater sparing effect on the tumour as compared with the dose-limiting normal tissue. A treatment with fractionated doses of X- or gamma-rays would then result in a relatively small effect on the tumour, i.e. the tumour would be classified as radioresistant. In this case the use of fast neutrons which would eliminate a large part of the shoulder of the survival curve of tumour cells, as well as that of cells of the normal tissue, might provide an advantage as compared with X- or gamma-rays. However, at present no methods are available to distinguish clinically tumours with cells which have survival curves with wide shoulders.

With respect to the effect of hypoxia, evidence has been obtained during the past years, that various types of tumours in animals contain anoxic or severely hypoxic cells. From similarities in architecture between various types of human and animal tumours it has been inferred that human tumours might also contain a proportion of hypoxic and viable cells. Hypoxic cells are known to be more resistant to X- and gamma-rays than well-oxygenated cells by a factor denoted $(OER)_x$. As a consequence it is possible that after irradiation to the tolerance of the dose-limiting normal tissues, some hypoxic cells in the tumour have retained the capacity for unlimited proliferation and cause a regrowth of the tumour. Because the $(OER)_n$ for fast neutrons is smaller than $(OER)_x$, the relatively high resistance of hypoxic cells in tumours as compared

to well-oxygenated cells of normal tissues will be partly eliminated if neutrons are employed instead of X-rays or gamma-rays. The radiotherapeutic gain with respect to the effective dose to the tumour cells as compared to normal tissues, to be expected on the basis of the oxygen effect, may be smaller for a fractionated treatment than for single doses, because cells which are hypoxic at the time of administration of a first dose fraction, may become better oxygenated during the interval, before a subsequent dose fraction is given. This reoxygenation has been demonstrated for a number of tumours in animals [7, 8]. It is possible that the presence of anoxic cells and their relatively slow reoxygenation forms part of the rationale of the clinical application of relatively small daily dose fractions of X- and gamma-rays, i.e. that the relatively slow process of reoxygenation necessitates the use of small daily doses. Because of their low $(OER)_n$, the use of fast neutrons might partly eliminate this necessity of using small daily dose fractions and might provide more flexibility for the application of other treatment schedules which might conceivably be found to take advantage of other factors influencing tumour radiosensitivity, e.g. cell repopulation and changes in age distribution.

2.3. *RBE values for damage to experimental tumours and normal tissues irradiated with 15 MeV neutrons*

In a large series of experiments RBE studies of 15 MeV neutrons have been carried out for a number of effects on tumours and normal tissues in experimental animals; notably an osteosarcoma in the mouse [8], a rhabdomyosarcoma in the rat [9, 10], rat skin and intestinal epithelium and the haemopoietic system in the mouse [11]. Data have been obtained for different single doses and treatments with five fractions per week. The results show that the RBE decreases significantly with increasing size of the dose for treatments with single doses and with the dose per fraction for treatments with daily fractions. This dependence is clearly related to the differences in shapes of survival curves and in repair of sub-lethal damage [2]. It is of interest to present the RBE as a function of the dose or of the dose per fraction, as shown in fig. 3. The characteristics of the different curves in this figure will not be discussed in detail, but it can be concluded that the RBE values of 15 MeV neutrons for effects on the two tumours investigated, are larger than the corresponding RBE values for effects on normal tissues. For the application of fast neutrons

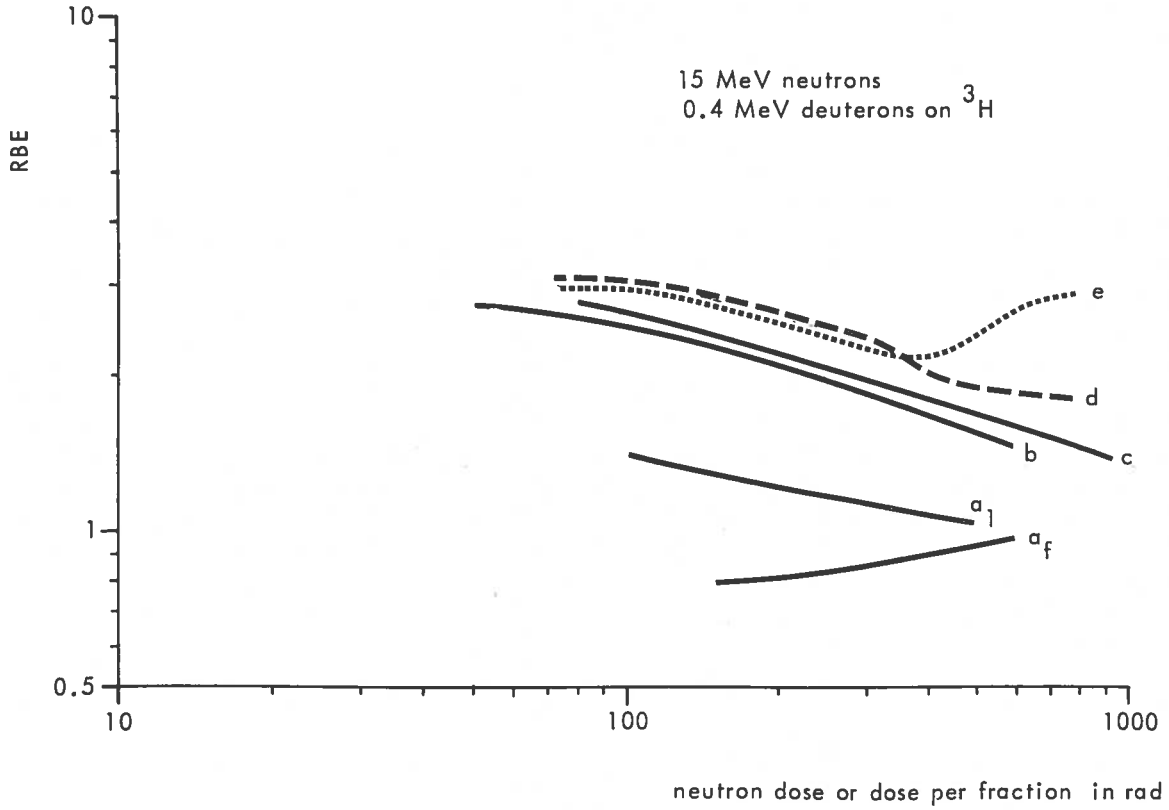


Fig. 3. Relations between the RBE of 15 MeV neutrons and the neutron dose or daily dose fraction, relative to 300 kV X-rays. Curve a_1 is derived from data on survival of mouse haemopoietic stem cells after single doses; curve a_f is derived from data on survival of mouse haemopoietic stem cells after 5 fractions given at daily intervals [11]; curve b is derived from data on survival of cultured cells of human kidney origin for single and fractionated exposures [6]; curve c is derived from data on damage to epithelial tissues in mice and rats for single and fractionated exposures [11]; curve d is derived from data on survival of cells in a mouse osteosarcoma after single and fractionated exposures [8]; and curve e is derived from data on survival of cells in a rat rhabdomyosarcoma after single and fractionated exposures [10].

in radiotherapy, the RBE values in the region between 50 and 250 rads are presumably the most important, because with very small dose fractions a large number of fractions would be required for attaining eradication of tumours, while dose fractions in excess of 250 rads would be expected to be too large to be tolerated by normal tissues. Curves b, c, d and e are in this region approximately parallel and the RBE increases with decreasing dose or dose per fraction. This suggests that the influence of differences in repair on the RBE, is approximately equal for the cultured cells, the epithelial cells of skin and intestinal tract and for the cells of the two types of tumours investigated. The curves for haemopoietic cells show significantly lower RBE values as well as a difference between single and fractionated doses, ascribed to the special proliferation kinetics of this system [11.] It can be concluded from the RBE values derived for various experimental systems at doses or dose fractions between 70 and 250 rads of 15 MeV neutrons, that the overall gain factor $RBE_{tumuor}/RBE_{normal\ tissue}$, relative to X-rays, does not exceed a factor of 1.2 to 1.3. This might still provide a significant advantage in clinical applications, if the dose distributions in the patient are equivalent to those obtained with high energy X-rays.

3. Motivation of clinical model studies

Investigations of effects of fast neutrons on tumours and normal tissues in animals are indispensable for an analysis of basic phenomena relevant to their application in radiotherapy. However, for an evaluation of possible advantages of fast neutrons for treatments of human tumours, data from animal studies cannot be extrapolated to man without knowledge about the influence of various characteristics in which tumours and normal tissues in man and animals differ. One important characteristic, which might influence the response to irradiation, is the growth rate of tumours in man as compared with the animal tumours mostly used for experiments.

Volume doubling times (T_d) of these rodent tumours generally range from 12 hours to a few days, while for tumours in man T_d values ranging from 10 to 300 days and more have been measured. In addition considerable differences exist with respect to the degree of differentiation in tumours in man as compared to the types of tumours used for experimental studies.

The two main differences between the biological effects of fast neutrons and X-rays, described in the preceding paragraph, cannot be expected to have a similar influence on the RBE values for effects on all types of tumours and normal tissues. As a consequence it is unlikely that neutrons will provide an advantage over X-rays or gamma-rays for the treatment of all tumours and for all locations in patients involving different dose-limiting tissues. In addition, even for the types of tumours where an advantage of fast neutrons over conventional radiations may exist, the gain factor cannot be expected to be very large. Therefore it is important that before a large scale trial of fast neutrons is initiated, a selection of the types of tumours and of the location in patients can be made for which a significant gain relative to X-rays can be expected. If infavourable types of tumours were included in a series, the mean gain factor might not be significantly different from 1 for a large series of tumours.

