PATHOLOGY

SOME RECENT DEVELOPMENTS IN THE FIELD OF CANCER CAUSATION

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Cancer is a group of diseases and not a single disease entity. The group includes a wide variety of morbid states which differ from each other in causation, manifestation, and rate of progress. The term 'cause' has little meaning in relation to most forms of the disease: only rarely is it reasonable to regard a single factor as entirely causative. In most cases a number of factors are jointly responsible for the genesis of a particular neoplasm. It is important that those who investigate cancer causation should appreciate that this is the true nature of the problem. This is equally true whether they are concerned primarily with cancer in man or with cancer in any other animal species.

Identification of factors and mechanisms involved in cancer causation is basic to the development of ways of preventing neoplasia. Study of the natural progress of a cancerous disease, from the pre-neoplastic state to frank neoplasia, may provide a basis for early diagnosis of the disease which, in turn, may enable deaths from cancer to be prevented; but such study is not strictly relevant to prevention of the disease process itself. With few exceptions the discovery of causative factors and mechanisms has not so far led to rational and effective methods of treating cancer. However, it is reasonable to hope that research on cancer-inducing viruses and on certain immunological aspects of neoplastic disease will lead in this direction.

The present paper deals first with some aspects of the prevalence of cancer and of the detection of carcinogens, and then with certain recent developments in relation to the induction of cancers by chemical, physical, and viral agents.

The Occurrence of Cancers

All species of animal are liable to develop cancers: none is exempt. At first sight it may seem that cancers are seen more frequently in man than in other animals, and more frequently in 'advanced' than in 'backward' peoples. Closer analysis shows that these statements are true only in a strictly limited, and rather misleading, sense. Most types of cancer in most animal species occur late in life and the risk of their appearance increases with age. With the exception of man and a few species of domestic animals, the majority of animals die or are killed before they reach those ages at which cancer is most likely to appear. Death may be due to infectious disease, inadequate food supply, slaughter in the case of farm animals, or attack by natural enemies. The first two factors also operate amongst a population of humans living in a backward or primitive community. The average expectation of life is much shorter in such a community, and the proportion of the population which

belong to those age-groups in which cancer is most common is low. Theoretically, the most meaningful way to measure cancer incidence would be in terms of 'attack' rates at different ages. In practice, however, this is difficult because it is rarely possible to know the exact time at which a cancer starts, and the interval between the first appearance of the disease and death from it is widely variable. It is more usual, therefore, to measure cancer incidence in terms of the ratio of the number of deaths from a particular form of the disease to the number of individuals of the same age and sex alive, and therefore at risk of dying from it.

The accuracy of age-standardized death-rates for any particular form of cancer depends on, amongst other things, accurate diagnosis. Even for man in the most sophisticated countries low necropsy rates limit the accuracy of death-rate data. Their inaccuracy is increased by antiquated and misleading systems of death certification (thus non-fatal cancers tend to be lost sight of), and by disagreements between pathologists with regard to the correct classification of particular cancers. In primitive communities, where the size of the population at risk is unknown, where post-mortem examination is rare, and where the possibility of correct diagnosis is low, the extent and nature of the cancer problem may remain almost entirely unknown.

Theoretically it should be possible to collect useful and interesting data in respect of farm animals. In practice most of the available data are lost, and, as pointed out above, most animals are slaughtered whilst relatively young. Despite this an increasing incidence of cancers among intensively farmed chickens (Campbell and Appleby, 1966) is giving cause for concern. In Britain and several other countries pathology has, until recently, been the Cinderella of veterinary medicine, so that, even in the case of domestic pets, such as the dog and the cat, which man permits to live to ages at which cancer is common, very little useful information has been accumulated.

The most reliable information has been derived from the study of laboratory animals, especially rats and mice (Cotchin and Roe, 1967). In these species it has been possible, by inbreeding, to produce many genetically homogeneous strains which differ markedly both in natural death-rates from particular forms of cancer and in the way they respond to exposure to carcinogenic stimuli.

By the use of these laboratory tools, many important facts about cancer causation have been discovered. In particular, the importance of genetic constitution and of vertically transmitted viruses (i.e., viruses transmitted from parent to offspring at conception, via the placenta, or immediately after birth via the milk or other route) has been established (Bittner, 1942; Gross, 1951). Similarly it has been shown that many environmental factors influence cancer incidence. Thus exposure to a wide variety of chemical and physical agents may increase the risk of development of particular types of cancer (Clayson, 1962).

