THE EFFECTS OF ENVIRONMENT ON TUMOUR INCIDENCE AND INDUCTION

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Man is an animal. The difference between human and other animal species is, for the purpose of the present discussion, less important than the distinction between the animal in its natural environment and the laboratory-bred and laboratory-reared animal.

By 'tumour', I mean benign or malignant neoplasm. The appearances and characteristics of 'induced' neoplasms are, in general, more dependent on the features of the tissue cells from which they are derived than on the nature of the stimuli which seemingly 'induce' them. The kinds of tumours that arise in response to deliberately applied stimuli are, with few exceptions, indistinguishable from the kinds of tumours that arise 'spontaneously'. There are no grounds for regarding the aetiology of 'spontaneous' neoplasms as fundamentally different from the aetiology of 'induced' neoplasms. The most important difference between 'spontaneous' and 'induced' neoplasms does not lie in their histological appearances or other features but in the 'risk' that they will appear at all.

Measurement of risk of tumour development

Virtually any type of tissue cell may be the origin of a variety of types of neoplasm. It is convenient to group the many types of neoplasm that occur in any species into categories, each of which includes lesions that may differ from each other in time of appearance after exposure to a stimulus, growth rate, invasiveness, chromosome number, antigenic structure and many other features. In most tests with 'carcinogens' on laboratory animals, tumours are encountered in untreated control animals as well as in 'carcinogen'-treated animals. In both, the chance of finding tumours at necropsy increases with the age of the animal and, in the case of 'carcinogen'-treated animals, it also increases with the time since first exposure and with dose.

Response to a weak 'carcinogenic' stimulus may only be seen very late in the lives of treated animals – there being a long latent interval between exposure to the effective stimulus and the onset of neoplastic growth. In such cases many control animals may die from unrelated causes before the first tumours arise in treated animals.

The quantitative interpretation of the results of such experiments is difficult or impossible unless actuarial analysis is used to compare the risk of development of neoplasms of specified types at specified ages or times after first exposure to the test stimulus in treated animals and controls. We have described a suitable method, based on the use of a computer for doing this (Pike and Roe, 1963; Mantel, 1967).

It must be obvious that such a refined method of analysis is only justified in the case of properly conducted experiments that involve the randomization of comparable animals between test and control groups, daily (seven days per week) observation of animals to enable a close to 100% necropsy rate, strict criteria for killing sick animals during the experiment, the use of a standard and searching necropsy procedure, and strict criteria for classifying a lesion as a benign neoplasm

or malignant neoplasm of a particular type. In my experience, the most important part of post mortem examination is macroscopic observation of tissues and accurate recording of findings by highly trained staff. Histological examination of 'blind slices' of tissues taken by individuals ill-trained in necropsy procedure is useless and may be positively misleading.

Because most types of neoplasm are likely to occur late in life, it goes without saying that the use of disease-free animals is a great advantage, if not essential, in relation to testing for 'carcinogenicity'. To use diseased stock for carcinogenicity tests is about as sensible as to study human ageing in a backward community where the average age at death is 33.

Distinction between 'carcinogenicity' and 'co-carcinogenicity'

A 'co-carcinogen' is an agent that increases the risk of tumour development in response to a 'carcinogen'. But what is a 'carcinogen'? In this dissertation, so far, I have been careful to encase 'carcinogen' and 'carcinogenicity' in single inverted commas. I would accept that a 'carcinogen' is an agent that 'induces' cancer without the mediation of another agent. But this begs the question because we do not know what 'induce' means, nor can we be sure that, in any laboratory animal or other model system, 'another agent' is not present. In other words, if the only evidence that exists refers to the 'induction' of neoplasms in a single experiment in a single species, it is impossible to distinguish between a 'carcinogen' and a 'co-carcinogen'.

On a basis of probability rather than proof, I am prepared to regard a substance which has been shown to increase the risk of development of a variety of neoplasms in a variety of species and under a variety of experimental conditions as either a 'carcinogen' itself, or as the 'precursor of a carcinogen'. Hereafter this is the only sense in which the term is used. These arguments have been developed more fully elsewhere (Roe, 1968, 1969).