With respect to normal tissues, data obtained by the group at Hammersmith Hospital in London have indicated that RBE values of fast neutrons may be closely similar for man and experimental animals. On the basis of this experience at Hammersmith Hospital a series of tumours in the head and neck region is now being treated with 1440 rads of cyclotron neutrons, given in 12 fractions over 4 weeks. This dose is close to the tolerance dose for skin. The early results of these treatments on the tumours are encouraging but long-term results with respect to recurrences are not yet available [12].

For a direct quantitative evaluation of effects of fast neutrons in comparison with photons on tumours in patients, we have selected to start first a series of investigations of effects on pulmonary metastases. Blood-born metastases in the lung fulfill many requirements for studies of differences in responses to neutrons and X-rays by different types of tumours [13]. They are produced from a variety of types of tumours, differing in place of origin and in histological type. They all grow under similar conditions in a tissue that causes little mechanical resistance and provides ample blood supply. On subsequent radiographs of the chest changes in volume of solid metastases can be measured accurately. If two or more lung metastases are present, they usually have equal growth rates and after treatments with different radiations the volume decrease and subsequent regrowth can be used for a quantitative analysis [13]. The comparison of responses of two pulmonary metastases from the same primary tumour in a patient treated with neutrons and X-rays or gamma-rays respectively, offers the possibility to derive directly an RBE value within a few weeks or months. Volume doubling times of these metastases range from about 10 to 300 days or more. It can be concluded that the lung metastasis model provides the possibility to obtain quantitative data for a variety of types of tumours and to study the dependence of the RBE on the growth rate. These data should enable an analysis of the possibilities for the selection of the types of tumours to be included in further clinical applications.

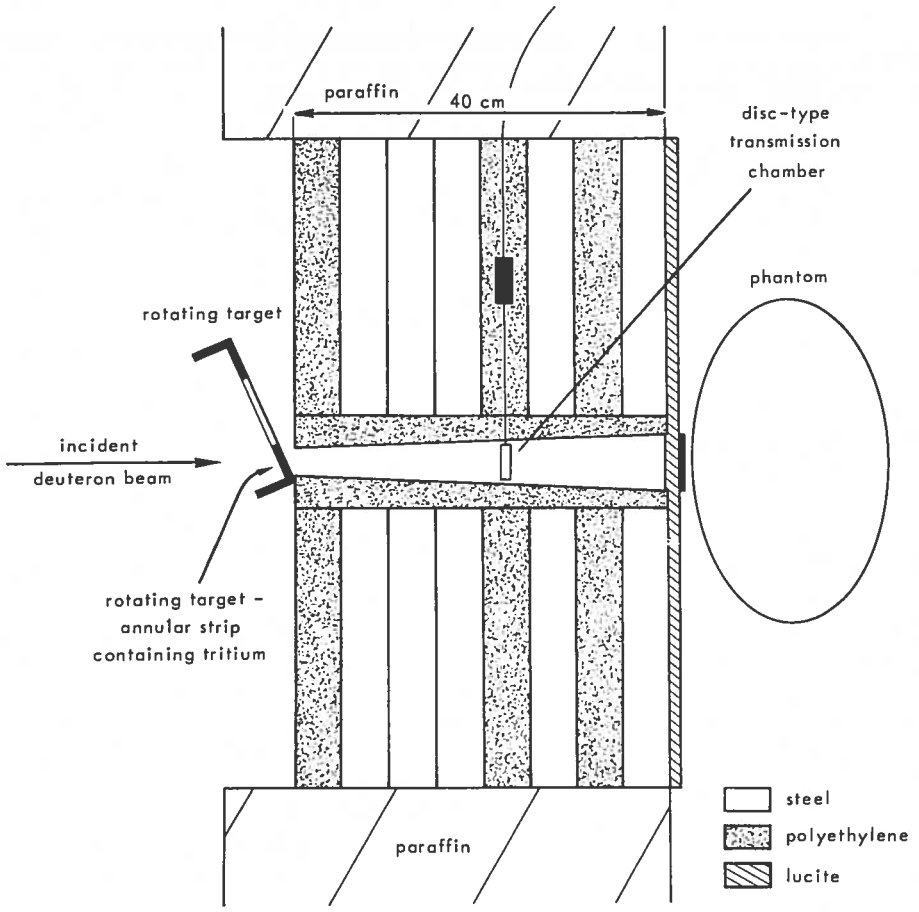
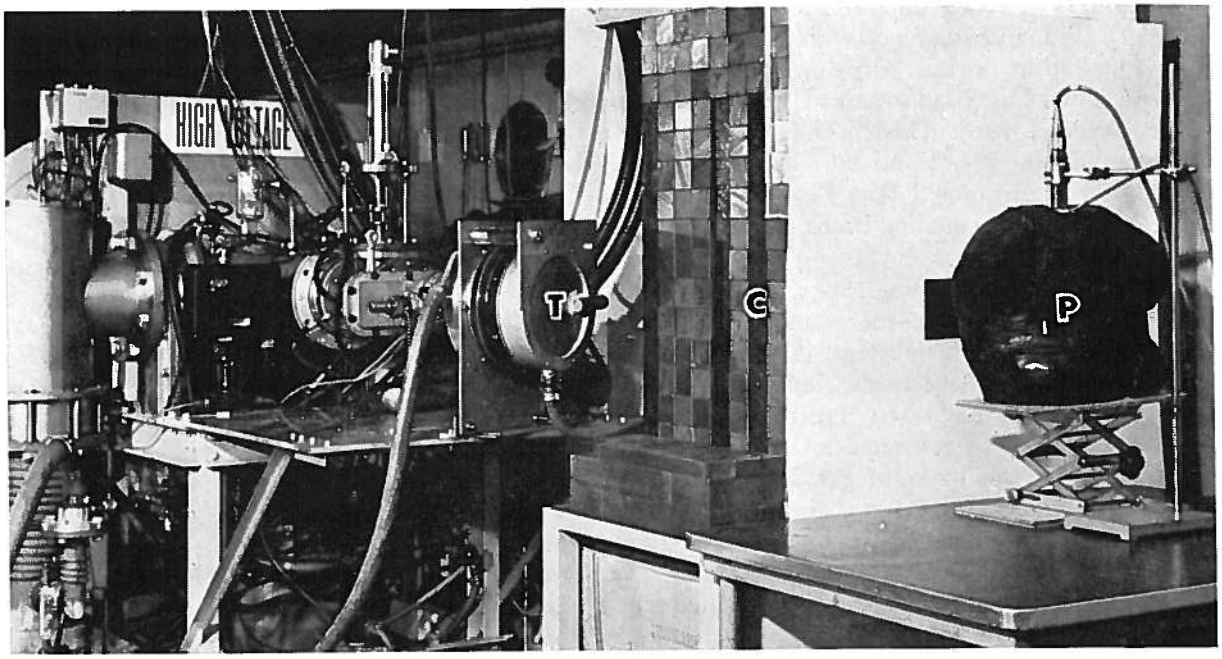


Fig. 4. Experimental arrangement at the Radiobiological Institute TNO, employed for pateint irradiations. A schematic diagram of the collimator consisting of alternate layers of steel and polyethylene is given in fig. 4a. The last steel layer was covered with a 1 cm thick lucite plate to decrease the low energy neutron contribution, whilst a 3 mm thick Teflon layer was positioned at the patient entrance field in order to obtain a maximum skin-sparing effect.

Fig. 4b shows the neutron generator with rotating target (T), part of the collimator (C) and the fluid rubber phantom (P) used for the depth dose measurements.



4. Characteristics of fast neutron beams for clinical studies

Since it has been demonstrated that the neutron energy has little influence on the oxygen enhancement ratio in the range between 1 and 15 MeV, the choice of the most suitable beam for radiotherapy is to a large extent determined by the depth dose characteristics of the neutrons and the intensity which can be produced by neutron generators. For the production of collimated beams of fast neutrons, the best practical possibilities are offered by bombardment of targets of low Z with accelerated positive ions. The neutrons used in the present studies, which are produced by the $^{3}H(d,n)^{4}He$ reaction (often referred to as D-T reaction), are emitted approximately isotropically and have energies in excess of 14 MeV. This reaction offers the advantage that the neutrons can be produced with positive ions of a few hundred keV energy, employing relatively small and inexpensive accelerators; however, the attainment of yields required to give adequate dose rates at target-to-skin distances (TSD) in excess of 100 cm, presents technical problems, which have not yet been solved completely [14].

The 15 MeV neutron generator employed in our evaluation studies, is a modified Texas Nuclear model 9909 S accelerator, with a separate oil insulated power supply, operated at the performance specifications of 270-280 kV accelerating voltage and 6-7 mA positive ion current. A special type of rotating target has been employed, which contains an annular strip of titanium loaded with tritium activities between 80 and 100 Ci. The target is water-cooled and rotated at 260 rpm in front of the deuteron beam which has a diameter of 15-20 mm. A total neutron yield of 6.10^{11} neutrons/sec produced by the D-T reaction is obtained at operation conditions of 270 kV and 6 mA deuteron beam current. Although experience with the rotating targets is limited at present, a preliminary half-life of 100 mA.hours has been attained for the targets which we have used. This half-life is lower than that obtained by Booth and Barschall [15]. However, it should be noted that we used an unanalyzed beam which contains both monatomic and diatomic ions.

