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General Toxicological Considerations of Extrapolation from Animals to Man

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The time has surely come for a serious and searching reappraisal of the use of tests on animals in the prediction of toxicological hazard for man and in the evaluation of probable safety of chemical and other agents for man's use.

The title of my talk is a general one and covers all manifestations of toxicity. However, my personal experience is mainly in the field of cancer and it is therefore from this field that I propose to draw examples to illustrate the points I shall make. In my opinion carcinogenicity is simply one manifestation of toxicity, and I can find no substantial grounds for regarding the assessment of carcinogenicity separately from the general assessment of toxicity. It is sometimes argued that carcinogens are distinct because their effects are irreversible. But the irreversibility of the effects of carcinogens is no more striking than the irreversibility of the effects of, say, agents that cause emphysema, testicular atrophy, or cirrhosis. Moreover, I shall refer to evidence which suggests that the effects of carcinogens may not be as fundamentally irreversible as has been generally supposed.

The question of the irreversibility of the effect of carcinogens or other toxins is, of course, a crucial one. If exposure to a chemical agent produces irreparable damage, then no level of exposure to it, however small, can be regarded as safe. The effects of repeated, individually insignificant, exposures to the same agent, or of exposure to a number of agents which produce the same kind of damage, may build up to the point at which there is a real threat to health or life. If, on the other hand, the damage caused by a small dose of a chemical can be made good, either by repair or by replacement of the damaged cells or tissue, then one may conceive of there being a threshold level of continuous or repeated exposure to the agent below which there is no hazard—this level being set by the capacity of the host for repair. (See Fig. 1).

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In the case of toxic chemicals, including carcinogens of types that have long been in the environment, such as aflatoxin or 3,4-benzpyrene, it would be surprising if a capacity for making good damage produced by small doses of them had not evolved. But for man-made toxins that have only been introduced into the human environment during the past few decades, there are no *a priori* grounds for expecting repair mechanisms to have evolved.

Evidence for Reversibility

In 1972 my colleagues and I reported (Roe *et al.*, 1972) that during the course of 12 months the skin-tumor initiating effect of a single dose of 7,12-dimethylbenz(a)anthracene (DMBA) was largely lost (Table 1). The finding was surprising because ever since the classical experiments of Berenblum and Shubik in the late 1940's it has been assumed that the tumor-initiating stage of carcinogenesis is irreversible. However, a reexamination of their experiments and of our own previous work showed in both cases that some loss of initiating effect may have occurred. Further studies of this kind in which relatively small doses of tumor initiator are given are urgently needed.

The Significance of Evolution

It is, of course, appropriate to consider the principles of extrapolation from animals to man in the context of natural evolution. The evolutionary process provides the *continuity* between species as different as laboratory rodents and man. The evolutionary process is commonly conceived as a tree with individual branches, leaves and twigs which *differ* quite remarkably from each other. The art of extrapolation from animals to man in toxicology lies in the accuracy of judging whether the response of a particular species to a chemical or other agent is dependent on some aspect of the general structure of the evolutionary tree which is a common feature of all its branches,

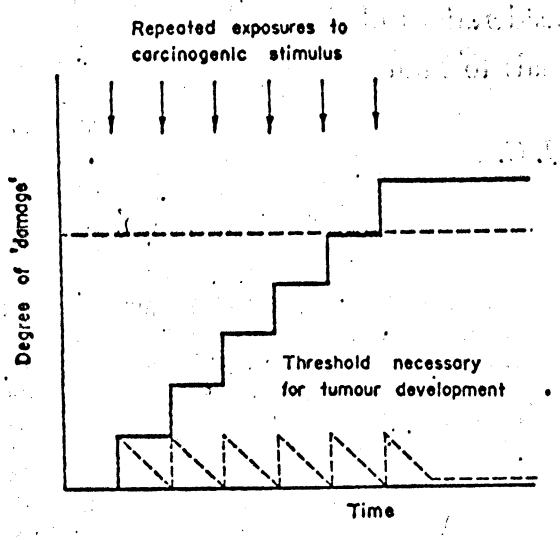

Fig. 1: —— Accumulation of "damage" if there is no repair; ---- non-accumulation of "damage" if repair takes place.

leaves and twigs, or a phenomenon peculiar to only one branch, one twig or one leaf of the tree.