Environmental *versus* genetic 'causes' of cancer

The medical student is taught that disease is either genetic or acquired as a result of exposure to environmental factors. This is an oversimplification in the sense that factors of both kinds frequently conspire. Nevertheless, we may look at the whole phenomenon of neoplasia in man and other animals and ask the general question, how much of this is genetic, and how much is environmental, in origin? It is immediately apparent that the division is, or may be, quite different for the laboratory animal than for the animal (including man) in the wild. Available evidence suggests that neoplasms may occur in high incidence in wild rats and mice (McCoy, 1909; Woolley and Wherry, 1911; Andervont and Dunn, 1962; see Roe, 1965, for review).

All species have a reproductive capacity in excess of that needed to maintain the species. The status quo between species is maintained by a complex of negative feed-back systems. Susceptibility to various forms of disease, including virus-induced neoplasia, is a factor in this complex. Insofar as neoplasia occurs late in life, it may benefit the species by killing off reproductively-effete individuals. But genetically-determined high susceptibility to the development of lethal cancer early in life would, in the wild, be bred out as a result of natural selection. So would high susceptibility attributable to the vertical (parent to offspring) transmission of cancer inducing (oncogenic) viruses.

The situation in the laboratory is quite different because of enforced inbreeding and the development of high cancer strains.

We know that vertically-transmitted viruses, as well as genetic factors, are often involved in the aetiology of neoplasms in high cancer strains. Examples in the case of mice are the Bittner virus (milk-factor) in high mammary-tumour strains, and Gross passage A virus and a variety of other viruses in high-lymphoma strains.

In other words, some inbred strains of laboratory animal have been selected (by man) because of a high tumour incidence which in the wild would have been disadvantageous and scheduled for extinction by natural selection. But even when selection for inbreeding in the laboratory is based on favourable rather than unfavourable attributes, inbreeding itself and high density housing is bound to favour the build up of viruses, some of which may be oncogenic. During the past 15-20 years, an increasing interest in tumour viruses and *in vitro* cell culture methods, and an increasing use of techniques involving the introduction of biological materials into newborn animals, may well have led to the untreated laboratory animal becoming even less like its wild counterpart. Such considerations are immensely important in any consideration of the role of environmental factors in relation to the risk of tumour development.

In Table 1 are listed examples of vertically-transmitted 'oncogenic' viruses in laboratory animals, and in Table 2, four examples of tumours that occur in high incidence in some strains of laboratory animals, but for which no virus aetiology has yet been demonstrated.

The relative roles of genetic and environmental factors in the aetiology of neoplasms

Clearly it is impossible to make any meaningful statement or guess with regard to the relative importance of genetic and environmental factors in relation to the causation of tumours in laboratory animals. The answer would be different for every strain and for every laboratory. For the reasons given above, genetic factors

TABLE 1

Some vertically-transmissible and neonatally transmissible 'oncogenic' viruses of mice

Virus	*Type(s) of neoplasm*	*Reference*
Bittner virus ('milk factor', 'mammary tumour agent')	Mammary gland neoplasms	Bittner, 1942; Bern and Nandi, 1961
Lymphoma (leukaemia) viruses		
(a) Gross passage A type	Lymphatic leukaemia	Gross, 1951
(b) Moloney type	,, ,,	
(c) Friend type	Malignant lymphoma with enlargement of spleen but not lymph nodes	See Rowson, 1966, and Salaman, 1967 for reviews
(d) Rauscher type	Mixed features of Moloney and Friend types	
Mouse sarcoma virus (Harvey and Moloney strains)	Connective tissue sarcoma	Harvey, 1964; Moloney, 1965, 1966
Polyoma virus	Wide variety of neoplasms of different organs and tissues	Gross, 1953; Stewart, 1953; Stewart et al, 1958; Huebner et al, 1962; Roe (unpublished)

TABLE 2

Common neoplasms of mice and rats for which virus aetiology not yet demonstrated

Mice	Pulmonary adenoma and adenocarcinoma
	Liver-cell tumours (hepatomas) (e.g. in C3H and CBA strains)
Rats	Mammary tumours
	Lymphoma (N.B. Evidence of virus aetiology in some cases)

and vertically-transmitted viruses are likely to play a more important role than in animals in the wild.

In man (Fig. 1), differences in cancer incidence between different countries favour the conclusion that most cancer is environmentally-determined. Death-rate studies on people who migrate from an area where the risk of development of a particular form of cancer is low (or high) to an area where it is high (or low) strongly suggest that environmental factors are, in general, more important determinants than genetic factors (see Tables 3 and 4).