Fig. 4 a and 4 b show the experimental arrangement including a collimator consisting of multilayers of steel and polyethylene, employed for the patient irradiations. Due to the limited yield of the neutron generator employed, the thickness of the collimator had to be restricted to a total thickness of 40 cm. On the basis of previous attenuation studies [16], it can be estimated that for this shield, the relative transmission of the total dose amounts to 4%. The inside surface of the collimator hole, which is lined by a tapered polyethylene layer, defines a 6 × 8 cm field at 45 cm from the target and converges to a 3 × 3 cm opening at the target side. A disc-type transmission chamber was inserted through the collimator and positioned in the beam line to serve as a reference ionization chamber during the patient irradiations.

Dosimetry studies have been carried out using different phantoms of the upper part of the human body, in order to obtain information on the depth dose characteristics of the fast neutron beam. The total absorbed dose at various sites was measured using tissue-equivalent ionization chambers. An estimate of the gamma-contamination was derived using an additional chamber which was relatively insensitive to neutrons. Detailed information on beam profile and iso-dose curves within patients can be found elsewhere [17]. As reported previously [18] the penetration characteristics for 15 MeV neutrons are comparable to those of ^{137}Cs gamma-rays. These measurements have been extended for different target-to-skin distances using a tissue-equivalent liquid phantom. From the depth dose curves presented in fig. 5, it can be deduced that penetration characteristics comparable to those of ^{60}Co gamma-rays can only be obtained at TSD values in excess of 100 cm. For the conditions of the actual patient irradiations, carried out at a TSD of 45 cm, depth dose measurements have shown that the 50% iso-dose line is at approximately 9 cm from the surface of the inhomogeneous phantom containing lung-equivalent material and tissue-equivalent liquid.

For each patient, data of the tumour size and position relevant to the surrounding tissue were obtained from radiographs and sometimes from transversal planigrams. On the basis of this information a depth dose curve for the patient was made, employing the relevant data from the phantom measurements. For the majority of the patients the irradiations were carried out bilaterally, primarily to reduce the dose to the skin, but also to provide a better dose distribution over the tumour. The dose rates at the site of tumours situated approximately in the centre of the patients, varied between 4 and 6 rads/min. Sulphur activation detectors attached onto the skin of the patients, have been employed to check the actual dose received by the tumour.

5. Preliminary results on responses of lung metastases in patients

Since October 1971 a small number of lung metastases in patients have been irradiated with single doses of 15 MeV neutrons and the responses were compared with those of approximately equivalent doses of ^{60}Co gamma-rays on the same metastases or on other metastases in the same patient. The range of doses employed in the pilot series, 150 to 200 rads of 15 MeV neutrons and 300 to 500 rads of ^{60}Co gamma-rays, was chosen to attain a suffi-

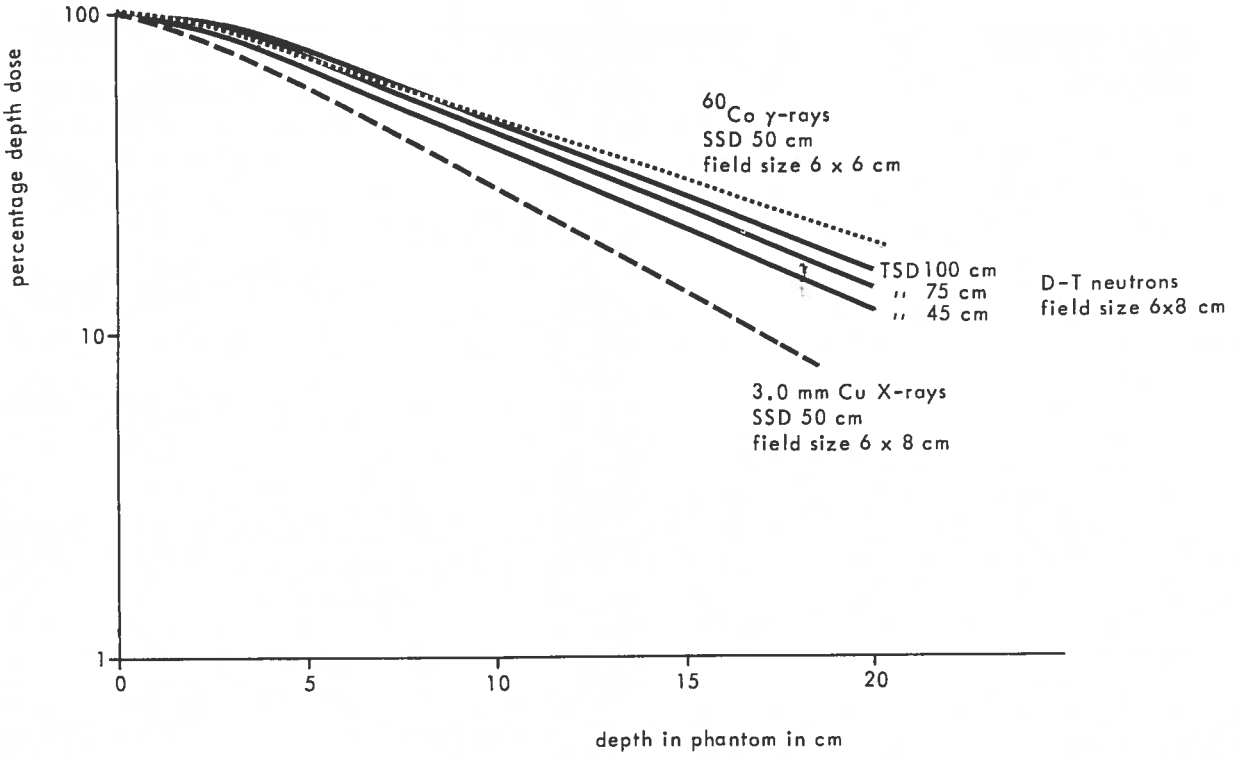


Fig. 5. Central axis depth dose curves of 15 MeV neutrons for various target-to-skin distances in comparison with those for ^{60}Co gamma-rays and X-rays.

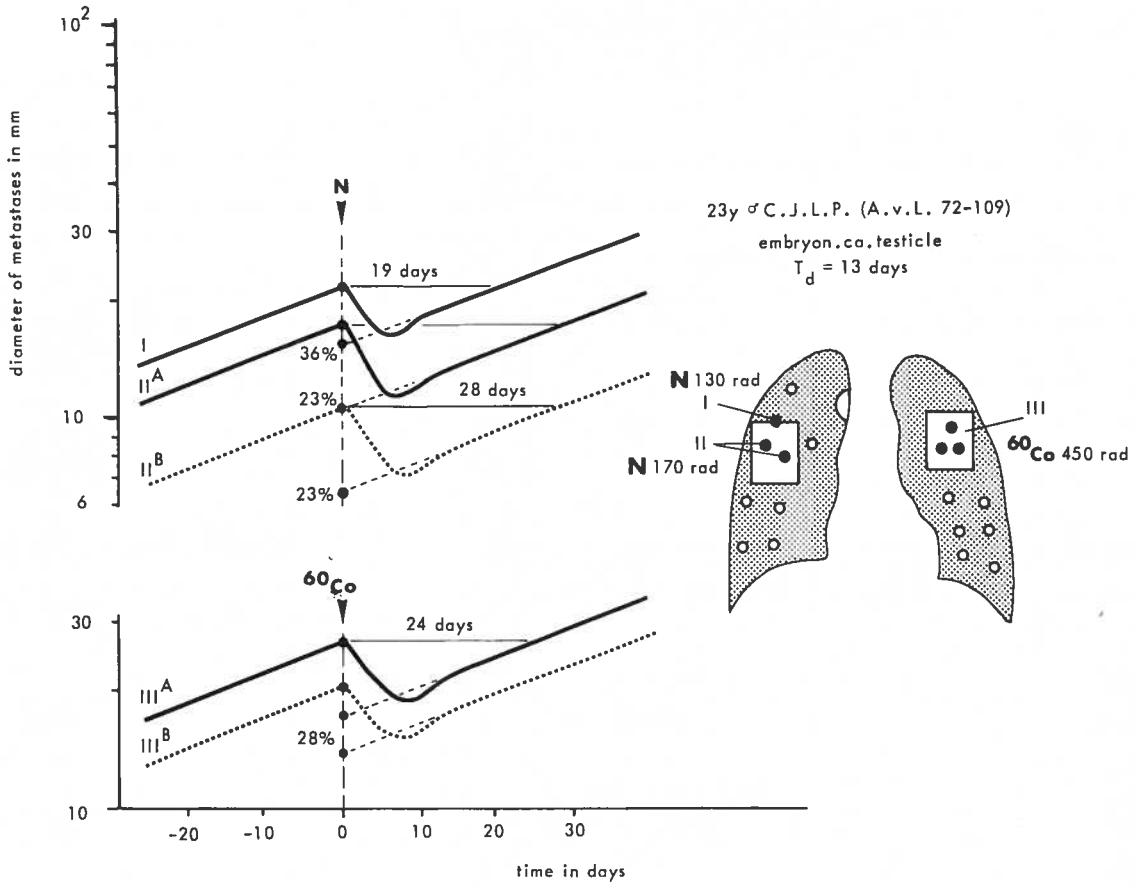


Fig. 6. Growth curves of pulmonary metastases in a patient irradiated with single doses of 130 rads (curve I) and 170 rads (curves II^A and II^B) of 15 MeV neutrons and 450 rads of ^{60}Co gamma-rays (curves III^A and III^B). Numerical values of the growth delay and the relative volume decrease are indicated in the figure. For the evaluation of the RBE values with reference to orthovoltage X-rays, employed in the radiobiological studies, the gamma-dose has to be multiplied by a factor of 0.85.

ciently large response of the metastases, while these doses were not large enough to interfere greatly with subsequent fractionated treatments with photons. The type of data obtained is illustrated in fig. 6 [19-21]. The growth delay i.e. the number of days in which the metastasis has reached its pre-irradiation volume, or the „effective" volume reduction obtained from extrapolation of the growth curve after the return of the growth rate to the pre-irradiation value, can be employed to derive RBE values. For this derivation a number of factors has to be taken into account which will not be discussed. The results obtained from the first ten irradiations indicate that considerable differences in the RBE values occur by a factor of at least 1.8. These differences might be related to the histological type of tumour and to its growth rate. Although no RBE values are known for the normal dose-limiting tissues of the same patients, it is most unlikely that the RBE for normal tissues in patients varies in dependence of whether their tumours grow fast or slow. This implies that the gain factor will also vary considerably depending on the type of tumour and its growth rate. The aim of further studies is to measure more RBE values for groups of metastases with different volume doubling times and different histological characteristics.