Despite the much greater difficulties in collecting accurate data in respect of man and non-laboratory animals, there is abundant evidence that some of the factors shown to predispose to cancer in laboratory animals have the same effect on man and other non-laboratory species. Thus exposure to tobacco smoke, industrial air-pollution, asbestos, nickel, radioactive materials, and

certain aromatic amines are known to predispose to cancer both in laboratory animals and man.

Perhaps the most significant inference to be drawn from several types of study, both epidemiological and experimental, is that exposure to environmental factors is likely to be the determining cause in many forms of cancer. The wide variation between species, and between humans in different areas of the world (Lea, 1967), in the spectrum of cancers with which they are afflicted points in this direction; so do observations on migrants whose risk of developing particular forms of cancer changes when they move to a new environment (Eastcott, 1956; Haenszel, 1961; Staszewski and Haenszel, 1965). Environmental factors are also implicated where studies over a period, too short to have genetic significance, show that, in a defined community, the age-standardized risk of developing a particular form of cancer changes (Case, 1956).

Detection of 'Carcinogens'

In theory man is concerned that he does not expose himself to cancer-inducing agents. In practice, a number of factors, including tradition, habit, self-indulgence, ignorance, and necessity, combine to lead or force him to ignore the risk. Thus the clear demonstration that tobacco smoke predisposes to cancer has had little effect on tobacco sales. Be this as it may, in most countries the legislature is concerned with the elimination of cancer-inducing agents from industry, from food, and from other parts of the human environment. Food manufacturers are, accordingly, required to study the effects of chemicals, which they propose to add to food, in laboratory animals. No substance which is shown in such studies to induce cancer may be used.

Fulfilment of this apparently simple principle, in practice, poses a number of problems. Firstly, cancer occurs spontaneously in all laboratory animals, so that exposure to an exogenous chemical may seem no more than to increase the incidence of, or hasten the appearance of, cancers which would have arisen, in any case, either 'spontaneously' or in response to exposure to factors in the background environment. A second difficulty is that in any single experiment it is not possible to distinguish between a carcinogenic and a co-carcinogenic effect. Thus, it is now fairly certain that a form of lymphoma in mice is invariably due to the action of a virus (Roe and Rowson, 1968). Before 1951, however, it was not known that a virus was implicated, so that the increased incidence of lymphoma in response to treatment of mice with oestrogens (Gardner, 1937; Lacassagne, 1937) was regarded as evidence that oestrogens are 'carcinogenic'. Today it would certainly be more appropriate to regard their activity, in this particular system, as co-carcinogenic.

Because of this difficulty of distinguishing between carcinogens and co-carcinogens, and because it is never possible to control experimental conditions absolutely, no agent should be regarded as 'carcinogenic' unless it has been shown to predispose to cancers in more than one experimental system.

Viruses which cause cancer when introduced into animals have a characteristic effect on cells grown in tissue culture (Stoker, 1965). This effect which is known as 'malignant transformation' and which is characterized by

changes in cellular morphology and behaviour is closely associated with the malignant behaviour of cells *in vivo*. Thus, on injection into animals, transformed cells tend to give rise to malignant tumours whereas normal, non-transformed, cells do not. There have been claims that exposure of tissue cultures to chemical carcinogens also results in malignant transformation (Berwald and Sachs, 1963; Sanders and Burford, 1967). However, these claims are not yet well established, and the hope that one may be able to distinguish between true carcinogens and co-carcinogens by a simple *in vitro* test cannot at present be realized.

Despite these uncertainties there exists a long list of chemical substances which may justifiably be regarded as true carcinogens. These substances give rise to cancers in a wide variety of species under a wide variety of conditions. Characteristically, the response to exposure to such agents is directly related to dose, and it is never possible to identify a threshold dose below which exposure is without carcinogenic effect. This point is illustrated in *Fig.* 5.