The environment of the laboratory animal

Many factors which influence the risk of cancer development in man are already known (Table 5). Most of them, such as the industrial causes, sunlight and smoking, do not apply to laboratory animals, but some, such as air pollution and poor personal hygiene, may apply with equal force. In addition, the laboratory environment entails some special hazards of its own, such as exposure to creosote which increases the risk of the 'spontaneous' development of skin and lung tumours in mice and the response of mice to the 'tumour-promoting' effects of croton oil (Rous, 1956; Boutwell et al., 1957; Shubik et al., 1957; Boutwell and Bosch, 1958; Roe et al., 1958; see Roe, 1965, for review). Another example is the increased risk of development of liver-cell tumours in guinea pigs at a time when aflatoxin-containing ground nut meal was used as an ingredient in their diet (Schoental,

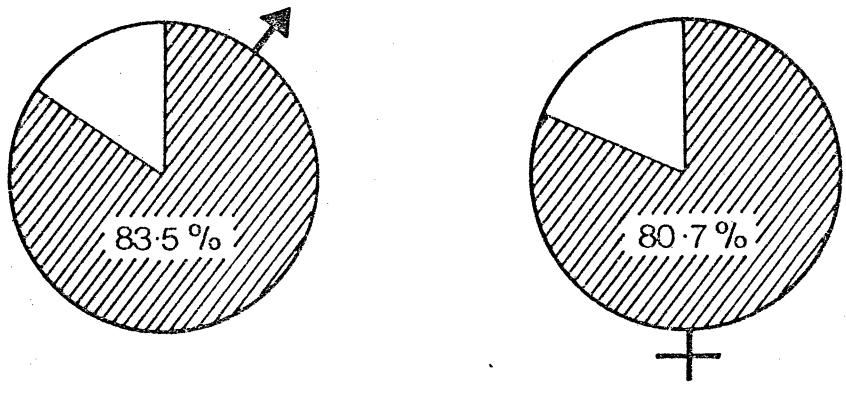

Figure 1. Minimum proportion of deaths from cancers that are of environmental origin.

A. If mortality data from different countries are accurate and comparable.

B. If differences in death rates between countries are wholly attributable to environmental differences.

(Calculations based on age-adjusted death rates for neoplasms of selected sites (accounting for 6/7 of all cancer deaths) for 1960-61 in 24 countries – Segi and Kurihara, 1964.)

TABLE 3

Changes in cancer risk for Japanese Men who migrate to U.S.A.

Standardized mortality ratios (Japan = 100) for:	*Japanese-born*	*Japanese-born migrants to U.S.A.*	*U.S.A. born Japanese*	*U.S.A. white*
Stomach	100	72	38	17
Colon	100	374	288	489
Lung	100	306	166	316
Leukaemia	100	314	–	265

(From Haenszel and Kurihara, 1968)

TABLE 4

Changes in cancer risk for Japanese Women who migrate to U.S.A.

Standardized mortality ratios (Japan = 100) for:	*Japanese-born*	*Japanese-born migrants to U.S.A.*	*U.S.A. born Japanese*	*U.S.A. white*
Stomach	100	55	48	18
Colon	100	218	219	483
Breast	100	166	136	591
Ovary	100	337	–	535
Cervix uteri	100	52	33	48

(From Haenszel and Kurihara, 1968)

1961). The possibility that natural 'carcinogens' derived from moulds or other microbes may have been present in laboratory animal diets, clouds the whole picture of research on liver tumour induction in relation to nutritional factors, and in some cases there is a need to repeat old experiments because of uncertainties of this kind.

Effects of specific environmental factors on risk of tumour development

The whole of this and many other papers could have been devoted to cataloguing environmental factors that influence the risk of the development of tumours of different animals. I have deliberately eschewed such cataloguing in favour of a more philosophical approach. Table 6 is included as the laboratory animal counterpart of Table 5.

Extrapolation from experiments on laboratory animals to man

I hope I have said enough earlier and in Table 6 to enjoin extreme caution in concluding that a test agent is 'carcinogenic' on the basis of the result of a single animal test.