6. Concluding remarks

A new method of cancer treatment by radiation cannot be evaluated but with extreme caution. Extensive data on tumours and normal tissues in laboratory animals have been obtained as a preliminary to pilot studies of responses of metastases in patients to different doses of fast neutrons. The results of these first applications show a sufficiently wide variation in values of the relative biological effectiveness for different types of tumours, to justify the expectation that these studies will yield indications about which types of tumours might best be treated with fast neutrons. Due to the limited number of patients with metastases suitable for these studies, it will take considerable time before an adequate evaluation can be completed.

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Experimental Treatment of Leukemia: Attempts to Improve the Results of Extracorporeal Irradiation of the Blood by Cell Mobilizing Agents*)

Behandeling van leukemie d.m.v. extracorporale bestraling van het bloed

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Summary

A review is given of a research project concerned with the treatment of leukemia by extracorporeal irradiation of the blood. The effectivity of this treatment is limited by the rate of influx of malignant cells from their production sites into the peripheral blood. A number of chemical compounds, all belonging to the group of polymeric anions, has been found to mobilize leukemic cells and their biological properties are being investigated in animal models of leukemia and other forms of cancer.

Samenvatting

Dit overzicht beschrijft een onderzoekprojekt betreffende de behandeling van leukemie door middel van extracorporale bestraling van het bloed. De resultaten van deze behandeling worden tot dusverre beperkt door een te geringe influx van leukemische cellen van de weefsels waar ze gevormd worden naar het perifere bloed. Een aantal chemische verbindingen die alle behoren tot de groep van polymere anionen, bleek de eigenschap te bezitten om de bovengenoemde uitwisseling van cellen te versnellen. De biologische eigenschappen van de meest effectieve verbindingen worden onderzocht in diermodellen van leukemie en van andere vormen van kanker.

Introduction

Extracorporeal irradiation of the blood (ECIB) was introduced in 1962 by Lajtha et al. [1] as a possible treatment of certain cases of leukemia. It had been known for many years that leukemic cells are quite sensitive to ionizing irradiation, but because of the disseminated nature of leukemia, a curative radiation would have to be administered to the whole body. This remains clearly impossible, since sterilization of the total tumor mass — usually between 10^{11} and 10^{12} leukemic cells — would require dosages far in excess of what can be tolerated by the intestinal tract, so that the intestinal tract, so that the patient would die from the intestinal syndrome of radiation sickness. This has been fully substantiated by experiments with leukemic mice as well as by the results of clinical whole body irradiation. Even when a dose of 1000 rads of whole body irradiation was administered — which had to be followed by bone marrow transplantation because such a dose eradicates the hemopoietic tissues of the patient — leukemia recurred between 3 and 9 months following the treatment. Therefore, theoretically, whole body irradiation and bone marrow transplantation can only be expected to cure leukemia if the malignant cell population has been reduced to relatively small proportions by prior treatment with cytostatic agents. For leukemic patients who do not respond to chemotherapy and for those in whom chemotherapy is contraindicated, ECIB seemed to offer a possibility for providing at least temporary palliation.

The Radiobiological Institute became involved in this treatment in 1966 when it appeared that the ^{137}Cs source of the Institute was the only source available in the country which could be easily adapted to the requirements of prolonged irradiation of an extracorporeal current of blood (Fig. 1). In collaboration with the Isolation Unit of the Institute for Radiopathology and Radioprotection at Leiden, a few patients were treated with ECIB, but the results were disappointing in that although a reduction of the peripheral blood count could be obtained, the patients died with excessive leukemic involvement of the central tissues. Subsequently, a

* This research project is jointly carried out by the Radiobiological Institute TNO and the Department of Radiobiology of the Medical Faculty Rotterdam. Certain parts of the program have been pursued in close collaboration with Dr. S. Ormai (Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest) and with the Medical Department of Brookhaven National Laboratory, Upton, N. Y. Part of this review is derived from a paper submitted by the author for publication in the Proceedings of the International Symposium on Chemotherapy of Cancer Dissemination and Metastasis, Milan, May 1972.

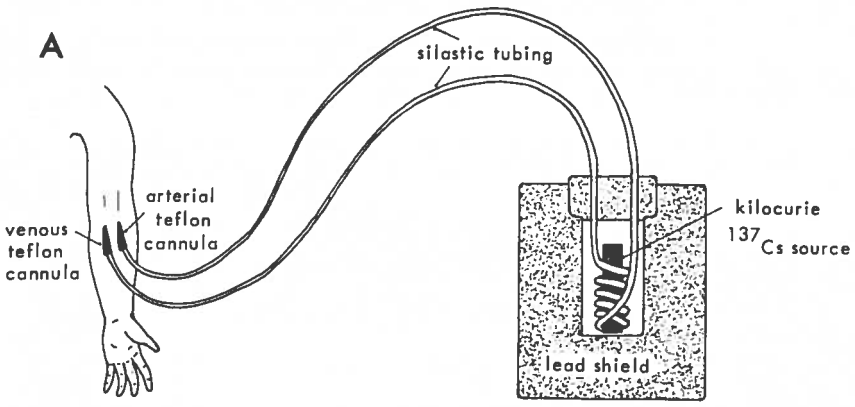
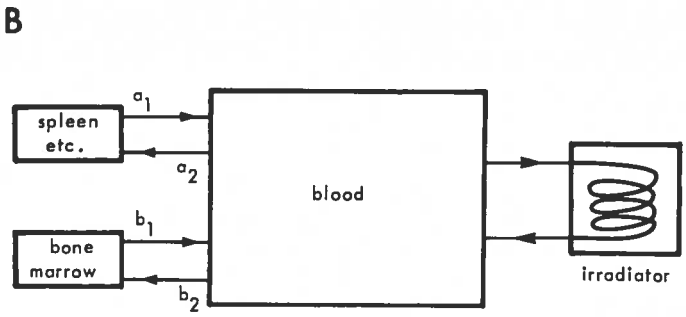


Fig. 1. Schematic representation of extracorporeal irradiation of the blood
A = Blood circuit and irradiator
B = Exchange of blood and leukemic cells between the various compartments



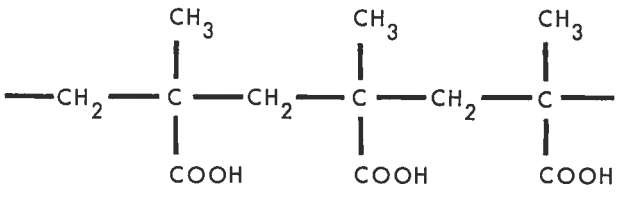
clinical apparatus for ECIB was developed at the Radiobiological Institute for use in the Leiden clinics. This has recently been replaced by a commercial radiation source, which is available for clinical groups interested in treating leukemia by this technique.

Following the first clinical experience it became soon clear that the main factor limiting the effects of the treatment is the poor exchange rate of the leukemic cells from the central tissues into the peripheral blood (indicated by arrows a_1 and b_1 in Fig. 1 B).

Since a single session of ECIB can deliver sufficient radiation to the leukemic cells in the whole circulating blood volume to kill nearly 100% of these cells, it is obvious that the rate of delivery of new viable cells to the blood should exceed the production of leukemic cells during the same time interval, if the subsequent irradiations are really to diminish the central masses of malignant cells [2]. Because this is apparently not the case, ECIB has remained of limited value and is presently only used in a more or less exploratory way in the treatment of chronic lymphatic leukemia, where the production of leukemic cells seems to be slow enough for ECIB to produce long-lasting remissions.

In order to improve the exchange of leukemic cells between central tissues and the peripheral blood, attempts have been made to mobilize cells from the lymphatic tissues and the bone marrow by chemical means. Cronkite et al. [3] described such an action of heparin and Sasaki et al. [4] reported on a lymphocytosis that could be induced by polysaccharide sulphates in experimental animals.

In 1968 our attention was drawn to observations by De Clercq at the Rega Institute in Louvain, in the course of a study on the interferon induction by chemical agents. One of their (weakly) active compounds was polymethacrylic acid (PMAA), the same chemical structure as that of the well-known plastic material perspex (Fig. 2). It was noted that the injection of PMAA into rats caused a lymphocytosis to occur within a few hours and this observation has been substantiated and extended in our Institute [5]. Subsequent investigations have revealed that PMAA mobilizes granulocytes, normal hemopoietic stem cells, and leukemic cells as well.



Direct anti-viral activity

Indirect - via interferon induction

Lymphocytosis

Fig. 2. Chemical structure of the polymethacrylic acid (PMAA) chain.

