CANCER HAZARDS IN OUR ENVIRONMENT: THE USE OF ANIMAL EXPERIMENTS IN THEIR DETECTION AND EVALUATION

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Introduction

Cancer, like other diseases, could be eradicated either by prevention or by cure. We have just passed through a decade during which most of the available resources have been devoted to the search for cancer cures. Valuable advances in palliation have been made, but fundamental cure, other than by radical surgery, has not yet been achieved. During the same decade relatively scant attention has been given to cancer prevention, but there are signs that this is to become the most important approach in the future.

Because of this change of emphasis it is necessary to consider to what extent the results of tests in animals can and should be applied to the human cancer problem. The inevitability of the fate of the patient with inoperable cancer makes it justifiable to try out any drug which offers the faintest hope of benefit, so that it has not been necessary to examine too closely the validity of applying the results of animal tests to man. But when we turn to the growing field of cancer prevention, the question of the validity of this application becomes far more important.

Theoretically, cancer may be prevented either by removing from man's external environment (the term is used in the widest sense and includes foods, food additives, food contaminants, air pollutants, ionising and other forms of radiation, heat, cold, humidity, infective agents, stress of any kind etc. etc.,) factors which induce cancer, or by making good deficiencies in the environment which favour cancer induction. There are not many examples of factors of the latter category (e.g. cancer of the esophagus in the Plummer-Vinson Syndrome due to iron deficiency) and we are concerned only with the former. Already, research in certain of the fields of cancer prevention has advanced to the point where it is desirable *to change the environment of people who as yet have not so far developed cancer.* In attempting this, we can expect opposition both from the general public who are averse to change of any kind (e.g. opposition of public to pasteurization of milk or flouridation of drinking water), and also, not infrequently, from commercial interests who tend to fight changes by which they will lose financially (e.g. the lack of co-operation from the dyestuff industries in some countries when it first became known that bladder cancer was associated with the handling of benzidine and β-naphthylamine). In order to meet both kinds of opposition it is essential that the case for bringing about a particular change in the human environment is supported by adequate, relevant and sound data and, in particular, by a knowledge of the extent to which the results from animal experiments can be applied to problems of cancer-aetiology in man. In the present article an attempt is made to establish a basis from which the validity of animal tests in this respect can be judged.

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II. The Need and Advantages of Using Animals in the Detection of Human Cancer Hazards.

Direct studies of human cancer causation by environmental influences are in practice more or less limited to the search by statisticians for associations between particular forms of cancer and specific environmental factors. Sometimes investigations of this kind have brought to light correlations of such high probability that no-one could reasonably doubt a cause-and-effect relationship. For instance, no-one can reasonably doubt that the incredibly high incidence of cancer of the nose and cancer of the bronchus in workers in the nickel industry (Doll, 1958) is caused by exposure to chemical substances used in the processing of nickel. The argument that a cause-and-effect relationship had not been proved and that it is *just as likely* that men who are peculiar in wanting to work in the nickel industry, are also peculiar in having a high expectation of developing nose and lung cancer, ~~then the statistician concerned~~ must be regarded as ridiculous if common sense is to play any part in this general field. (Compare R. A. Fisher's arguments re smokers and lung cancer—Fisher, 1959). More frequently, however, there are alternative explanations of apparent correlations and conclusions from retrospective studies need to be supported by data from prospective statistical studies, such as those of Hammond and Horn (1958) and Doll and Bradford Hill (1956) on smoking and lung cancer.

Prospective statistical studies are expensive and not always practicable. Because of the tendency for a long latent interval between exposure and tumor-induction it could take over twenty years to obtain adequate data from this type of study. Sometimes the overall incidence of the particular type of human cancer is very low, perhaps 1 or 2 per thousand of the population, and in order to show a statistically significant difference, between those exposed and those not exposed to a suspected carcinogenic agent, enormous population samples would have to be studied. It is rarely possible to control adequately other possibly relevant environmental differences between the two groups. The pattern of human living is infinitely variable, so that no questionnaire can cover all the relevant issues; human memories are inaccurate, and there are often reasons for not wishing to disclose all the facets of one's personal life on an apparently anonymous (though perhaps secretly identifiable!) questionnaire form.

