© 1987 Elsevier Science Publishers B.V. (Biomedical Division) Interactions between drugs and chemicals in industrial societies. G.L. Plaa, P. du Souich, S. Erill, editors.

DRUGS AND CHEMICALS: CARCINOGENS, PROCARCINOGENS AND PROMOTERS

FRANCIS J.C. ROE

Consultant in Toxicology, 19 Marryat Road, Wimbledon Common, London SW19 5BB, England.

INTRODUCTION

People of all races and nations are prone to develop cancers and to die in consequence. However, there is considerable geographical and inter-racial variation in the incidence of, and mortality from, cancers of particular sites and kinds. The fact that migrants from one area or culture to another tend to lose the cancer-incidence characteristics of the country of origin and adopt those of the country to which they migrate has led to the theory that over 80% of human cancers are environmental in origin and, therefore, in principle, preventable.

There are several reasons why the exploitation of this concept is unlikely to reduce dramatically the overall death rate from cancer. Advances in medicine, partly brought about by the pharmaceutical industry, are reducing death rates from causes other than cancer. However, the risk of developing most of the more common forms of cancer increases logarithmically with age. Consequently, reductions in cancer incidence in people under the age of, say, 60 are more than offset by increases in the numbers of people living beyond the age of, say, 70. The true age-standardized risk of developing most forms of cancer is either stationary or declining. Among some populations in whom exposure to tobacco smoke was still increasing 20 years ago, the age-standardized incidence of death from lung cancer is still increasing today. However, in Britain, where the tar delivery of cigarettes began to be reduced over 20

years ago, the age-standardized mortality from lung cancer is now falling rapidly except in the very oldest age groups.

As far as drugs are concerned, although there are a few which have been incriminated of increasing cancer risk in man, the overall contribution of drugs to the human cancer burden, according to Doll and Peto, is probably less than 1% in the United States and there is no evidence that it is increasing (1).

Despite this, Regulatory Authorities responsible for the safety of medicines attach considerable importance to the need to demonstrate that prospective drugs will not cause cancer in man. For this purpose, they demand mutagenicity tests and life-time carcinogenicity tests in animals for all drugs with which humans are likely to be treated for non-life-threatening ailments. In this paper, I discuss certain problems involved in the extrapolation to man from the results of such laboratory studies.

DEFINITIONS

TABLE 1 defines some of the terms that are currently The two-stage theory of carcinogenesis proposed in use. by Peyton Rous and developed by Berenblum and Shubik led to the popularity of the terms INITIATION and PROMOTION. However, promotion is, in reality, just one specific form of CO-CARCINOGENESIS and the term should certainly not be used unless there is convincing evidence that an agent only enhances cancer risk after prior exposure to a genotoxin. Indeed, the time has probably come when we should stop using the terms initiation and promotion altogether. I say this, firstly, because there is known no agent capable of initiating cancer, that is not, in higher dosage, a complete carcinogen, and, secondly, because there is known no agent capable of promoting cancer which is not, in higher or more prolonged dosage, a complete

102

TABLE 1

DEFINITIONS

- CANCER Any invasive autonomous growth (NB Non-invasive growths are not cancers but they may be autonomous and may progress to cancers)
- CARCINOGEN Any agent that increases the agestandardized risk of cancer
- PROCARCINOGEN A NON-CARCINOGENIC CHEMICAL WHICH CAN BE METABOLIZED TO A CARCINOGEN IN THE BODY (I.E. BY METABOLIC ACTIVATION)
- CO-CARCINOGEN An agent which enhances carcinogenesis by another agent (<u>NB</u> numerous mechanisms)
- ANTICARCINOGEN An agent which inhibits carcinogenesis by another agent (<u>NB</u> numerous mechanisms)
- INITIATOR An agent which damages the genetic information of cells in a way that favours cancer development
- PROMOTER An agent which enhances carcinogenesis by stimulating the proliferation of <u>previously</u> initiated cells
- GENOTOXIC CARCINUGEN SAME AS FOR "INITIATOR"
- NON-GENOTOXIC CARCINOGEN An agent which increases the age-standardized risk of cancer but does not damage DNA or chromosomal integrity.

carcinogen. For these reasons, I now prefer the term GENOTOXIC CARCINOGEN to initiator and have more or less abandoned the term promoter in favour of NON-GENOTOXIC CARCINOGEN.

NATURAL PROCARCINOGENS AND CARCINOGENS AND ENDOGENOUS ELECTROPHILES

Some 20 or more years ago it was widely assumed that the 80% of human cancers attributable to environmental factors were all caused by exposure to man-made genotoxic carcinogens or procarcinogens in the environment or, at least, that they were all initiated by exposure to such agents. Consequently, the development of quick, cheap and sensitive tests (such as the Ames test) for genotoxic activity led to chaos because it was soon found that many important everyday chemicals gave positive results in one or more such tests. Furthermore, this was true for several chemicals which had previously given negative results in long-term animal tests. Some saw resolution of these problems in denigrating the reliability of the short-term tests. Others, such as John Cairns (2), suggested that genotoxicity is a necessary but not sufficient attribute of chemical for the purpose of rendering it carcinogenic. Thus, exposure to such a chemical has not only to result in damage to DNA but it has to do this in stem cells. Also, DNA damage has to be of a relevant kind - interference with chromosomal integrity being far more important than a point mutation or frameshift in a single chromosomal strand. Others postulated, that in the cases where apparently non-carcinogenic chemicals had given negative results in previous tests for genotoxicity, the reason was that the animal tests had not been stringent enough. Therefore, they argued, all carcinogenicity tests in animals should involve groups exposed to maximum tolerated doses (MTD). Accordingly, in the United States, the National Cancer Institute (NCI) and subsequently the National Toxicology Program (NTP) embarked on a massive programme involving the testing of selected chemicals, including several pharmaceutical agents, in rats and mice at the MTD and half the MTD. Broadly speaking, the results of this programme to date have, far from resolving anything, simply multiplied the chaos. Certainly, some genotoxins previously thought to be non-carcinogenic have been found under more stringent test conditions to be carcinogens, but so have many non-genotoxins (e.g. butylated hydroxy anisole, BHA)! Furthermore, in many cases in which positive results have been obtained in both types of