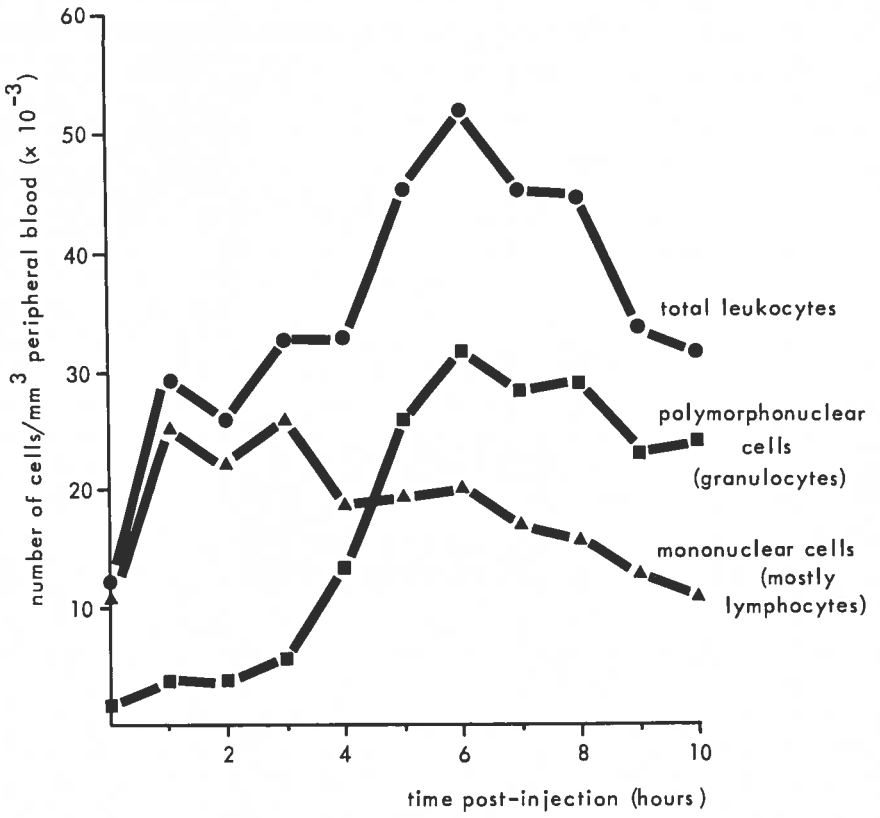


Fig. 3. Increase in leukocytes in peripheral blood of the rat after 40 mg/kg PMAA (i.v.) — data obtained by Ross et al.

Following the administration of PMAA the rise of lymphocytes in the peripheral blood occurs earlier than that of the granulocytes (Fig. 3). Most of our investigations have been concerned with the ability of the compound to mobilize lymphocytes and we have accordingly introduced the term lymphocyte mobilizing compounds (LMC). Since it could be demonstrated that PMAA removes lymphocytes from the spleen and the lymph nodes, this compound seemed of possible practical value in conjunction with extracorporeal irradiation of the blood in the treatment of leukemia. The main objective of our research program has been to collect the information required for the clinical application of PMAA and of analogous compounds with a similar or more pronounced LM activity and preferably with less toxicity. For this purpose, we have recently initiated a screening program in which a variety of polymeric anions is being investigated.[1]) Recently, several observations have indicated that PMAA inhibits the occurrence of metastasis from transplanted tumors. These studies have not yet been completed and the present paper will review the available data.

Mobilization of nucleated cells by PMAA and other compounds

Some initial batches of PMAA were kindly provided by the Rega Institute (Louvain), where it had been synthetized by polymerization of methacrylic acid in the presence of benzyl-peroxide [6]. The molecular weight ranged between 25,000 and 1,175,000. In addition, PMAA has been prepared by two different procedures:

a) Polymerization of methacrylic acid in the presence of hydrogen peroxide (courtesy of the Central Laboratory TNO); a PMAA-water gel, containing 16.5% PMAA of M. W. 750,000 was produced, which could be dissolved in phosphate buffered saline before injection.

b) Polymerization of methacrylic acid in water solution or in the pure form, by irradiation with ^{60}Co γ-rays. From the watery solution, a similar gel is obtained as with the first method. The pure monomer yielded a solid polymer, which was powdered and dissolved in saline.

There was no difference in the physiological properties of the two (solid and gel). The radiation induced polymerization can be employed to produce other polymeric anions and has the advantage that it does not require the addition of catalysts which are difficult to remove completely and which may be toxic.

PMAA was tested in doses of 40 mg/kg body weight in three different transplantable leukemias: a mouse lymphosarcoma which originated in our own Institute, a mouse lymphoma (L5178Y) and the Shay chloroleukemia of the rat [7]. In all three models this treatment caused a significant increase of tumor cells in the peripheral blood ranging from

[1]) This project is supported in 1972 and 1973 by a grant from the Netherlands organization for the advancement of pure research to the Medical Faculty Rotterdam.

2 to 3 times control values. We have confirmed these findings numerous times in the Shay leukemia in the course of experiments on the combined effects of PMAA and ECIB. PMAA was recently also found to increase the peripheral tumor cell count in rats carrying transplants of a chemically DMBA induced leukemia.
In preliminary experiments, S. K. Lahiri (unpublished observations) has demonstrated an increase in the number of hemopoietic stem cells in the peripheral blood of mice, as determined with the spleen colony assay.
PMAA causes a lymphocytosis in all other animal species so far investigated; these include rats, mice, dogs, monkeys, and calves.

The kinetics of lymphocyte mobilization in normal rats by PMAA

By employing thoracic duct cannulation in normal and in splenectomized rats, the mobilization of lymphocytes via the direct (spleen-blood) circulation and the indirect route (lymph nodes-thoracic duct-peripheral blood) could be quantitatively evaluated (Fig. 4). PMAA caused an increase in the lymphocyte counts of the thoracic duct output in both normal and splenectomized animals. Peripheral blood lymphocytosis occurred in non-thoracic duct cannulated rats, both splenectomized and normals. However, when splenectomized rats were thoracic duct cannulated, injection of PMAA did no longer cause an increase of peripheral blood lymphocyte counts, indicating that PMAA mobilizes lymphocytes from both the spleen and the lymph nodes simultaneously (Table 1). These experiments demonstrate that mobilization of lymphocytes from the lymph nodes takes mainly place via the indirect route and that from the spleen via the direct route.
By counting the nucleated cells in suspensions of collected lymph nodes or the spleen of animals treated with PMAA, the overall cell loss from these tissues could be determined (Ormai et al., to be published). In the mesenteric lymph nodes the lowest cell number — corresponding with a 36% decrease of cells — was observed at 2 hours, in combined axillary and inguinal lymph nodes the peak loss was 33% at 3 hours. Thereafter, the cell numbers rapidly normalized. A maximum cell loss of 23% was observed in the spleen at 4 hours after injection, followed by an increase in cell numbers

Table 1. *Changes in lymphocyte counts in blood and thoracic duct lymph in rats following injection of PMAA 40 mg/kg b. wt**

		increase of lymphocytes in	
	---	peripheral blood TD cannulated	thoracic duct lymph TD cannulated
intact	++	++	++
splenect.	++	—	++

* data from S. Ormai et al. (to be published)

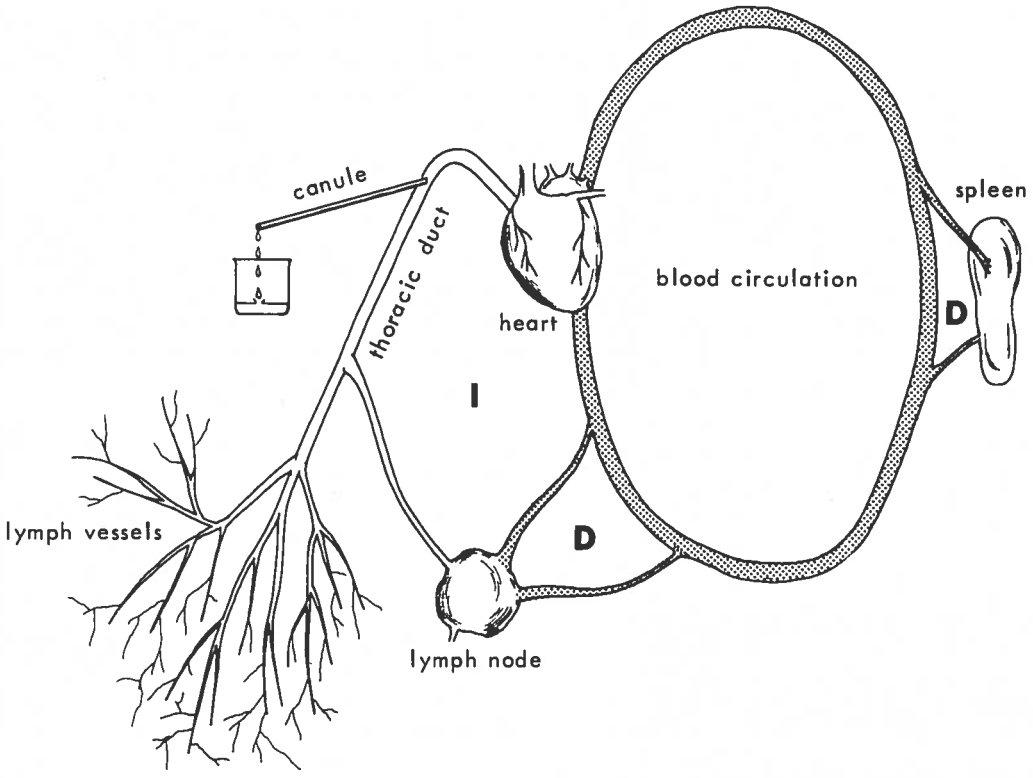


Fig. 4. Schematic representation of blood and lymph circulation in relation to the spleen and the lymph nodes, the main sources of circulating lymphocytes in adults.
I = Indirect route of circulation of lymphocytes
D = Direct route of circulation of lymphocytes

with a peak at 5 hours. Thereafter, normalization also occurred.