If neither retrospective nor prospective statistical surveys can provide a clear-cut answer, then the possibilities of direct approach are exhausted; for we cannot deliberately test substances for carcinogenic action on man, nor can we study the mechanism of cancer induction in him. As in almost every other branch of medical research, therefore, we must turn to tests on *other* animal species. Indeed, experiments on laboratory animals provide the very basis of modern concepts of human physiology and of general medical therapeutics.

In general, man and laboratory animals suffer from a similar range of diseases, and apart from man's ability to describe subjective symptoms, it is hard to find human diseases which are not simulated in at least one other species. In most cases, the kinds of cancer seen in man, organ for organ, and cell-type for cell-type, have every one of them, parallels in other species of animal; in many instances it is impossible to distinguish a human tumor from an animal tumor simply from its microscopic appearance. On the other hand, these sometimes striking similarities can never be taken to indicate similar

causation. It is known from animal experiments that a variety of different stimuli (i.e. chemical, physical, viral, etc.) may give rise to histologically identical tumors, and that *the microscopic appearance of a tumor depends more on the nature of the tissue from which it arose than on the specific nature of the stimulus which caused it to do so.* For instance, fibrosarcomata of identical appearance may be induced in mice either by the polyoma virus or by one of a variety of different chemical substances.

Even where satisfactory statistical tests on man are practicable, carefully selected animal tests can usually provide an answer very much more quickly. Both human and animal data indicate that malignant tumors arising towards the end of the life span of a species can be the result of exposure to carcinogenic stimuli much earlier in life. For instance, in the dye-stuff industry where workers were exposed to benzidine, or to naphthylamine, between 15 and 25 years usually elapsed from the beginning of exposure to the time when the first cases of bladder cancer were seen, and in many cases exposure to the chemical agent ceased long before the cancer arose. A negligible number of cases occurred during the first 5 years from the beginning of exposure (Case *et al.*, 1954). The average induction time in relation to a particular form and rate of exposure was approximately one-quarter to one-third of the average life span of man. By treating mice with the same substance it is possible to produce a high incidence of bladder tumors in approximately the same proportion of the life span (i.e. 25 to 40 weeks) with these substances (Bonser *et al.*, 1956).

III. The Design of Animal Tests.

The three most commonly used routes by which substances are tested in animals for carcinogenicity are (1) by application of the substance to the skin after removal of the hair, (2) by subcutaneous or intramuscular injection and (3) by mouth, either in the food or drinking water, or by stomach tube. A fourth method, particularly applicable to the study of the induction of bladder cancer, has been the injection or implantation of substances into the bladder. Less commonly used have been inhalation and intravenous injection. Many other routes have been developed and used from time to time, sometimes in relation to the study of cancer of a particular organ, e.g. injection of benzopyrene into the prostate gland to induce prostatic cancer (Moore and Melchionna, 1937) or the implantation of pellets into the brain to induce a variety of gliomas and other tumors (Zimmerman and Arnold, 1941).

Faced with this wealth of method it is necessary to formulate certain general principles upon which to base the selection of tests:—

1. Each substance should be considered separately: all that is known about its chemical structure and pharmacologic action should be taken into account before the tests for carcinogenesis are designed.
2. Sufficient animals should be studied for a sufficient part of their natural life span before a negative response can be accepted. In all species cancer is essentially a disease of the latter half of life and this is the interesting period in all animal tests for carcinogenesis.
3. It is often impossible to design a fully adequate battery of tests on a new substance without more information than is available from preliminary tests: a second line of tests may have to follow the first.
4. Flexibility in design is essential. Cumulative toxicity may make it necessary to reduce dosage of a substance part-way through a long-

term experiment. Alternatively, the development of tolerance may enable the dosage to be increased.