An important point of difference between the kind of data derived from epidemiological studies on man and that from animal experiments needs to be stressed. Most human data relate to deaths from malignant neoplasms. Many data from laboratory animals relate to neoplasms, some of them non-malignant, discovered at routine necropsy. Cancer in man would seem a very different disease if

TABLE 5

Some factors that increase the risk of cancer in man

OCCUPATIONAL	
(a) *Lung cancer*	
Arsenic	(See Bidstrup, 1967, for review)
Nickel refining (old process)	
Bichromate manufacture	
Asbestos dust	
Coal gas manufacture (old process)	
Isopropyl alcohol manufacture (old process)	
Uranium mines	
(b) *Mesothelioma*	
Asbestos dust	(See Harington, 1967, for review)
(c) *Skin cancer*	
Arsenic	(See Ingram and Comaish, 1967, for review)
Coal tar and pitch	
Mineral oils	
Ionizing radiation (X-ray martyrs)	
Sunlight (out-door occupations)	
(d) *Urinary bladder*	
Aromatic amines including β-naphthylamine, benzidine, *o*-tolidine, *o*-dianisidine (chemical industry, rubber industry, hospital laboratories, rat catchers)	(See Scott, 1962, for review)
(e) *Leukaemia*	
Ionizing radiation (radiologists)	(See Mayneord, 1967, for review)
PHARMACEUTICAL AND IATROGENIC	
Phenylbutazone – leukaemia	(e.g. Woodliffe and Dougan, 1964)
2-Chlornaphazin – bladder cancer	(Thiede et al, 1964)
Radioactive iodine – thyroid cancer	(Doniach, 1950,
Goitrogenic drugs – thyroid cancer	Lindsay et al, 1966)
Diagnostic irradiation – leukaemia in children of women X-rayed during pregnancy	(Stewart, 1967)
Implanted plastic – sarcoma	(Carter and Roe, 1969)
FOOD (including constituents, additives and contaminants)	
None proved	
Suspected	
3-4 benzopyrene and other polycyclic aromatic hydrocarbons in smoked foods – stomach cancer	
Nitrosamines in alcoholic beverages – oesophageal cancer and possibly cancer of liver	(McGlashan et al, 1968)
Aflatoxin, toxin from *Penicillium islandicum* – cancer of the liver and possibly other sites	(Roe and Lancaster, 1964)
Cycasin – for general reference see	(Roe, 1967)
GENERAL	
Atmospheric pollutions – lung	(Waller, 1967)
Tobacco smoke – lung, larynx, oral cavity and possibly urinary bladder	
POOR PERSONAL HYGIENE	
Penis	(Griffiths, 1967)
Uterine cervix	(Elliott, 1964)

we counted the latent carcinomas of the prostate described by Franks (1954), and yet this may be more or less what the experimentalist is doing when he counts ademomatous tumours in the lungs, or benign parenchymal-cell tumours in the livers of mice.

As indicated in Table 6, we have virtually no information on the influence of advanced age on susceptibility to 'carcinogens', because the experiment has not been done. Today, the availability of disease-free animals makes such studies possible. The results might be especially relevant to man in whom cancer is predominantly a disease of the middle and older age groups.

TABLE 6

Some factors which increase the risk of tumour development in laboratory animals

Vertically or neonatally transmitted viruses

See Table 1 for examples

Genetic constitution

e.g. Strain differences in susceptibility to 'spontaneous' pulmonary tumours in mice (see Heston, 1948)

Hormonal status, temperature, exercise, number of animals per cage

e.g. (i) Oestrogen-treatment enhances risk of lymphoma in mice (Lacassagne, 1937; Gardner, 1939).

(ii) Lemonde (1964) reported that pregnancy delayed occurrence of malignant lymphoma in AK strain mice.

(iii) Sex hormones influence response to 'hepatocarcinogens' (see Morris, 1970, for review).

(iv) Finkel and Scribner (1955) found risk of spontaneous tumour development higher in mice housed in plastic cages than in metal cages. The lower thermal conductivity of plastic may be responsible.

(v) Physical exercise reduces risk of spontaneous development of mammary tumours in mice (Mühlbock, 1951).

(vi) Risk of development of mammary tumours and neoplasms of reticuloendothelial system increases inversely with number of mice per cage (Andervont, 1944; Mühlbock, 1951).

Immunological status

(i) Neonatal thymectomy significantly increased responsiveness of mice to repeated application of 'carcinogen' mouse skin (Grant et al, 1966).

(ii) Anti lymphocyte serum appears to increase risk of development of various neoplasms in 'carcinogen'-treated mice (Grant and Roe, 1969b).

(iii) Under certain circumstances interference with the immunological status of an animal in relation to a virus may markedly alter its risk of developing cancer in response to a chemical carcinogen (see Roe and Rowson, 1968, for review).