As to the site of lymphocyte depletion in the lymphatic tissues, S. Ormai and M. Palkovits (personal communication) have measured the size of Malpighian corpuscles (the lymph follicles — sites of lymphocyte accumulation — in the spleen) and found a significant volume decrease of about 30% at 5 hours after injection of PMAA. Apparently, PMAA dislodges cells from the follicles which may explain the peripheral lymphocytosis, while the lymphocytes subsequently return to *other* locations in the same organs. It is of interest in this context that Cottier et al. [8] have reported a depletion of the thymus dependent areas in the lymphatic tissues following prolonged ECIB and thoracic duct drainage in calves. It has to be investigated whether mobilization by PMAA is affecting mostly the non-thymus-dependent areas, or instead the same areas as are depleted by ECIB, in other words, whether PMAA and ECIB are complementary or not. Obviously, similar studies will have to be performed in the tissues of leukemic animals.

The mechanism of action of PMAA and other LM agents

The fact that PMAA has been found to effectively mobilize malignant (leukemic) cells is obviously of interest not only because of its possible application in ECIB, but also because any interference with the behaviour of malignant cells is worth pursuing in the present stage of our knowledge of the cancer process.

The influence of PMAA treatment of rats on the electrophoretic mobility of normal thoracic duct lymphocytes has been studied in a preliminary way by Dr. G. Haemmer il (Department of Cancer Research, Aussenstation, University of Zurich, Switzerland) in order to investigate whether PMAA mobilizes cells by binding to the cell membrane, which would be likely to result in changes in electrical charge. However, at 3-5 hrs after treatment no significant difference with cells from control animals could be observed. Our present approach is to study the distribution of PMAA in the various tissues of the rat using labelled compounds. Results have been obtained with a ^{125}I-labelled styrene derivative of PMAA P (20M: IS^{125}I). Localization occurs primarily in the liver, kidneys, and spleen, and to a smaller extent in the adrenals, thymus and lymph nodes (Fig. 5). If the concentration of the label relates to the biological activity it would seem that PMAA acts mainly on the spleen to cause lymphocyte mobilization. So far there seems to be little difference in the localization of the radioactive compound between normal and leukemic rats. These investigations have to be extended with ^{3}H or ^{14}C-labelled PMAA, not only to confirm the distribution pattern revealed with the ^{125}I styrene derivative, but also to study the

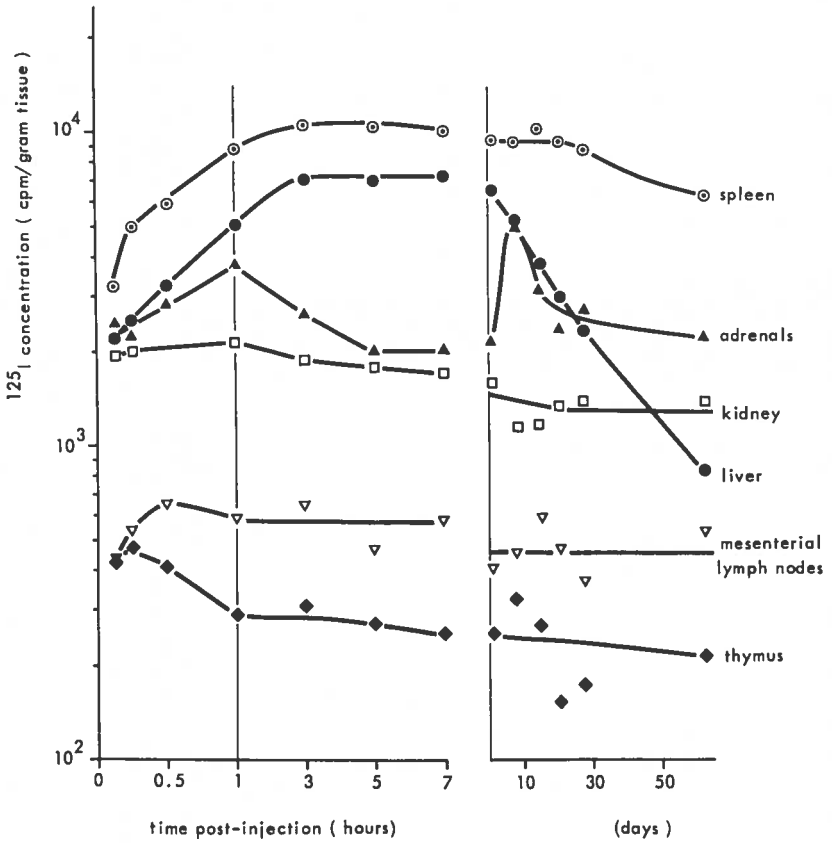


Fig. 5. Distribution with time of ^{125}I-PMAA (5% styrene) in rats — data from Ross, Robev et al.

localization of the compound at the microscopical level.

A large range of biological effects has been reported for a variety of polyanions among which were heparin, heparinoids and synthetic polymers. Some of these compounds have antitumor activity, many of them exhibit some anticoagulant effect which has been related to their ability to precipitate proteins such as thrombin and fibrinogen and in certain systems the occurrence of tumor metastasis was found to be inhibited [9].

Our information on the systemic effects of PMAA and on its toxicity is still fragmentary. Prolonged administration of PMAA to rats (5 daily administrations of 60 mg PMAA/kg) has induced anemia and thrombocytopenia. Hemorrhagic foci in the lungs have been observed, as well as liver cell necrosis. This pathological picture is reminiscent of descriptions in the literature of toxic effects of several other polyanions.

Preliminary evidence on dissemination of the Lewis lung carcinoma in mice under influence of daily treatment with PMAA (5-20 mg/kg i.p.) was obtained by Franchi who observed a pronounced inhibition of the number of lung metastases [10]. In a model of lymphatic spread of tumor metastases employing intratesticular implantation of a transplantable osteosarcoma in mice, which was developed in this Institute by Van Putten, a moderate but significant decrease of the incidence of lymphatic metastasis was observed following a 5-day treatment course with PMAA.

The mechanism of the metastasis inhibiting effect is as yet unknown as is the case for other polyanions with a similar action. It is tempting to speculate about a common mechanism for this effect, for the thrombopenic action (or anticoagulant effect) and for the ability to mobilize lymphocytes and other blood cells. Such an interrelation could be studied by comparison of the activities of a series of compounds which process LM activity.

Conclusion

The relatively simple phenomenon of a mobilization of lymphocytes and granulocytes into the peripheral blood by PMAA has been found to be part of a complicated scala of biological activities of this compound and of related ones. The LM activity which is expressed in leukemic animal models, seems practicable for application in ECIB. Unfortunately, a practical animal model to study ECIB in leukemic animals is not readily available, because extracorporeal blood circuits are difficult to maintain in rodents, while in larger animals, which are more suitable for such procedures, transplantable leukemias are not available. Thus far, data have been obtained in Shay leukemic rats treated with partial body irradiation (designed to kill circulating cells rather than central leukemic deposits) in conjunction with PMAA administration indicating that the antileukemic effect of irradiation of the blood is increased by PMAA treatment. However, in view of possible direct antileukemic effects of PMAA and because of certain toxic effects observed following repeated administration of large doses of the compound, further investigations in animals are clearly required before PMAA can be introduced in the clinical treatment of leukemia.

Some of its effects on solid tumor systems so far observed, in particular the metastasis inhibiting effect of PMAA, seem to justify a more detailed study of the biological properties of this polyanion and its derivatives.

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The Relationship between Tumour Vascularisation and Response to Radiotherapy

Het verband tussen vascularisatie van de tumor en de gevoeligheid voor radiotherapie

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Summary

The blood supply of most tumours seems to be insufficient. This restricts the growth rate as well as some therapeutic modalities such as radiotherapy and chemotherapy. In this review some methods are discussed that may be used to obtain a better insight into the various aspects of the microcirculation of tumours.

Samenvatting

De bloedvoorziening van de meeste tumoren is inefficiënt. Dit vormt een beperking voor zowel de groei van tumoren als de therapie. In dit artikel worden enkele methoden beschreven die ons een beter inzicht kunnen verschaffen over de verschillende aspecten van de micro-circulatie van tumoren.

Introduction

Tumours present some peculiarities in their blood supply in which they clearly differ from normal tissues. This has attrackted attention during the last decades, as some aspects are of importance for the diagnosis of tumours (arteriography). Also of importance is the oxygen tension for the radiosensitivity of tumour cells. Moreover, some recent evidence indicated that tumours may produce a substance that specifically induces proliferation of blood vessels. As the introduction of modern techniques made it possible to gain more information concerning the vascularization of tumours, the insights changed accordingly. At the turn of the century the general opinion was „tumours have an abundant blood supply". This was based mainly on the observation that tumours tend to „ooze" blood. This opinion, however, gradually yielded to the opposite conviction that tumours have an inferior blood supply. Such findings present a challenge for further investigations regarding the origin of any possible abnormalities.
Investigations in any field make sense only if the results will lead to progress. In this case, this means progress with regard to the therapy of tumours. There are indeed a number of aspects of tumour vascularization that are of importance to medical science. In the first place, is the growth of tumours dependent to a large extent upon their vascular supply? Secondly, it has been shown that tumours are deficient in their vascular supply. Because of this, radiotherapy and chemotherapy are seriously handicapped.
This paper will deal mainly with the latter aspects, the microcirculation of tumours. Other aspects, however, are also of importance and, therefore, these will be discussed first.