5. Most important of all, interpretation of results is not a matter of simply recording positive and negative responses. Cancer-induction by a substance is not necessarily confined to the site of application. Expert knowledge and experience may be necessary to distinguish cancer from other pathological lesions. A significant incidence of certain non-cancerous lesions in particular test groups may be of considerable interest and importance in its own right. Thus, tests for carcinogenicity should not be divorced from tests for other forms of chronic toxicity and every animal experiment should be designed and conducted to yield a maximum of information.

6. For the above reasons it is clear that there can be no inflexible standardized test regime for testing substances for carcinogenic action and that tests should never be conducted without the advice and, preferably, supervision of a highly-trained biologist or pathologist. The choice and design of tests should be essentially his responsibility. In general, he will be bound to use one or more of the well-tried test systems by which carcinogenic action can be revealed. In addition, if not included in the above, he may try to test substances by the same routes as those by which man is likely to be exposed. This is not always practical and is not necessarily more helpful than standard test systems about which so much more is known.

7. Most authorities agree that substances should be tested near the maximum tolerated dosage (e.g. Hackmann, 1959). In most experiments carcinogenic effect increases with dosage, though not always proportionately. In no case has a positive result been missed because of super-optimal dosage, except where the dosage used also affected survival. On the other hand there are many examples of positive results having been missed by the use of too small dosage.

Subdivision of total dosage into a number of smaller doses either has no effect on carcinogenesis (Druckrey, 1954) or enhances it (Saffiotti and Shubik, 1956). As a general rule it is advisable to use more than one dose-schedule.

8. Obviously all tests must be properly controlled. Adequate numbers of untreated animals, and animals treated with solvents only, must be observed for tumors. Cancer is a naturally occurring condition in animals, just as it is in man. Therefore tumors should not be regarded as having been caused by a test substance unless the probability of their not having arisen spontaneously is known, and known to be significantly lower. Incidentally, tumors that occur apparently spontaneously do not necessarily have a different cause from those induced experimentally. Shubik *et al.* (1957) recorded a high incidence of skin tumors in mice obtained from a particular breeder. It transpired that the mice had been reared in wooden boxes the wood of which had been preserved with creosote. Mice of the same strain bred in metal cages did not develop such tumors (Boutwell and Bosch, 1958). Later it was shown that this casual exposure of infant mice to creosote led also to a high incidence of lung tumors (Roe, Bosch and Boutwell, 1958). In point of fact creosote may be one of the most potent carcinogens in our environment.

A negative result in a test for carcinogenic action cannot carry the same weight as a positive one: it can always be argued that had the

test conditions been different so might the result. The value of negative results is reduced further by the absence of comparable positive controls. Occasionally substances are tested by methods which have never been known to give a positive result using a known carcinogen. Such results are almost valueless as tests for the carcinogenicity of the substance, though they may be of value in the search for new methods.

IV. Precautions in Interpretation of Experimental Results.

1. *Purity of the substance under test.*

It is necessary to be sure that the material under test is the same as that to which man is exposed. Accidental contamination of the test material with even a trace of a potent carcinogen has in the past given a false positive result.

2. *Statistical significance.*

It must be known, at an acceptable level of probability, that the apparent difference in incidence between the test and control groups did not occur by chance alone. A 1 in 20 possibility that the difference arose by chance is barely adequate for a firm conclusion: but if there is only a 1 in 100 possibility that it did so, the result is usually acceptable. In this connection it would be wise to suspect over-ingenious use of statistical tests. Dr. Armitage of the London School of Hygiene once pointed out that if the results in two identically treated groups of animals are analysed by 20 different statistical tests, it is possible that, by chance alone, one would find a significant difference, with a probability of 1 in 20, by one of the tests!

A paper by Boyland (1957) provides useful information on the size of experimental groups necessary to give significant results.