The efficacy of some of the environmental chemicals, such as pesticides and antibiotic drugs actually *depends* on the different susceptibility of different parts of the evolutionary tree to the toxic effects of the chemicals concerned. One would hardly use a parasitic worm for evaluating the safety for humans of an antihelminthic drug specifically developed to kill it. I often wonder whether some members of the toxicological fraternity do not sometimes come close to doing just this when they blindly apply routine toxicological tests to chemical agents regardless of the purpose for which the latter have been developed.

Susceptibility to Disease and Death

In the case of short-term tests on animals, that is to say, tests which are completed during only the early part of the natural life-span of the species, it is usually easy to decide whether exposure to particular agents has adversely affected the health of a group of animals under a particular set of laboratory conditions. In the case of longer-term studies, however, it may be less easy to reach a decision. There are examples of agents which increase the risk of disease or death from one cause but decrease it from another.

It is therefore short-sighted to look at individual toxic effects in isolation in any species, be it man himself or a laboratory rodent. Toxicity should be assessed in terms of the effects of a test material on the whole of the animal and on the whole of the life of the animal. Inevitably this means that one needs to have a good knowledge of the diseases and natural causes of death of animal species used for toxicological tests. Moreover, without this background information it may be very difficult to judge whether an apparent effect—either toxic or beneficial—is likely to be a species-specific phenomenon or a warning that a similar effect will be seen in other species, including man, if they are exposed to the agent under test.

The Naturalness of Life

It is very easy to assume that normal humans live "natural" lives but in this context the word "natural," I suggest, begs definition. Let us take for example the natural sex-hormone status of women. As far as we can judge from the study of present-day primitive socie-

Table 1
Loss of Skin Tumor-Initiating Effect with Time

Initiating Treatment (day 0)	Interval (days)	Promoting Treatment (×2 weekly)	% Tumor-bearing mice at 15 weeks
100 μg DMBA* in acetone	21	3.12 μg TPA** in acetone	85
//	350	//	4
//	21	Acetone only	0

* 7,12-dimethylbenz(a)anthracene.
** Croton oil factor A_1.

tics, the natural status of a woman from the time of the menarche until the menopause is that she is either pregnant or lactating, with lactation continuing for two or more years after the birth of each child depending on the level of food supplies available to the community of which she is a member. Taboos keep the male at bay during the lactation period while the male indulges in polygamy and, of course, fighting to reduce the population of his male competitors. It must have been as much a matter for concern for a primitive lady to menstruate as it is for a modern lady to fail to do so!

In two other ways modern life is quite unnatural in the sense that it is quite different from the conditions under which human life evolved. Firstly the conquering of many infectious diseases and the discovery of ways to prevent early death from genetically-determined diseases, such as diabetes, have resulted in a considerable prolongation of the average life-span. Secondly, for a proportion of the world's population there exists a super abundance of food which is responsible for widespread overnutrition.

The life-style of laboratory animals, especially of the small rodents—the mouse, the rat and the hamster—is also very different from that of the forebears of those species. Firstly the conditions under which most long-term animal studies are conducted involve the complete deprivation of animals of their normal sexual activity. Females are never allowed to be pregnant or suckle young. Males have perpetually over distended seminal vesicles and develop proteinaceous casts which block their urethras and bladders. In many laboratories, animals under long-term experiment are provided ad libitum with a highly nutritious high-fat, high-protein diet on which they become profoundly obese. Thirdly, and worst of all, they are subjected to a measure of inbreeding so that genetic defects and genetic susceptibility to vertically-transmitted viruses, some of which are oncogenic, are concentrated in them. The result of inbreeding can be that any one strain or subline of a species is quite unrepresentative of the species as a whole. In other words, it may not be valid to extrapolate from the results of studies on one genetically distinct strain to other strains of the same species, let alone to other species such as man.