The architecture of blood vessel patterns in tumours

When body cells become malignant and begin to proliferate into a tumour, or when malignant cells are transported to other parts of the body to form metastases, a supplying vascular system must develop at the same time.
It is very likely that the early tumour cells are supplied by pre-existing vessels of normal tissues; for instance, of the lung or the brain. In situations in which the normal cells are replaced by the malignant cells, it is even very likely that the blood vessels of that organ adapt to serve the new occupants. If no surrounding vessels are available, the capillaries supplying the malignant cells initially serve as the substratum for a complete tumour blood vessel system.
Irrespective of whether a tumour rebuilds the blood vessels of the host organ into its own system,

or whether this system develops initially, from tumour capillaries, the result is that a tumour inevitably ends up with a vascular system that obviously differs from that of the normal tissues.
This is of importance for diagnosis. The modern X-ray procedures for diagnosing tumours frequently make use of arteriography. In this procedure, a radio-opaque dye is injected into an artery leading to the process requiring examination. During the passage of the blood (including the dye) through the area, a series of X-ray films is taken. In this way, not only can a good impression of the vascular architecture be obtained, but also the rate of passage. The arteriograms of some of the larger tumours may have so many characteristics that the arteriography alone suffices for establishing the diagnosis.
Apart from all possible characteristics of tumour blood vessels such as turtuous or dilated vessels, a typical observation is that much dye, and thus blood, passes directly from the arteries into the veins. In addition, many tumours contain areas in which hardly any dye shows up; these are the „avascular" areas. Another typical characteristic is that in some vessels the blood flow is very sluggish, leading to a „pooling" of dye.
From the foregoing, it will be clear that the blood supply of tumours is not as systematic as that of normal tissues. Aside from the fact that the typical aberrations seen on the arteriograms are useful for diagnostic purposes, they also have consequences for the tumour therapy.
If a tumour is to be treated with chemotherapeutic agents, it is essential that the drugs reach all tumour cells. An inefficient tumour blood supply means that not all malignant cells receive an adequate amount of drug. The same applies to oxygen. It has been known for some time that, in order to obtain the maximum effect of X- or γ-rays in the sense of cell killing, the cells have to contain a certain amount of oxygen. However, the majority of experimental tumours investigated up to now contain a sizable proportion of hypoxic cells. As hypoxic cells are much more radioresistant than well-oxygenated cells, the radiation response of a tumour is to a large extent determined by its hypoxic cells.

A recent approach to overcome this handicap is the use of radiations with other physical properties, e.g. by using fast neutrons. A treatise on this subject will be found in this issue on page .
Imagine that we could influence the vascularization of tumours, what would we wish to do? There are two changes that may benefit the patient, but they are in some ways contradictory.

a) During therapy we would like to see an *improvement* in the blood supply, so that all tumour cells become better oxygenated and therefore more radiosensitive. In the case of chemotherapy, this would mean that all cells would become exposed to the drug.

b) In case no active therapy is performed, we would like to inhibit tumour growth in so far as is possible. If we could prohibit the induction of the vascular supply, then this would also arrest tumour growth.

Angiogenesis

In what ways does a tumour induce its vascular supply? This subject has recently been a matter of discussion even in the newspaper. This was due to a publication of Folkman (Harvard) who isolated a substance that is thought to be responsible for the induction of blood vessels throughout the tumour tissue. The existence of such substances was inferred some years before by Greenblatt et al., who found that, if tumour tissue was isolated from the surrounding tissues by means of a „millipore" membrane, the vessels of the surrounding tissues transformed to tumorous type blood vessels. By means of a complicated method Folkman extracts from tumour tissue a substance called by him, the T.A.F., or „Tumour-Angiogenesis Factor". When applied to a suitable substratum, this substance induces vascular proliferation. Folkman propagandizes that one can make antibodies against T.A.F. If this would be possible, Folkman reasons, then one could inhibit tumour growth by eliminating the factor that new vessels induce growth in the tumour.
If there is a chance that indeed the growth of tumours may be arrested by antibodies against T.A.F.? It is questionable whether T.A.F. is the only factor that induces the growth of tumour blood vessels.
Everyone knows situations in which blood vessels proliferate. This occurs e.g. after woundings or etching. In some experimental situations, capillary proliferation has been induced by the application of lactid acid. A likely explanation for this phenomenon is that, in the case of wounding, many capillaries are cut and because of this the cells in the tissue become hypoxic. Such cells switch to anaerobic metabolism, in which (among other things) lactid acid is produced. Other weakly acidic substances also appear to be capable of inducing capillary proliferation.
One should, therefore, consider the possibility that the typical shape of tumour blood vessels is caused by the T.A.F., but that the anaerobic catabolic products of the hypoxic tumour cells can also induce vascular proliferation. In that case, the use of T.A.F. antibodies would be, at the most, only partially effective.

Tumour microcirculation

In what ways can the microcirculation of tumours

be studied? In the first place, the investigator will have to consider in what aspect of tumour vascularization he is interested. Is it in the morphology for diagnostic purposes, or rather the physiology, directed towards therapy? With regard to the latter, one has to also make up his mind as to which physiological parameter is to be evaluated. If it is the distribution of metabolites such as oxygen, then not only is the vascular density of importance, but also the flow rate of the blood in the various areas of the tumour tissue.

Morphological analysis, i.e. the study of the vascular architecture, can obviously never yield information about matters such as the local oxygen supply of the tumour tissue. Angiography hardly brings us any further than that we become convinced that the microcirculation of tumours must be an exceedingly intricate matter! This means that the majority of the investigations on the oxygen supply of tumours are carried out with sampling techniques that unfortunately do not take into account the localization of the sampling site. Examples are: the assay of cell survival after irradiation, in which hypoxia will manifest itself in increased survival; and, polarographic determination in „vivo" by means of thin electrodes that are implanted in the tumour tissue.

However, for a proper understanding of the role that the microcirculation plays in causing localized hypoxia in tumours, and whether this may be influenced by physiological or pharmaceutical means, we need methods that indicate the *sites* of the hypoxic foci in tumours. The great advantage in making such a system available is that it would form a link between the "cellular radiobiology" and histology. The latter is one of the few methods available for obtaining information on human tumours and their response to therapy. In other words; a topographically oriented "in vivo" system is just what the pathologist and the tumour radiobiologist would bring together for the design and guidance of treatment plans for radiotherapy, chemotherapy, or a combination of both. It is for these reasons that we are putting considerable effort into the development of such systems.

During the last few years some new methods have been developed, for the study of the microcirculation in "vivo", in which (experimental) tumours are forced to grow in a sheet-like fashion; the "sandwich" tumours. With regard to the details one has to be able to observe in these preparations, one has to know the extent to which oxygen can diffuse into tissue. This can be calculated by the aid of mathematical models. It has become clear that there may be a "critical distance" of about 150μ from the capillaries at which the oxygen tension in the tumour tissue is zero (fig. 1) This is due to the fact that oxygen diffusing into the tissue from the capillaries will have been metabolized by the cells at that distance. This value has been calculated for

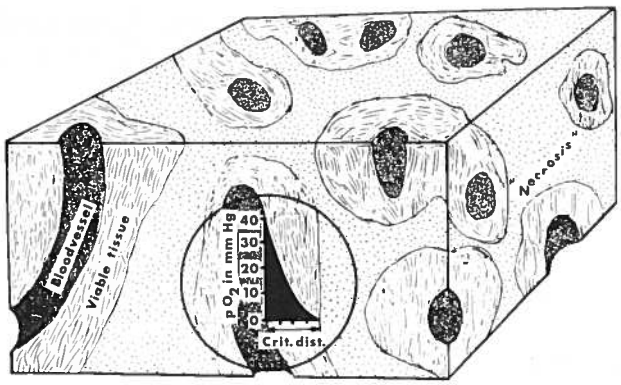

Fig. 1. Diagram of the spatial relationships in tumour tissue. The blood vessels are located in the center of cords of viable tumour tissue. The oxygen tension in these cords decreases with distance from the capillary.

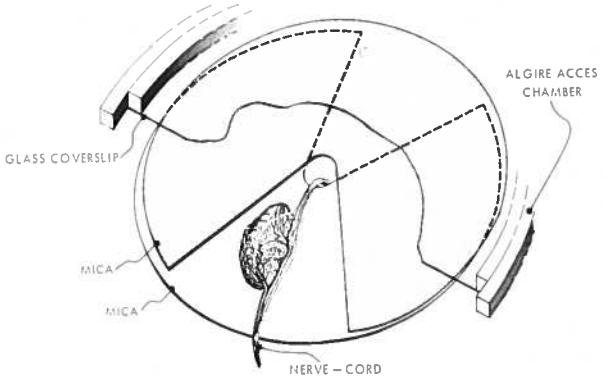

Fig. 2. The "sandwich" tumour system. A window is fitted in the skin of the mouse. A small piece of mica separates a cord containing nerves and blood vessels from the underlying skin. In this cord a piece of tumour tissue is implanted. A second piece of mica prevents the tumour from being squared. Tumour thickness 50 to 100 μ.