3. *Peculiarities of species and test tissue.*

Although the use of genetically pure animals has been of great benefit in cancer research, it is important to remember that certain peculiarities have been bred into them. These peculiarities may include unusual susceptibility of one or more tissues to carcinogenic stimuli. A clue to the existence of unusual susceptibility of this kind is often given by a high incidence of, so-called, spontaneous tumors of the same kind. It is, for instance, much easier to induce lung tumors in strains of mice which have a high spontaneous incidence of these tumors than in those which have a low spontaneous incidence (Lynch, 1926).

A special precaution is necessary in the induction of sarcomata in the subcutaneous tissue of rats and mice. This tissue is extraordinarily sensitive to the induction of such tumors. Sarcomata have been reported following the subcutaneous injection of normal body constituents such as glucose in this species (Nishiyama, 1938), and all the solvents in which substances have been administered to rats by the subcutaneous route have given rise to malignant tumors on their own. It is now generally felt that evidence of carcinogenesis should never rest *solely* on the demonstration of sarcoma-induction in this site (see Report of Panel on Carcinogenic Risks in Food Additives and Pesticides, Ministry of Health, London, 1960).

Sometimes in animal experiments positive results seem to depend upon quite exceptional circumstances. For instance, if male mice of certain strains are exposed to minute traces of chloroform by any route, they develop a severe inflammatory reaction in the kidneys. For reasons unknown, the kidneys of females of these strains, and of both sexes of many other strains are completely unaffected by exposure even to much higher doses (Deringer *et al.*, 1953). Similar peculiar species and sex differences exist in susceptibility to cancer induction. For instance, the kidney of the male Syrian Golden Hamster is peculiarly susceptible to the induction of renal tumors by oestrogens (Kirkman and Bacon, 1949; Horning and Whittick, 1954). In the early stages cessation of oestrogen treatment is followed by regression of the tumors. The kidneys of no other species so far studied react in this way to administration of oestrogens.

4. *Criteria of malignancy.*

Perhaps the most important, certainly the most difficult and controversial, precaution concerns the criteria for the diagnosis of malignant cancer. There is a tradition in medical education according to which the post-mortem room is the High Court of Justice, and the Pathologist, the Lord-Chief thereof. If he says "It's malignant", then it *is* malignant. No doubt in most cases he is right, but it is always wrong to confuse opinion with truth. In this case the truth is that there is no clear-cut distinction between benign and malignant: a whole series of grades exist between these two extremes. Where human material is concerned, marginal lesions are often called malignant on the basis of "If it were mine, I'd have it out". In animal research one can afford to be more objective. The growth of a tumor can be observed, its growth-rate recorded, and it is not necessary to remove it before it has invaded muscle or metastasised to local lymph glands and other organs. Every tumor can be examined microscopically in a fresh state, since no permission from a mouse's relatives is necessary before post-mortem examination is carried out! Unfortunately, advantage is not always taken of these facts, and lesions are called benign or malignant without any statement of the criteria of diagnosis. It should be a general rule that substances are only described as "carcinogenic" if they have given rise to tumors of unquestionable malignancy, and malignancy should always be questioned if the criteria of diagnosis are not stated.

A commonly used aid in the diagnosis of malignancy is transplantation of part of the suspect tumor to other animals of the same species. It is generally agreed that if a tumor transplant will grow progressively then the tumor is malignant. However this test is of limited value since many undoubtedly malignant tumors fail to thrive in new hosts. In any case caution must be exercised in that a progressively growing swelling in the region of the graft may be entirely inflammatory, so that even at this stage microscopic examination by a competent pathologist is essential. Another source of error occurs when inbred strains of mice are used: normal tissues and benign tumors can survive indefinitely after transplantation to a genetically identical host. In this case it is necessary to be sure that the graft has grown progressively, and has infiltrated surrounding tissues.

Significance of induction of benign tumors.