Examples of Effects of Abnormal Hormonal Status, Over Nutrition and Stress on Tumor Incidence

Before I proceed further I should like to give examples of effects attributable to the unnatural environment in which we keep our experimental animals. In a recent routine carcinogenicity test with which I was involved, the findings in untreated control Sprague-Dawley rats were as shown in Table 2. No less than 40 out of 50 rats developed one or more neoplasms before the 123rd week from the start of the experiment, when the 23 surviving animals were killed. Twenty-nine (58%) developed mammary tumors, 21 (42%) developed pituitary tumors, two had thyroid tumors, two had parathyroid tumors and one

Table 2
Tumor Incidence among 50 Untreated Female Sprague-Dawley Rats which Formed a Control Group in a Carcinogenicity Test

Tumor incidence	0–60 wks	60–80 wks	80–100 wks	100–120 wks	120–123 wks	0–123 wks
Number of deaths	3	2	9	12	24	50
Mummary gland and s. c. tissues	0	1	4	8	16	29
Pituitary	0	1	5	5	10	21
Thyroid	0	0	1	0	2	3
Parathyroid	0	0	0	0	2	2
Adrenal	0	0	0	0	4	4
Malignant lymphoma	0	0	2	0	0	2
Other sites	0	0	2	2	1	5
1 or more benign or malignant tumor of any site	0	1	9	10	20	40
1 or more malignant tumors of any site	0	0	4	1	0	5

Table 3
Reduced Incidence of Mammary Tumors in Female Rats Stressed by Exposure to Tobacco Smoke

	Rats with mammary tumors	
	Observed	Expected
Smoke-exposed (×10 weekly)	37	57
Sham-exposed (×10 weekly)	40	29
Untreated	42	30
Significance $p<0.01$		

(See Davis *et al.*, 1975)

had an adenoma of the adrenal cortex. In virtually all the animals, areas of haemorrhagic necrosis were seen in the adrenal cortex.

As many of this audience will know this incidence of neoplasms in untreated laboratory rats is by no means unusual. Indeed it is such a regular finding that we have come to accept it without thinking what it means. It is my belief that the high incidence of mammary, pituitary and other endocrine gland tumors is an indication that the hormonal status of laboratory female rats is completely out of balance, partly because of over-feeding and lack of exercise and even more so because of enforced sexual inertia. Common sense suggests that animals in which tumors of certain kinds develop so readily cannot and should not be regarded as appropriate models for distinguishing between exogenous chemical agents which may or may not influence the incidence of endocrine-related cancers in man. For instance one could not judge whether oral contraceptive drugs are likely to increase or decrease the incidence of breast cancer in women from the results of studies on laboratory rats.

Influence of Stress and Dietary Intake

In the course of an experiment designed to investigate the effects of inhaled tobacco smoke on female rats, Davis *et al.* (1975) found, to their surprise, that exposure to tobacco smoke was associated with a highly significant reduction in the incidence of mammary tumors (Table 3) compared with untreated control animals. Smoke exposure was also associated with reduced body weight gain and it is debatable whether the effect on mammary tumor incidence was secondary to some effect on the nutritional status of animals—either they ate less or their basal metabolic rate was higher—or secondary to stress.