Gut flora and microbiological status

(i) The carcinogenic activity of cycasin is seemingly dependent on its conversion by gut flora to the proximate carcinogen methyl azomethanol (Laqueur and Matsumoto, 1966).

(ii) Germ-free status reduces risk of development of liver cell tumours in untreated and in 'carcinogen'-treated C3H male mice (Grant and Roe, 1969a).

Nutritional status

(i) Caloric restriction reduces risk of 'spontaneous' development of tumours and response to carcinogens (Tannenbaum and Silverstone, 1953).

(ii) High fat or high protein diet increase development of neoplasms of some types but not of others (Tannenbaum and Silverstone, 1953; Elson, 1958; Walters and Roe, 1964).

(iii) In general diets deficient in vitamins inhibit tumour development but riboflavin-deficiency enhances liver tumour induction by azo dyes (Tannenbaum & Silverstone, 1953).

Miscellaneous factors

(i) Inadvertent exposure to creosote (see text).

(ii) Inadvertent exposure to aflatoxin (see text).

(iii) The possibility that advanced age *per se* alters responsiveness to background environmental 'carcinogens' or to deliberately administered carcinogens has not been adequately tested.

Deliberate exposure to carcinogens and co-carcinogens

(i) All agents shown in Table 5 except arsenic and wood dust.

(ii) A wide variety of agents never shown to increase risk of cancer development in man (see Clayson, 1962).

Conclusions and Summary

1. In man environmental factors seem to play a more important role than genetic factors in determining the risk of development of neoplasia.
2. In the artificial environment of the laboratory, genetic factors, vertically-transmitted viruses, and possibly 'laboratory' viruses (especially prevalent or virulent because of *in vitro* cultivation or neonatal injection) are apt to play a far more important role.
3. The laboratory assessment of 'carcinogenicity' should be based on a comparison of risk of development of specified neoplasms in test and control animals calculated, if necessary, by an actuarial method and by the use of a standardized necropsy procedure.
4. In certain circumstances, viruses, hormones, changes in immunological status, nutrition, temperature, stress in various forms and specific toxins in the diet have each been shown to influence both the incidence of spontaneous neoplasms in untreated control animals and the response of test animals to putative carcinogens. Background interference by such factors would normally be detected in a properly designed test for 'carcinogenicity'. Nevertheless, the possibility that such factors play a part – possibly a dominant part – in the mechanism by which a test agent increases the risk of development of a particular type of tumour, must be taken into account in the *interpretation* of a 'carcinogenicity' test. There are many recorded examples where the risk of development of tumours in response to an otherwise 'latent' virus may be enhanced non-specifically because of interference with hormonal or immunological status.
5. It follows that it is difficult – or impossible in the case of a single experiment – to distinguish between a 'carcinogenic' and a 'co-carcinogenic' effect.

Discussion

Professor MAISIN: Why do you think that germ-free animals are more resistant to the induction of cancer by chemical agents than animals maintained under ordinary conditions?

Dr ROE: At the present time I do not know the mechanism, but there are several possible explanations.

It is possible that, as in the case of cycasin, enzymes produced by micro-organisms in the gut lumen are necessary for the conversion of 7,12-dimethylbenz(a)anthracene (DMBA), the polycyclic hydrocarbon used in our study, to the agent which predisposed to liver tumour formation. For this to be true, it must be assumed that the DMBA found its way from a subcutaneous injection site into the lumen of the gut, probably via the bile. This in turn would suggest that the metabolite of DMBA that increases the risk of liver tumour formation may be different from that or those responsible for the increased risk of tumour formation in other tissues.

A second possibility is that a virus is involved in liver tumour formation and this is absent from or only rarely present in germ-free mice.

Thirdly, it could be argued that in the germ-free state the immunological defence systems do not have to combat microbial invasion and are therefore able to devote more effective attention to the destruction of cells transformed into cancerous cells by chemical agents.

Fourthly, although our germ-free and 'conventionally'-maintained mice were fed on the same diet, their nutritional status throughout the experiment would have differed as a result of the enzymic activity of the gut flora in conventional animals. I suspect that this difference in nutritional status is the crucial one.

Professor MAISIN: What do you think is the relationship between chemically induced cancer and latent virus infection? Could it be that the carcinogen activates a latent virus which is the true carcinogen?

Dr ROE: That is certainly one possibility, but there are many others. Dr K. E. K. Rowson and I recently reviewed this subject (Roe and Rowson, 1968). It is theoretically possible for the reverse to be true insofar as several viruses have been shown to diminish the capacity of animals to mount immunological responses.