Experience indicates that substances capable of inducing benign tumors are often capable of inducing malignant ones also, though the induction time is longer and the incidence much lower. Therefore the induction of benign tumors, although inadequate evidence of carcinogenicity of itself, should lead to a strong suspicion of such activity. Further work, perhaps on a larger scale and with a longer period of observation, may well reveal the ability of the substance to induce malignant tumors.

These considerations apply equally to metaplasia, and to carcinoma in situ. *Metaplasia* is generally considered to be a precancerous change, but as such its induction should not be regarded as equivalent to cancer-production in a test for carcinogenesis. If it really is precancerous, then it should progress to cancer during a further period of observation, at least in a proportion of cases. *Carcinoma in situ* covers a range of the many stages between undoubted benignity and undoubted malignancy. In human morbid anatomy the term is applied to a variety of lesions, the malignancy of which is uncertain. It has a more or less definite meaning in surgery and prognosis, but has no place in experimental pathology.

5. *Confirmation by repetition of the experiment.*

Finally, it is almost a general rule that, before the result of a carcinogenic test is acceptable, it must have been repeated, preferably in a different laboratory. Past experience indicates that this is a wise precaution, so many are the pitfalls in this type of research.

V. Extrapolation of Results of Animal Experiments to Man.

It has often been pointed out that there are considerable species differences in susceptibility to carcinogens. Therefore, it is argued, the results of tests in animals should not be applied to man. In fact, data for species other than rats and mice are usually entirely inadequate in order that apparently negative responses can be regarded as conclusive. In the past some cancer investigators, while apparently fully aware of the statistical requirements of experiments when using rats and mice, seem to have lost this awareness progressively as the size of the species under test increases: a worker who would not dream of reporting a result obtained on less than 20 mice has, seemingly, been quite content to report experiments on 6 rabbits or 4 dogs or 2 monkeys!

In the case of the most potent carcinogens, e.g. 3,4-benzopyrene, 9,10-dimethyl-1,2-benzanthracene, 1, 2, 5, 6-dibenzanthracene, 20-methylcholanthrene, positive results have been obtained in almost all species tested (Hartwell, 1951; Shubik and Hartwell, 1957 (2)). Admittedly man has not been deliberately tested but, on the other hand, it was because of the demonstration of cancer-induction in man by chimney soots (Pott, 1775) and other coal tar products that research in the field of chemical carcinogenesis was begun; and it is from coal tar, creosote, and chimney soots etc. that substances such as 3,4-benzopyrene and 1, 2, 5, 6-dibenzanthracene were originally isolated. Therefore, it is unlikely that man is insusceptible to carcinogens of this type. In the case of other types of substances where there is less or no direct information of man's susceptibility, the likelihood that he is susceptible increases progressively if:—

1. Cancer can be induced in more than one tissue and species.
2. Cancer can be induced by a realistic method of exposure.
3. Cancer can be induced in the same tissue, and of the same histological type as that which the agent is suspected of inducing in man (such suspicion being based on retrospective and prospective statistical surveys of the human disease).
4. Cancer can be induced by realistic doses of the material in question. The meaning of realistic in this connotation is discussed in the next section.

Realistic Dose.

The ratio of the weight of a man to that of a mouse is approximately 2000:1. For drugs which act *systemically* the L D_{50}, expressed as weight of drug per unit of body weight, is similar in the two species. In other words, if a certain weight of drug kills 50 per cent of a group of mice, then 2000 times as much would be required to kill 50 per cent of men.

In the case of substances which act *locally at the site of administration* the body weight is not necessarily particularly relevant. It is generally accepted that every cancer finds its origin in a single altered body cell. Now the body cells of man are not 2000 times the size of those in mice, on the contrary they are of a very similar size. Hence at the site of injection, a particular volume of material would come into contact with approximately the same number of cells in the two species. Given equal susceptibility at the cellular level, therefore, the resulting tumor incidence would be related directly to the volume of the material injected, irrespective of body weight.