Ross and Bras (1972) found that dietary restriction reduced the incidence of various kinds of tumor, including mammary tumors, in rats and a similar effect of dietary restriction on mammary tumor incidence has been reported for mice by Rowlatt *et al.* (1973). The suggestion that stress might reduce susceptibility to mammary tumor development in rats is at variance with a recent report by

Table 4

Feeding	Total tumors by 18 mos	Liver tumors	Lung tmmors	Lymphoreticular neoplasms	Other neoplasms
4 g diet/day 1 mouse/cage	4	1	1	2	0
5 g diet/day 1 mouse/cage	4	2	0	1	1 testis
Diet ad libitum 1 mouse/cage	32	15	2	11	2 testis 1 kidney 1 thyroid
Diet ad libitum 5 mice/cage	23	8	6	9	0

Riley (1975) on the effect of stress on mammary tumor incidence in mice. Riley observed C3H mice that carried the Bittner mammary tumor virus maintained under different levels of stress. Non-parous mice protected from stress had an incidence of mammary tumors of only 7% at 400 days compared with an incidence of 63% in non-parous mice not protected from stress.

Mary Tucker, as reported in Roe and Tucker (1974), observed a remarkable effect of dietary intake on the incidence of a variety of kinds of neoplasm in mice (see Table 4). Ad libitum fed mice of an outbred Swiss strain consumed on average 5.77 g of an ordinary pelleted diet per day. Restrictions of their intake to 5 g or 4 g a day led to an eight-fold reduction in tumor incidence. It is not clear whether this effect on tumor incidence reflected a direct effect of reduced intake or an indirect effect of the stress of being faced with an empty food hopper during a part of each day.

Conclusions

At a meeting of the European Society for the Study of Drug Toxicity in Zurich in 1973, Mary Tucker and I (Roe and Tucker, 1973) made a plea for higher standards of conduct of toxicological tests, particularly carcinogenicity tests. Table 5 illustrates some of the common design faults in the conduct of such studies. These still merit the attention of toxicologists in some organization.

Obviously to extrapolate from a badly-designed or badly-executed test on laboratory animals to man could be entirely misleading. A chemical substance which is in fact hazardous for man could be judged safe or vice versa. But the main point I should like to make today is that it is also possible to draw false conclusions from what would, according to modern day standards, be regarded as well-designed and well-executed tests.

There is very considerable background noise in the systems most commonly used for the evaluation of carcinogenicity. Rats and mice of most laboratory strains are highly susceptible to the spontaneous development of one or more kinds of neoplasm. Exposure to known or putative carcinogens often has the effect of increasing the incidence of such neoplasms, but it sometimes has the opposite effect. Non-specific factors, such as level of dietary intake and stress, may also profoundly influence the incidence of these commonly-occurring neoplasms. The level of background noise is increased by inbreeding, over-feeding and the maintenance of animals in a perpetual state of hormonal imbalance.

Personally, I see no more reliable way of testing environmental agents for carcinogenicity than by conducting long-term tests in laboratory animals. The results of short-term tests, such as mutogenicity tests using artificially sensitive bacterial systems, can, if considered in isolation, be even more misleading than the results of long-term tests. But it is not sufficiently widely realized that the results of even well-designed and well-executed long-term tests on animals are open to misinterpretation.

During recent years this area of toxicology has been invaded by politicians, lawyers and statisticians while the knowledge, experience, scientific work and conclusions of biologists, toxicologists, biochemists and others have tended to be brushed aside. It is now long overdue that scientists of these various disciplines should reassert their authority to see that statisticians with no training in biology, physiology, pathology or toxicology do not assume that the interpretation of carcinogenicity tests is no more than a number-crunching game. The art of extrapolation from animals to man lies in making the fullest and most intelligent use of *all* the toxicological and other information available and in considering this information in the context of the evolutionary process.

Table 5
Carcinogenicity Testing: Common Design Faults

1.	Inadequate randomization
2.	Unintended variation—Position on racks —Room differences —Operator differences —Observer differences
3.	High loss of animals without postmortem examination
4.	Poor records of necropsy findings—position and size of lesions not recorded
5.	Non-standard postmortem technique—e.g. procedure less rigorous on Saturdays and Sundays than on weekdays
6.	Failure to match microscopic with macroscopic findings
7.	Failure to take survival differences into account in expressing results

WORKING GROUP B

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