Mr HOBBS: Do you think that the consumption of fresh bracken may be partly to blame for the high incidence of stomach cancer in Japan, since bracken has been shown to contain carcinogenic factors (Evans and Mason, 1965)?

Dr ROE: It is certainly a possibility that needs to be considered along with others such as hazard from the consumption of raw fish and of smoked fish. The Japanese eat many things that seem strange by western standards.

Mr HOBBS: Do you think that the milk or meat from cattle that graze on bracken may constitute a hazard for man?

Dr ROE: The urine from cows that graze on bracken has been shown to induce neoplasms of the bladder in mice (Pamukca et al., 1966). I know of no evidence that the milk or meat from bracken-fed cattle contains factors that favour the development of cancers. This may be an important field for research.

(*Note added in proof:* H. Leach (in "The chemical isolation of a toxic component of bracken", M.Sc. Thesis, University of Wales, Bangor, 1970) has reported the presence of a carcinogenic principle in 'Warabi', a preparation of bracken consumed by Japanese.)

REFERENCES

ANDERVONT, H. B. (1944). Influence on environment on mammary cancer in mice. J. Natl. Cancer Inst., 4, p. 579.

ANDERVONT, H. B. and DUNN, T. B. (1962). Occurrence of tumors in wild house mice. J. Natl. Cancer Inst., 28, p. 1153.

BERN, H. A. and NANDI, S. (1961). Recent studies of the hormonal influence in mouse mammary tumorigenesis. Progr. Exptl. Tumor Res., 2, p. 90.

BIDSTRUP, P. L. (1967). Bronchi and lungs – industrial factors. Chapter in The Prevention of Cancer, Ed. by Raven, R. W. and Roe, F. J. C. Butterworths, London, pp. 193-203.

BITTNER, J. J. (1942). Possible relationship of the estrogenic hormones, genetic susceptibility, and milk influence in the production of mammary cancer in mice. Cancer Res., 2, p. 710.

BOUTWELL, R. K. and BOSCH, D. (1958). The carcinogenicity of cresole oil: its role in the induction of skin tumours in mice. Cancer Res., 18, p. 1171.

BOUTWELL, R. K., BOSCH, D. and RUSCH, H. P. (1957). On the role of croton oil in tumour formation. Cancer Res., 17, p. 71.

CARTER, R. L. and ROE, F. J. C. (1969). Induction of sarcomas in rats by solid and fragmented polyethylene: experimental observations and clinical implications. Brit. J. Cancer, 23, p. 401.

CLAYSON, D. B. (1962). Chemical carcinogenesis. Churchill, London.

DONIACH, I. (1950). The effect of radioactive iodine alone and in combination with methylthiouracil and acetylaminofluorene upon tumour production in the rats thyroid gland. Brit. J. Cancer, 4, p. 223.

ELLIOT, R. I. K. (1964). On the prevention of carcinoma of the cervix. Lancet, 1, p. 231.

ELSON, L. A. (1958). Some dynamic aspects of chemical carcinogenesis. Brit. Med. Bull., 14, p. 161.

EVANS, I. A. and MASON, E. (1965). Carcinogenic activity with bracken. Nature (Lond.), 208, p. 913.

FINKEL, M. P. and SCRIBNER, G. M. (1955). Mouse cages and spontaneous tumours. Brit. J. Cancer, 9, p. 464.

FRANKS, L. M. (1954). Latent carcinoma. Annals R. Coll. Surg. Engl., 15, p. 236.

GARDNER, W. U. (1939). Further studies on the effects of estrogen on bone formation in mice. In Proc. from Conf. on Metabolic Aspects of Convalescence, 12th Meeting, Ed. by Reifenstein, E. J., Jr. Occasional Publ. Am. Assoc. Adv. Sci., 4, p. 67.

GRANT, G. A. and ROE, F. J. C. (1969a). Influence of germ-free status on hepatoma induction by 7,12-Dimethylbenz(a)anthracene in C_3H mice. Nature, 222, p. 1282.

GRANT, G. A. and ROE, F. J. C. (1969b). Effect of germ-free status and antilymphocyte serum on induction of various tumours in mice by a chemical carcinogen given at birth. Nature, 223, p. 1060.

GRANT, G. A., ROE, F. J. C. and PIKE, M. C. (1966). Effect of neonatal thymectomy on the induction of papillomata and carcinomata by 3,4-Benzopyrene in mice. Nature, 210, p. 603.