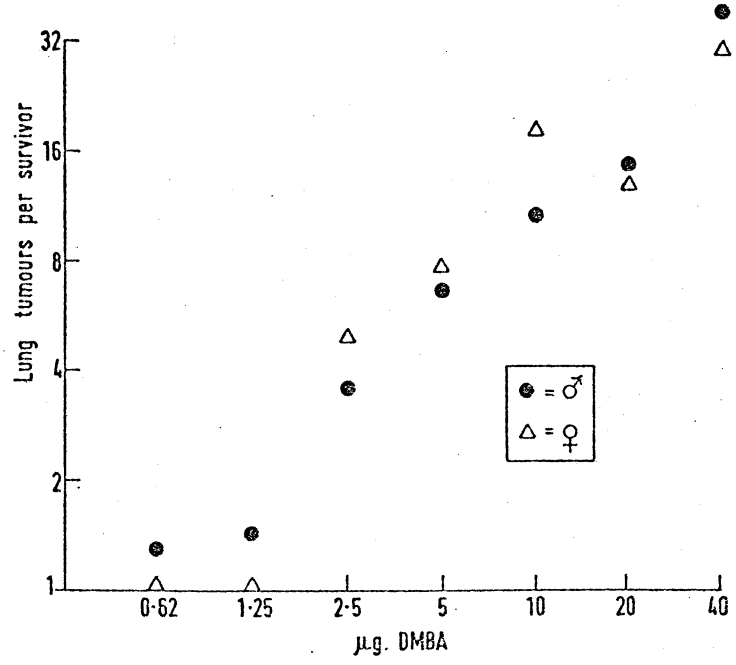

Fig. 5.—Groups of male and female mice of the BALB/c inbred strain were injected subcutaneously on the first day of life with various doses, measured in microgrammes, of the potent carcinogen 7,12-dimethylbenz(a)anthracene (DMBA). When they were killed some 12 months later the log of the average number of lung tumours per survivor in each group was directly related to the log of the dose of DMBA injected at birth. Even the smallest dose administered had a detectable effect in males.

In the laboratory it is rarely possible in one experiment to study the effect of a particular dose of a potential carcinogen on more than a few hundred animals. In most experiments, test groups consist of far fewer animals than this. It follows that, for comparable levels of exposure, it may be possible to detect the effects of a weak carcinogen in a population of thousands or millions of humans, but impossible to do so in a small group of laboratory

animals. It is customary, therefore, to require that materials should be tested in animals at much higher doses than those to which man is likely to be exposed. In this case difficulties arise if the ratio between the human exposure dose and the maximum dose that the test animal will tolerate is small. This is rarely the case with potential food additives (though ethyl alcohol is a notable exception), but often occurs in relation to drugs and to major constituents of the diet.

Cancer of the lung is the commonest fatal neoplasm in man in Britain. Most of these cancers are attributable to exposure to the inhalation of carcinogens. It is surprising, therefore, that so few institutions are equipped with facilities for the study of the long-term effects of inhalation exposure to suspect substances. There are, of course, many difficulties in the conduct of experiments involving the long-term exposure of laboratory animals to substances by inhalation (Roe, 1968). But the techniques have now been evolved, mainly by British scientists, for such experiments, and it is only the lack of facilities which delays useful experimentation of this kind.

Some New Developments in the Field of Chemical Carcinogenesis

In recent years there have been many significant discoveries in the field of chemical carcinogenesis. In this section, however, discussion is limited to just three: the nitrosamines, bracken, and asbestos.

The Nitrosamines.—The potency and special properties of this type of chemical carcinogen were first appreciated by Barnes and Magee in 1954. During the 14 years since Barnes and Magee's discovery, mainly as a result of extensive research by Druckrey and his colleagues (1963), a long list of carcinogenic nitrosamines has come to light. Many of the compounds in the list are so potent that a single exposure to them, by injection, by mouth, or by inhalation in the case of a volatile substance, is enough to induce cancer. However, the most interesting feature of this group of carcinogenic agents is the fact that many of them tend to induce cancers of particular organs or tissues irrespective of the route of exposure (Druckrey and others, 1967). This is unlike the carcinogenic polycyclic aromatic hydrocarbons which induce cancer preferentially at the sites in the body most exposed to them. Thus certain derivatives of nitrosourea give rise to tumours of the central nervous system irrespective of the route by which they are administered. Other nitrosamines induce cancers of the liver, kidney, oesophagus, stomach, peripheral nerves, etc. (*Plate XIII*). In some cases, the type of cancer induced varies with the species and age of animal exposed, and with the total dose and dose-schedule by which a particular nitrosamine is administered.