Part of the current controversy over the possible hazards of the clinical use of iron-dextran ("Imferon") (Richmond, 1959, 1960; Haddow and Horning, 1960: Golberg, 1960*a*; Golberg, 1960*b*), is basically concerned with this point:—Injection of certain doses of iron-dextran subcutaneously or intramuscularly into rats and mice induces sarcomata and histiocytomata. Some workers argue that on the basis of body weight the doses required to induce these tumors are enormous compared with those used clinically. On the other hand, the actual size of the doses used clinically are much the greater. Here is a dilemma which only time can resolve, for although no cases of sarcomata attributable to therapy with iron-dextran have been reported, in view of the much longer life span of man, it is too early to expect them.

Threshold dose.

Is there a threshold dose for each carcinogenic substance below which it is ineffective? There is no doubt that, in any set of experimental conditions, there is an apparent threshold: using groups of practical size (say 50 animals per group) it is always possible to select a dose of a carcinogen too small to produce any tumors. But if the size of the groups could be unlimited, would any dose, however small, be entirely ineffectual?

As far as man is concerned, a substance could not be regarded as harmless because it failed to induce any tumors in a group of 50 men. A cancer incidence of 1 in 1000 or 1 in 10,000 or even 1 in 100,000 would concern us if we knew that it could be prevented: an incidence of 1 in 50 or 1 in 100 in a civilized community would cause not only concern but alarm! The truth of this is evident from the response of the public to the campaign against poliomyelitis. At the time of the publication of the 1954 Francis Report (Francis *et al.*, 1955), vaccination was shown to reduce the total incidence of the disease

from 46 to 28 per 100,000 and the incidence of paralytic cases from 36 to 16 per 100,000. Despite the fact that only 20 out of every 100,000 vaccinated children appeared to have benefited from the injections, the differences in incidence were considered sufficient to warrant the extension of an enormously costly trial vaccination programme into a nationwide campaign.

Since it is impractical to test substances for carcinogenicity on groups of 1,000 or 10,000 animals, the usual practice is to expose smaller groups of animals to doses much higher than normally present in the environment. However, opinion is divided as to whether it is justifiable to conclude that *a positive result obtained with a large dose in a small group of animals indicates that a similar result would be obtained using a smaller dose on a proportionately larger group of animals.* Workers such as Druckney (1954) hold firmly to the view that such a conclusion is justified. Others argue that this view is based almost entirely on the experimental induction of liver tumors, and may not be true of cancer-induction in general.

Looking back through the literature of the last 25 years it is apparent that, as techniques have been refined, the dosage of a variety of different chemical carcinogens considered necessary to produce tumors has steadily declined. Take for example 9, 10-dimethyl-1, 2-benzanthracene: in the earliest experiments approximately 5-10 mg. were applied to the dorsal skin of mice before tumors arose (Bachmann, Kennaway and Kennaway, (1938); in 1941 tumors were induced by approximately 0.5 mg. (Law, 1941); and today carcinogenesis has been demonstrated with only 1.2 μg. (Klein, 1956).

In the final analysis the question whether there exists an absolute threshold is unanswerable; but most people would concede that there are practical thresholds, by which is meant dose-levels at which no effect can be seen in as large a group as it is possible to observe.

But the arguments concerning threshold dose do not end here. Exposure to a carcinogen is in no way similar to exposure to aspirin or to a barbiturate. Recovery from an overdose of either of the latter is as far as we know complete, and has no effect on subsequent tolerance. In the case of exposure to carcinogens however there is considerable evidence that *the effect of one exposure is irreversible* (Berenblum and Shubik, 1949) and that *the effect of several exposures is cumulative* (Roe and Salaman, 1954). Indeed some experimental results suggest that where the total dosage is constant several small doses are more effective than one large dose, (Saffiotti and Shubik, 1956; Salaman and Roe, 1956). If this evidence is accepted, then the size of any one dose of a carcinogen is irrelevant, and the sum of all the doses of a lifetime is the factor which has to be considered.