GRIFFITHS, J. D. (1967). Carcinoma of the penis. Chapter in The Prevention of Cancer, Ed. by Raven, R. W. and Roe, F. J. C. Butterworths, London, pp. 262-264.

GROSS, L. (1951). 'Spontaneous' leukaemia developing in C_3H mice following inoculation, in infancy, with AK-leukaemic extracts, or AK-embryos. Proc. Soc. Exp. Biol. Med. N.Y., 76, p. 27.

GROSS, L. (1953). A filtrable agent, recovered from AK-leukaemic extracts, causing salivary gland carcinomas in C_3H mice. Proc. Soc. Exp. Biol. Med. N.Y., 83, p. 414.

HAENSZEL, W. and KURIHARA, M. (1968). Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States. J. Natl. Cancer Inst., 40, p. 43.

HARINGTON, J. S. (1967). Mesothelioma. Chapter in The Prevention of Cancer, Ed. by Raven, R. W. and Roe, F. J. C. Butterworths, London, pp. 207-211.

HARVEY, J. J. (1964). An unidentified virus which causes the rapid production of tumours in mice. Nature, 204, p. 1104.

HESTON, W. E. (1948). Genetics of cancer. Adv. Genet., 2, p. 99.

HUEBNER, R. J., ROWE, W. P., HARTLEY, H. W. and LANE, W. T. (1962). Ciba Foundation Symposium on Tumour Viruses of Murine Origin. Churchill, London, p. 314.

INGRAM, J. T. and COMAISH, S. (1967). Occupational cancer of the skin. Chapter in The Prevention of Cancer, Ed. by Raven, R. W. and Roe, F. J. C. Butterworths, London, pp. 216-225.

LACASSAGNE, A. (1937). Sarcomes lymphoides apparus chez des souris longement traitées par des hormones oestrogènes. Compt. Rend. Soc. Biol., 126, p. 193.

LAQUEUR, G. L. and MATSUMOTO, H. (1966). Neoplasms in female fisher rats following intraperitoneal injection of methylazoxymethanol. J. Natl. Cancer Inst., 37, p. 217.

LEMONDE, P. (1964). Effect of pregnancy on spontaneous leukaemia in mice. Brit. J. Cancer, 18, p. 317.

LINDSAY, S., NICHOLAS, C. W. and CHAIKOFF, I. L. (1966). Induction of benign and malignant thyroid neoplasms in the rat. Induction of thyroid neoplasms by injection of 131-I with or without the feeding of diets containing propylthiouracil and/or desiccated thyroid. Arch. Path., 81, p. 308.

MANTEL, N. (1967). Ranking procedures for arbitrarily restricted observations. Biometrics, 23, p. 65.

MAYNEORD, W. V. (1967). Cancer hazards in diagnostic and therapeutic irradiation. Chapter in The Prevention of Cancer, Ed. by Raven, R. W. and Roe, F. J. C. Butterworths, London, pp. 53-59.

McCOY, G. W. (1909). A preliminary report of tumours found in wild rats. J. Med. Res., 21, p. 285.

McGLASHAN, N. D., WALTERS, C. L. and McLEAN, A. E. M. (1968). Nitrosamines in African alcoholic spirits and oesophageal cancer. Lancet, 2, p. 1017.

MOLONEY, J. B. (1965). Joint Zoological Society/WHO Symposium on Comparative Medicine. Academic Press, London.

MOLONEY, J. B. (1966). A virus-induced rhabdomyosarcoma of mice. Nat. Cancer Inst., Monograph, 22, p. 139.

MORRIS, H. P. (1970). Influence of sex and hormones in the development of liver tumours. Chapter in Metabolic Aspects of Food Safety, Ed. by Roe, F. J. C., Blackwell Scientific Publications, Oxford (in press).

MÜHLBOCK, O. (1951). Influence on environment on the incidence of mammary tumours in mice. Acta Un. Int. Cancer, 7, p. 351.

PAMUKCA, A. N., OLSON, C. and PRICE, J. M. (1966). Assay of fractions of bovine urine for carcinogenic activity after feeding bracken fern. Cancer Res., 26, p. 1745.

PIKE, M. C. and ROE, F. J. C. (1963). An actuarial method of analysis of experiment in two-stage carcinogenesis. Brit. J. Cancer, 17, p. 605.