The full practical significance of the discovery of the carcinogenicity of this group of compounds has yet to be realized, but it is already apparent that hazard from nitrosamines may be associated with a wide variety of aspects of the human environment. Nitrosamines are widely used as solvents and as intermediaries in the chemical industry; not only this but the possibilities of their accidental formation in the course of industrial processes are legion. Nitrosamines are formed by the interaction of nitrites or oxides of nitrogen with secondary or tertiary amines at relatively low temperatures (e.g., 70° C.). The conditions necessary for their formation are therefore easily met, e.g., in the smoking of tobacco, during ordinary cooking processes, etc.

Since the early 1950's it has been the practice in Norway for deep-sea herring fishermen to use sodium nitrite as a preservative. Its addition delays or prevents the conversion of trimethylamine oxide to trimethylamine and other simple amines which is a feature of decay in fish. However, if nitrite is added after some decay has already taken place, and if the nitrited fish is then subjected to heat, dimethylnitrosamine may be formed (Ender and others, 1964). From 1957 onwards deaths from liver intoxication began to occur among sheep and fur animals (i.e., mink and fox) on farms in Norway (Koppang and Helgebostad, 1966). In 1961 liver disease reached epidemic proportions in Norway and a large epidemic occurred also in Britain. The cause of the latter was eventually traced to the incorporation in a brand of fortified mink food of a consignment of Norwegian herring meal. It is now established that significant amounts of dimethylnitrosamine may occur in nitrite-preserved herring meal (Ender and others, 1964)—and also that the mink is exceptionally sensitive to liver intoxication by this substance (Roe, Carter, and Percival, 1968). Other species, such as the rat, however, are less sensitive to the lethal hepatotoxicity of this substance, and in such species liver and kidney cancers may arise as a late sequel to exposure (Magee and Schoental, 1964).

The whole question of the safety of using nitrites for the preservation of meat and other foods will now have to be reviewed. At the same time there is a need to investigate the possibility that nitrosamines may occur naturally in the environment. In his account of Captain Cook's 1768–71 voyage to the East Indies, Hooker (1896) described the symptoms and signs of acute intoxication in men who had eaten products of the cycad species. This species is an important source of starch (e.g., sago), but can only be used safely as a food if prepared by a complicated process, which is known traditionally by people who normally eat cycad products. One of the toxins in unprocessed cycads is a glycoside, cycasin, which has been shown to have a nitrosamine-like structure. Recent research has shown that cycasin may induce cancers of the liver, kidney, and intestines when fed to laboratory animals (Laqueur, Mickelsen, Whiting, and Kurland, 1963). Cycasin, however, is not itself a carcinogen; its tumour-inducing activity depends on its conversion by bacteria in the gut to methylazoxymethanol (Spatz, McDaniel, and Laqueur, 1966). In animals kept under strictly germ-free conditions, this conversion is not effected, and administration of cycasin does not result in the induction of cancer.

Bracken.—It is possible that the carcinogenic constituent of some species of bracken is also a nitrosamine, though this is not yet established. As long ago as 1954, Evans and her colleagues (Evans, Evans, and Hughes, 1954) reported pyrexia, petechial haemorrhages, and ulceration of the gut in cattle following ingestion of *Pteridium aquilinium*. More recently haemangiomatous cancers of the urinary bladder have been described in cows that have grazed on bracken, and the implantation into the bladders of dogs or mice of pellets containing an extract of the urine of bracken-fed cows has been shown to induce haemangiomatous tumours (Georgiev, Vrigasov, Antonov, and Dimitrov, 1963). The most recent development is the report of adenomas and adenocarcinomas of the intestinal tract in rats fed a diet containing bracken (Evans and Mason, 1965).