Even this is not all, for it is possible that the carcinogenic effect of two substances is additive, or perhaps, even synergistic, or that *co-carcinogenic* factors may enhance the effect of carcinogens. Experimental evidence strongly suggests that these are not merely theoretical possibilities. In the case of coal tar, for instance, the carcinogenicity cannot be explained quantitatively by the concentration of any one of the carcinogens in the tar, and it must be the result of the effect of more than one constituent. The role of co-carcinogenic factors has now been clearly demonstrated in rabbit skin (MacKenzie and Rous, 1941; Friedewald and Rous, 1944), mouse skin (Berenblum, 1941; Berenblum and Shubik, 1947*a*, 1947*b*), and mouse forestomach (Peirce, 1961); and recent work suggests that they may play an important part in the causation of human bronchial cancer by cigarette smoke (Roe *et al.*, 1959). Moreover, it is becoming apparent that a wide variety of co-carcinogenic substances

are present in the environment (e.g. phenolic substances, including phenol itself (Boutwell and Bosch, 1959); many surface active agents (Setala, 1960); certain citrus oils (Roe and Peirce, 1960); and latices from the stems of plants of the Euphorbia (Spurge) family (Roe and Peirce, 1961).

It may be concluded, therefore, that the existence of threshold dose-levels is of theoretical interest only, since even if a particular dose of a carcinogen is regarded as subthreshold, its effect may nevertheless be augmented to above the threshold by further exposure to the same carcinogen, or to other carcinogens, or to co-carcinogens.

VI. Action.

Interest in possible carcinogenic hazards has increased very much in recent years, but the problem as a whole is not new. *Already thousands of cases of human cancer have been prevented by the introduction of suitable measures*, particularly in connection with industrial processes (e.g. in the dye-stuff, nickel, and chromate industries). The task which lies ahead is the logical continuation of this approach, and its extension to less obvious and less potent carcinogenic hazards in the environment. As in the past, experiments on laboratory animals will play an indispensable role in the detection of these hazards. It is true that it cannot be proved absolutely by animal experiments that there is a cause and effect relationship between a particular environmental factor and a particular form of cancer in man. But this impossibility of obtaining absolute proof is not peculiar to this problem. it occurs in every situation in life. Important decisions are constantly made on the basis, not of proof, not even on probability which can be expressed mathematically, but on a balance of probabilities based on a common sense interpretation of all the relevant data. This is the very basis of judgment in courts of law*: there is no other.

Agreement that an environmental factor constitutes, or probably constitutes, a cancer hazard indicates the need for legislation. At this stage the scientist should not attempt to become the legislator but should be content to advise. Cancer is not the only hazard in life and from arbitrary high-handed action more harm than good may result. The public, and any industries involved, ought to be represented on the governmental committees which eventually decide the action to be taken.

Where it is possible for the substance which is considered hazardous to be eliminated from the environment, without serious economic loss or interference with established practice, there are no grounds for disagreement. But in other cases, because of lack of alternatives or because possible alternatives carry their own hazards or for serious economic reasons, etc., it is necessary to compromise by agreeing to *permissible levels* of exposure. However, a decision to tolerate a potential carcinogenic hazard up to a certain level should always be regarded as an interim solution only and complete elimination of the hazard should be the goal.

Much of the recent increase in prosperity and well-being in a great part of the world has been due to the development of modern agricultural methods and, in particular, to the use of chemical insecticides, herbicides, and fungicides. Such substances frequently contaminate human food. The manufacturers argue that unless new substances are continually introduced it is

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*"In civil actions . . . a contested case may be established by a balance of probabilities". Halsbury's "Laws of England" 3rd Edition, Vol. 15 p. 272 para 496. Butterworth, London, 1959.

probable that the development of resistance by weeds, insects and micro-organisms will nullify the advances already made. On the other hand the full-scale testing of large numbers of potentially useful chemicals for carcinogenic action poses an enormous problem for the industries concerned. This situation could be eased by:—

1. A very much greater measure of international agreement which would reduce the pressure of foreign competition.
2. The establishment, preferably under the aegis of Governments, or internationally, of centers for the purpose of testing on a large scale environmental substances for carcinogenic action. This would be of great benefit to those industries wishing to introduce new materials.