ROE, F. J. C. (1965). Spontaneous tumours in rats and mice. Fd. Cosmet. Toxicol., 3, p. 707.

ROE, F. J. C. (1967). Food. Chapter in The Prevention of Cancer, Ed. by Raven, R. W. and Roe, F. J. C. Butterworths, London, pp. 19-31.

ROE, F. J. C. (1968). Carcinogenesis and Sanity. Fd. Cosmet. Toxicol., 6, p. 485.

ROE, F. J. C. (1969). Mechanisms of carcinogenesis. Chapter in The Biological Bases of Medicine, Ed. by Bittar, E. E. and Bittar, N., Academic Press, London and New York. Vol. 5, pp. 487-504.

ROE, F. J. C., BOSCH, D. and BOUTWELL, R. K. (1958). The carcinogenicity of creosote oil: the induction of lung tumours in mice. Cancer Res., 18, p. 1176.

ROE, F. J. C. and LANCASTER, M. C. (1964). Natural, metallic and other substances, as carcinogens. Brit. Med. Bull., 20, p. 127.

ROE, F. J. C. and ROWSON, K. E. K. (1968). The induction of cancer by combinations of viruses and other agents. Int. Rev. Exp. Path., 6, p. 181.

ROUS, P. (1956). Influence of hereditary malformations on carcinogeneses in 'crew' mice and deer mice of hairless strains. Proc. Am. Ass. Cancer Res., 2, p. 143.

ROWSON, K. E. K. (1966). Viruses and cancer. Chapter in The Biology of Cancer, Ed. by Ambrose, E. J. and Roe, F. J. C. Van Nostrand; London, pp. 124-155.

SALAMAN, M. H. (1967). Virus-induced lymphoma in mice. Chapter in Pathology of Laboratory Rats and Mice, Ed. by Cotchin, E. and Roe, F. J. C. Blackwell Scientific Publications, Oxford, p. 613.

SCHOENTAL, R. (1961). Liver changes and primary liver tumours in rats given toxic guinea pig diet (M.R.C. Diet 18). Brit. J. Cancer, 15, p. 812.

SCOTT, T. S. (1962). Carcinogenic and chronic toxic hazards of aromatic amines. Elsevier, Amsterdam.

SEGI, M. and KURIHARA, M. (1964). Cancer mortality for selected sites in 24 countries, No. 3 (1960-1961), Dept. of Pub. Hlth., Tohoku Univ. School of Med., Sendai, Japan.

SHUBIK, P. SPENCER, K. and DELLA PORTA, G. (1957). The occurrence of skin tumours in untreated albino mice from dealer's stock. J. Natl. Cancer Inst., 19, p. 33.

STEWART, A. (1967). Epidemiology of childhood cancers. Chapter in The Prevention of Cancer, Ed. by Raven, R. W. and Roe, F. J. C. Butterworths, London, pp. 352-358.

STEWART, S. E. (1953). Leukaemia in mice produced by filtrable agent present in AKR leukaemic tissues with notes on a sarcoma produced by the same agent. Anat. Rec., 117, p. 532.

STEWART, S. E., EDDY, B. E. and BORGESE, N. (1958). Neoplasms in mice inoculated with a tumour agent carried in tissue culture. J. Natl. Cancer Inst., 20, p. 1223.

TANNENBAUM, A. and SILVERSTONE, H. (1953). Nutrition in relation to cancer. Adv. Cancer Res., 1, p. 451.

THIEDE, T., CHIEVITZ, E. and CHRISTENSEN, B. C. (1964). Chlornaphazine as a bladder carcinogen. Acta Med. Scand., 175, p. 721.

WALLER, R. E. (1967). Bronchi and lungs – air pollution. Chapter in The Prevention of Cancer, Ed. by Raven, R. W. and Roe, F. J. C. Butterworths, London, pp. 181-186.

WALTERS, M. A. and ROE, F. J. C. (1964). The effect of dietary casein on the induction of lung tumours by the injection of 9,10-Dimethyl-1,2-Benzanthracene (DMBA) into newborn mice. Brit. J. Cancer, 18, p. 312.

WOODLIFFE, H. J. and DOUGAN, L. (1964). Acute leukaemia associated with phenylbutazone treatment. Brit. Med. J., 1, p. 744.

WOOLLEY, P. J. and WHERRY, W. B. (1911). Notes on 22 spontaneous tumours of the rat. J. Cancer Res., 2, p. 39.