Asbestos.—It has been recognized for several decades that exposure to asbestos dust may cause a fatal fibrotic condition of the lungs known as asbestosis. In 1935 came the first reports of an association between asbestosis and the risk of lung cancer (Lynch and Smith, 1935; Gloyne, 1935). During the 30 years since this observation, measures for the suppression of dust in industries which produce or use asbestos have been introduced. As these measures have become more effective, the proportion of asbestos workers who develop asbestosis has decreased, and the average ages at which they become incapacitated by it, and die because of it, have risen (Buchanan, 1965). *Pari passu* with this improvement in the situation with regard to asbestosis, there has been an increasing risk of lung cancer development in persons exposed to asbestos. The explanation is simple. The induction time for most lung cancers is well in excess of 25 years. Before the introduction of dust suppression, men and women who handled asbestos tended to die from asbestosis in their fifties before they had time to develop lung cancer. Partially effective dust suppression, by delaying death from asbestosis, has increased the time during which lung cancer may develop. The latest figures indicate that more than 50 per cent of persons who develop asbestosis will also develop lung cancer. At present it is not known whether the fibrosis and lung destruction which accompany asbestosis are necessary precursors of cancer, or whether asbestosis and lung cancer are related independently to exposure of asbestos. If the relationships are to any extent independent of each other, the situation with regard to the general population may be serious. The use of asbestos for purposes of lagging, fire-proofing, and for building and construction generally has snowballed since the Second World War, with the result that in developed countries the proportion of the population exposed occupationally to asbestos has grown to a significant level. Not only this, but asbestos dust has become a general air pollutant in industrial areas and latest reports indicate that asbestos bodies may be found in the lungs of more than 50 per cent of cadavers in such areas.

There are three main types of asbestos—crocidolite, amosite, and chrysotile. Epidemiological evidence suggests that the first two of these may be more dangerous than the last. However, experimental studies indicate that chrysotile is not free from carcinogenic risk.

Since 1960 it has been appreciated that another type of cancer is associated with exposure to asbestos, namely mesothelioma (i.e., cancer of the pleura, peritoneum, or pericardium (Harington, 1967)). Reports from South Africa implicate exposure to asbestos in virtually every case of this rare form of cancer. During the past 8 years, reappraisal of the case histories of patients with mesothelioma in various parts of Britain has, in nearly all cases, indicated a strong association with exposure to asbestos. Indeed, it is possible that there is no other cause of this type of cancer. Again, the most worrying feature of these discoveries is that, in patients who developed mesothelioma, the degree of exposure to asbestos is generally too slight to give rise to asbestosis. During the past year we ourselves have reported (Roe, Carter, Walters, and Harington, 1967) that when asbestos fibres are injected subcutaneously into mice they migrate specifically to submesothelial tissues, where they give rise to an inflammatory and proliferative response and sometimes to mesothelioma (*Plate XIV*). Migration was observed in relation to all

three main types of asbestos, but mesotheliomas were seen more frequently in mice injected with crocidolite or amosite.

It is interesting to note that post-mortem examination of animals living in the vicinity of asbestos mines in South Africa has also revealed some cases of mesothelioma. This is as great a cause for concern as there being examples of mesothelioma in persons living near, but not working in, asbestos factories in London, or in the wives of asbestos workers whose only contact with the dust is in relation to washing the clothes of their husbands.

Conclusions

Clearly in the space available here it is not possible to review more than a small fraction of the recent discoveries relevant to the causation of cancers in man and other animals. Nevertheless, perhaps enough has been said to conclude this article on a philosophical note.

Man has no right to assume that the natural environment is safe from a carcinogenic point of view. Some of the recently discovered carcinogens occur in nature and no doubt contribute to the causation of 'spontaneously' arising cancer. The prevention of cancer by preventing exposure to these natural carcinogens is as fully justified as screening man-made chemical agents for cancer-inducing activity. Man is nowadays introducing potential carcinogens into his own environment, and into that of all living creatures, at a rate that vastly exceeds the capacity for research into their possible dangers. Even when the carcinogenic potential of factors is recognized, it is not always possible to exclude them from the environment.

At present, therefore, there is no alternative but to regard the environment as carcinogenically hostile—full of known and unknown, natural and unnatural, carcinogens. The best we can hope to do, either as individuals or as a race, is to avoid exposure to high doses of the most potent of these agents. To this end it is essential that research effort should be directed away from the less important, and towards the more important, potential hazards.