Progress towards international agreement is slowly being made through the World Health Organization; and some Governments have begun to organize testing facilities. But the rate of progress in these directions is not commensurate with the rate of the accumulation of problems, nor has account been taken of the enormous backlog of work in the form of *the innumerable untested, but potentially hazardous, substances already present in man's environment. It is among these that factors responsible for the existing cancer incidence must be sought.*

Of course every attempt should be made to prevent the further addition of hazardous factors to the environment, but at least equal attention should be paid to the evaluation and elimination of existing hazards. Consider, for example, 3, 4-benzopyrene: this is a ubiquitous carcinogenic substance of considerable potency. It is found in "polluted" air (Waller, 1952), in cigarette smoke (Cooper and Lindsey, 1955), in automobile exhausts (Lyons, 1959), in smoked foods (Gorelova and Deekoon, 1959, 1958), in coffee grounds (Kuratsune and Hueper, 1960), in fact it is produced by the pyrolysis or burning of almost any organic material (Gilbert and Lindsey, 1957). The existence of substances of this kind in almost every department of man's environment presents a very complicated problem of assessment and their elimination an enormous challenge to research workers in many fields, especially that of engineering.

The present tendency to puff up doubtful hazards into nation-wide cancer scares is to be deplored, not only because of the largely unnecessary anxiety which it causes, but also because it blinds the public to what are probably more serious cancer hazards. By all means let us be concerned about how the farmer sprays his cranberries, but let us also try to find out as soon as possible whether the creosote with which he sprays his fences is as great a carcinogenic hazard to him as it is to laboratory animals.

Summary and Conclusions

1. There is currently a shift of emphasis in cancer research away from cure to prevention. One method of prevention is to remove carcinogenic hazards from the human environment. This involves the testing of suspect substances for carcinogenic activity.
2. The necessity, advantages and disadvantages of using laboratory animals in research of this kind are discussed.
3. It is argued that, in the testing of environmental substances for carcinogenic activity, each substance should be considered separately in the light of all that is known of its pharmacological actions. These tests should be designed, supervised, and assessed by highly trained

workers and cannot be reduced to a standardized routine capable of application by the semi-skilled.

4. Before a positive result is accepted in a test for carcinogenic activity the following points must be considered:—
   (a) Chemical purity of the substance.
   (b) Peculiarities of the strain of animals used and of the test site.
   (c) The inclusion of both positive and negative controls.
   (d) The production of malignant, as well as benign, tumors confirmed histologically according to an acceptable standard.
   (e) Statistical analysis of differences in tumor incidence between test and controls.
   (f) Confirmation of the results by repetition of the experiment.
5. A positive result obtained in animals gains increasing significance for man if:
   (a) Cancer is induced in more than one tissue and species and by a realistic dose and method of exposure.
   (b) Cancer is induced in the same tissue and of the same histological type as that which the agent is suspected of inducing in man.
   (c) If a retrospective statistical study in man shows a significant association between exposure to the substance and development of the disease.
   (d) If a prospective study shows the same association.
6. None of these types of evidence constitutes absolute proof of a cause and effect relationship. Action can only be taken in the light of evidence based on the balance of probabilities.
7. There is an urgent need both for international agreement in this general field and for the establishment of centers for the large-scale testing of substances for carcinogenic action. Present progress in these directions is too slow.
8. At present attention is focussed on the testing of substances which it is proposed to add to the environment. Important though this work is, it cannot lead directly to a fall in the existing high incidence of cancer. Search for factors responsible for this should be made amongst *substances already present in the environment.*

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