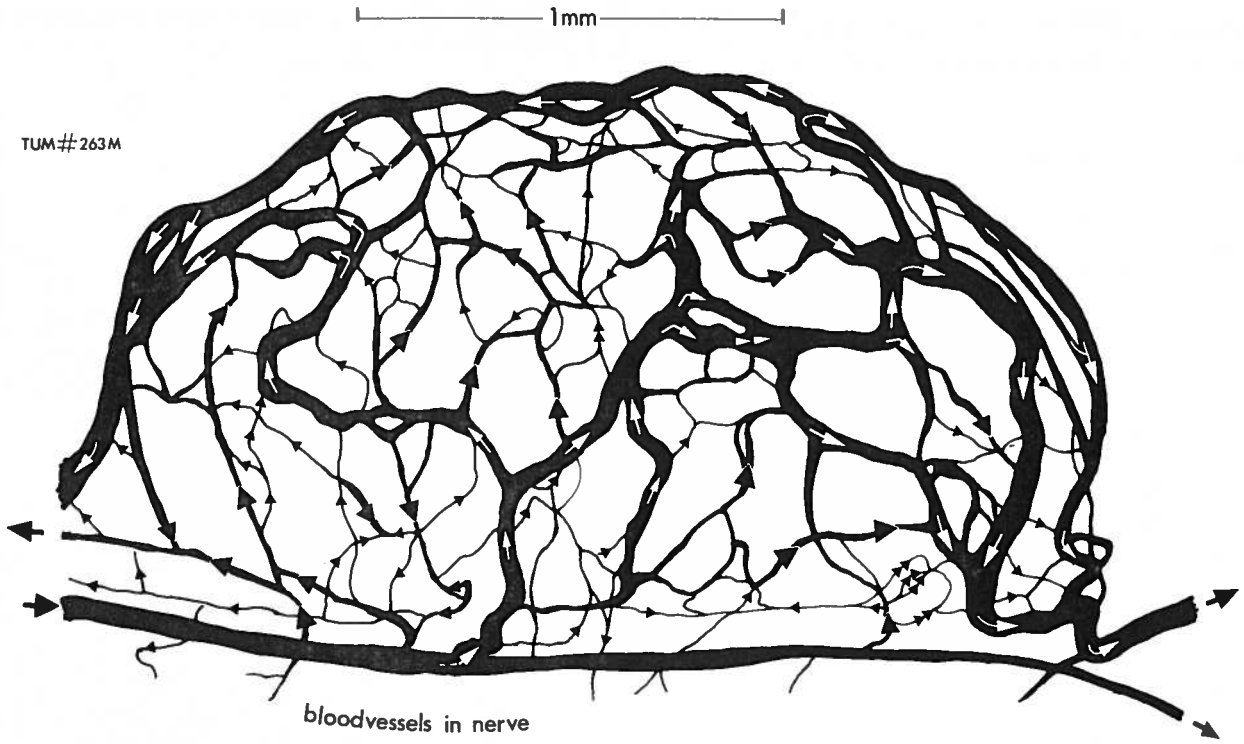

Fig. 3. A "sandwich" tumour. This diagram has been composed from photographs and observations. Note that the blood flows in many different directions, and that shunting occurs (double arrows).

oxygen, and for an oxygen concentration in the capillaries of 40 mm Hg. For other nutrients, such as glucose, the "critical distance" is much larger. These findings imply that any method that is designed to locate hypoxic cells in the tumour tissue will, of necessity, have to be a micro-method. A very promising method is that of the above mentioned "sandwich" tumours (fig. 2 and 3). By means of this system, one can obtain "in vivo" microangiograms with fluorescent dyes and record the distribution of the blood in the various parts of the tumour (fig. 4). Quantification of the microangiogram can be performed by using cartographic methods. By this means it could be demonstrated that the circulation in the tumour improved during a course of fractionated radiotherapy.
In addition, it has been shown that it is possible to obtain information on the oxygen supply of these tumours by means of an optical method. This method is the N.A.D.H. fluorescence. N.A.D. (formerly known as D.P.N.) is a nucleotide that converts into N.A.D.H. if the cell becomes less oxygenated. The fluorescence of N.A.D.H. is stronger than that of N.A.D. If one lowers the oxygen content of the inspired air of an animal carrying a "sandwich" tumour, the resulting NAD/NADH conversion can be measured microscopically by the aid of a very sensitive light measuring device; a micro-fluorimeter. In this way, it has been shown by means other than bio-assays, or polarographic electrodes, that these tiny "sandwiches" also contain hypoxic areas (fig. 5).
Our present investigations are designed to analyse to what extent such hypoxic areas may become more efficiently oxygenated as a means of general improvement of the microcirculation during fractionated radiotherapy.

Blood vessels of normal tissues

The microcirculation of tumours is not the only matter of concern in modern tumour radiotherapy. In the last few years, it has become evident that blood vessels of normal tissues that are included in the radiation beam may show serious alterations. The "hard" X-rays generated by the modern machines have a much higher penetration than in the days of the 250 kV X-rays. As a result, the severity of the skin reaction has ceased to be an indicator for "radiation tolerance". Damage to deeper lying structures are now of more importance, and this damage typically develops in one to two years following therapy. One of the most striking characteristics of heavily irradiated tissues is a fibrotic induration with many blood vessel abnormalities such as thickening of the arterial walls, and widening (teleangiectasies) of the capillaries. The blood vessel abnormalities are very likely instrumental in causing "the late tissue fibrosis".
This has formed the basis for extending our studies

on tumour blood vessels to designing methods to study the effect of radiation on blood vessels of normal tissues. Normal microcirculation is an intricate matter requiring not only evaluation by histology, but more so by physiological methods.
Blood flow, changes in permeability, cell division, and cell death of the endothelial cells forming the inner lining of the blood vessels, to mention a few, are subjects requiring urgent investigation. It is for these purposes, therefore that an European group, sponsored in part by the "EULEP" committee (European Late Effects Project Group), has recently initiated investigations into the various aspects of the response of the vascular system to ionizing radiation. Specialists in such fields as the effect of radiation on ultrastructure, microcirculation, blood flow, histology etc. from four European countries collaborate in a joint effort to elucidate the mechanisms of late vascular damage with the aim of improving radiotherapy treatment.
Blood vessels and microcirculation are probably not essential to the *cause* of cancer, but they are involved in so many aspects of tumour growth and therapy that they certainly deserve all our attention.

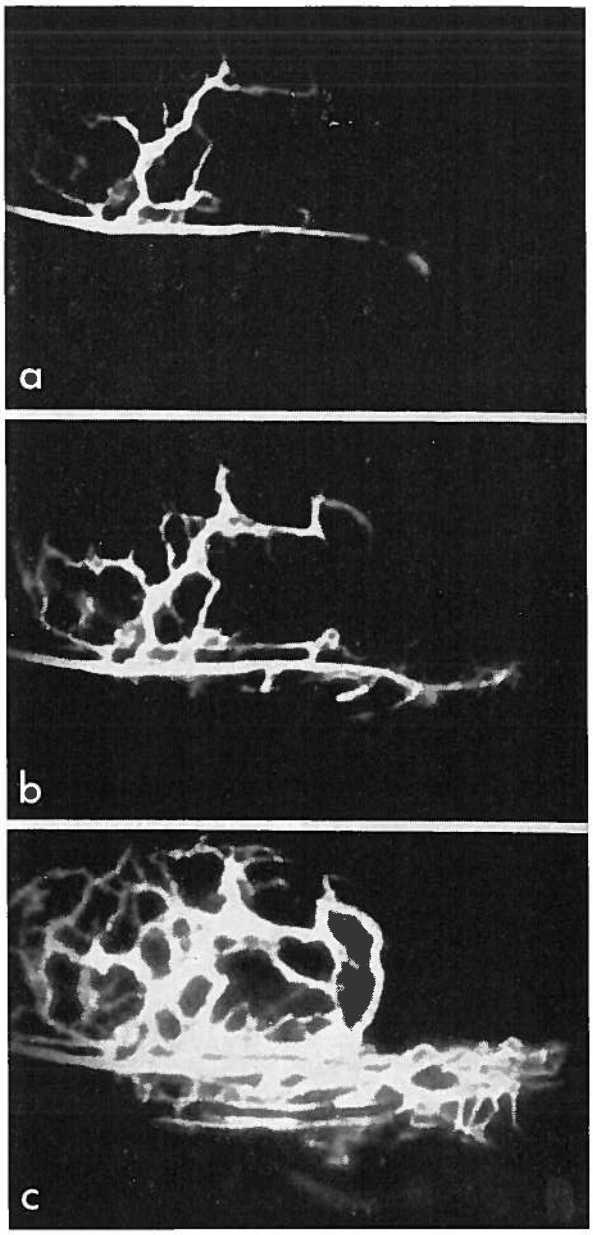

Fig. 4. Some frames from a micro-angiogram made with fluorescent dyes from a "sandwich" tumour. Frames at 1/2, 1, and 2 seconds. Quantitative analysis of such fluorescent micro-angiograms showed an improvement in the tumour "microcirculation" as a result of fractionated radiotherapy. Note the shunts from fig. 3.

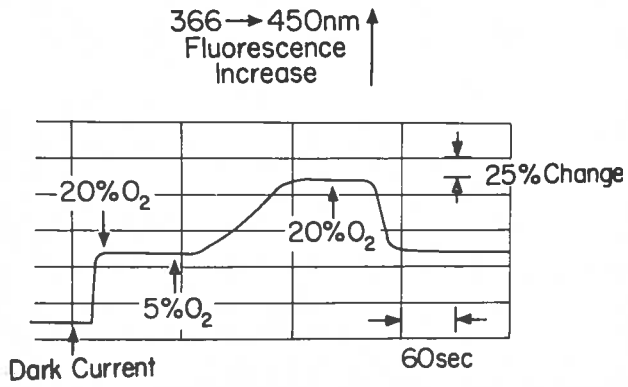

Fig. 5. Recording of the NAD/NADH conversion in a small area in a "sandwich" tumour as measured by "in vivo" micro-fluorometry. The fluorescence increases when the animal inspires 5% oxygen. Multiple sampling of such areas (not shown here) indicates that there are large differences in oxygenation of the cells in these tumours.

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