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NERVOUS DISEASES OF ANIMALS

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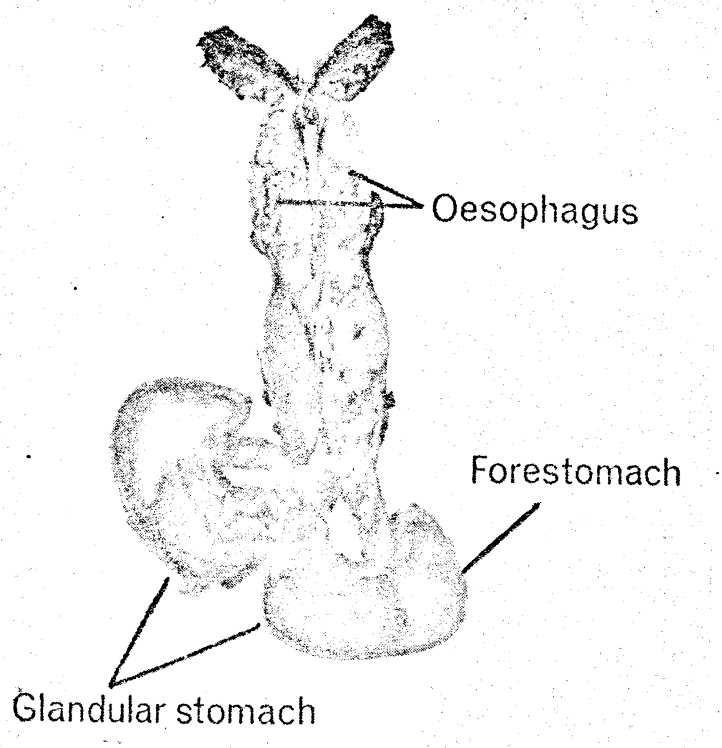
The purpose of this article is to give a short account of research into neurological diseases which has been carried out at the Cambridge Veterinary School over the past few years. It is not intended to be a thorough review of the literature.

SHEEP

Scrapie

Much of the recent literature on scrapie was reviewed by Pattison in this ANNUAL in 1966/7 (p. 167). Quite clearly most of the interest has been focused on the experimental disease as it occurs in laboratory animals and on the nature of the transmissible agent. An agent that has such unusual characteristics is bound to attract much scientific investigation. On the other hand, it is perhaps unwise to forget the natural disease as it occurs in the sheep, because it is still questionable whether the experimental condition in species other than the sheep and goat is in all respects the same as naturally occurring scrapie in sheep.

My own interest in scrapie is concerned with the strange neuropathological changes that occur in the brain of affected sheep. The significance of the vacuolated nerve-cell was subject to much debate, but its role has been finally accepted after many investigations (especially at the Moredun Institute). The vacuoles in the neurons often contain eosinophilic, round bodies and the progress of their formation can be followed. In the earlier stage of this degeneration the nucleus of the cell becomes eccentrically placed and the Nissl substance homogeneous. This is very similar to chromatolysis. Neurons



Multiple papillomas and squamous carcinomas of the oesophagus of a rat given 0·2 per cent nitrosopiperidine in the drinking water for 206 days.

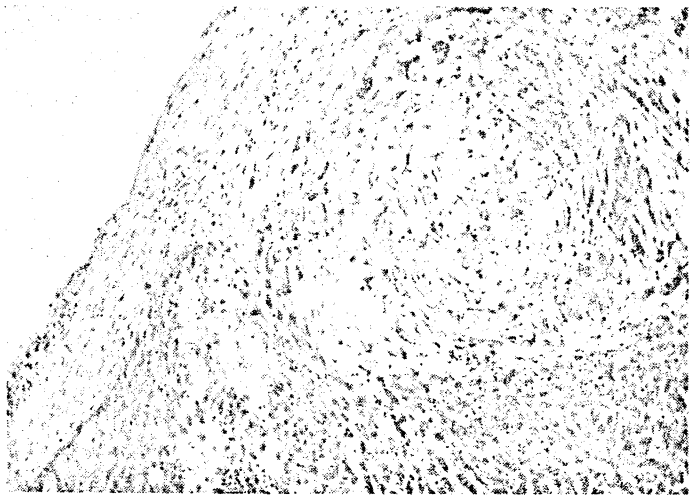


Fig. A.—Large deposit of fibres on the surface of the liver of a mouse previously injected subcutaneously with 60 mg. asbestos. Note the fibrous and proliferative reaction.

Fig. B.—Mesothelioma in a mouse treated as indicated in the legend to *Fig. A.*