test, there is no obvious relationship between the positive result in the one and that in the other. Not surprisingly, we are presently witnessing an outcry against the use of the MTD as a basis of choice of dosage in carcinogenicity tests, and, at long last, thought is beginning to begiven to the importance of comparative metabolic and pharmacokinetic data in the design and interpretation of toxicity tests on agents which appear to be acting as procarcinogens (3). Examples of the saturation of normal detoxification pathways and consequent increased generation or persistence of electrophilic metabolites under conditions of prolonged high exposure are increasingly finding their way into the literature.

This is a significant development, but an even more significant one is the recognition that electrophiles are constantly being produced in the body by the normal metabolic processes that are the essence of life. In 1983, Bruce Ames (4) stunned many cancer research scientists with a review in Science in which he listed numerous mutagens, potential carcinogens and anti-carcinogens that are present in commonly consumed natural foods. Thus, he wrote "There are large numbers of mutagens and carcinogens in every meal, all perfectly natural and traditional. Nature is not benign". То Ames' list, Japanese scientists are presently busily adding a further long list of mutagens and potential carcinogens that are formed during the cooking of foods (5,6).

Thus, we are presented with an entirely new scenario. No longer should we be deceiving ourselves that Nature is benign and that before the days of synthetic chemicals humans did not develop cancers. Instead we should regard the fact that any man survives until the age of three score years and ten without developing numerous cancers as a matter for wonderment. Every part of every cell in the body is under constant bombardment from endogenously generated electrophiles. DNA is constantly being damaged and repaired. Damaged cells are constantly being shed and replaced. Damage which cannot be repaired accumulates in the form both of degenerative diseases and of logarithmically increasing risk of cancer development.

It is against this rich and colourful background, and not against a pure white background, that we have to try to assess whether proposed new drugs are likely to increase the risk of any form of cancer.

LABORATORY TESTING OF DRUGS FOR CARCINOGENICITY

The dose level to be tested

The ideal drug would be one which (i) has only one form of biological activity, namely, the one required for therapeutic purposes, and (ii) can be administered by an appropriate route, in the right dosage and at the right Few drugs approach this ideal. Most have unwanted time. activities. In the past, carcinogenic potential has all too often been regarded simply as an undesired side effect which is not related to the therapeutic activity of the drug concerned. In practice carcinogenicity is usually an unavoidable consequence of disturbance of physiological status attributable to the pharmacological activity of the agent. The requirement to test drugs for carcinogenicity at the MTD more or less guarantees that they are given to laboratory rodents at dose levels which disturb their physiological status - often seriously. Testing at such high levels may or may not be appropriate depending on the safety margin between the therapeutic dose and the minimal toxic dose in man. If the normal clinical use of a drug can lead to disturbances in the physiological status of patients, then it is not unreasonable to require that tests for chronic toxicity/carcinogenicity in animals should include dose levels high enough to cause comparable disturbances. On the other hand, it makes no sense at all to insist on

106

tests being carried out at the MTD when this exceeds the clinical dose by a multiple of 10, 100 or even more and where no disturbance of physiological status is seen during the recommended clinical use of a drug. <u>The physiological status of untreated control</u> <u>animals in carcinogenicity tests</u>

The assumption that one can take an animals species out of the wild, breed it in captivity and maintain it in normal physiological status is, <u>a priori</u>, a bold one particularly if in the course of doing this there is interference with the genetic pool, if animals are seriously deprived of exercise, if they are perpetually bored, if they are completely deprived of sexual fulfulment, if they are obliged to eat a monotonous and overnutritious diet without choice, and if they are provided with free access to food throughout the 24 hours of each day.

At a recent meeting in the United States, Conybeare (7) listed the indicators of disturbance of physiological status that are almost universally seen in untreated control rats in 2-year or life-time carcinogenicity studies (TABLE 2).

Particularly relevant, of course, is the fact that disturbed physiological status predisposes to high spontaneous tumour incidence both in rats and mice. In rats, most of the tumours are of endocrine glands or hormone-sensitive tissues, whereas in mice all sites are affected but particularly the lung, liver and lymphoreticular system.

By restricting food intake it is possible partly to normalise the physiological status of caged rats and mice. But it is by no means yet certain whether complete normality can be achieved. Reduced caloric intake leads to striking reductions in the incidences of all the disturbances listed in TABLE 2, including big reductions in the incidences of most kinds of neoplasm.

TABLE 2

COMMONLY OBSERVED INDICATORS OF ABNORMAL PHYSIOLOGY (From Conybeare, 1986)

A			
Obesity		:-	Sluggish behaviour Poor coat condition
Premature	DISEASE	:-	Myocardial degeneration Polyarteritis Glomerulonephritis
Endocrine	GLAND AND ASSOC	ΙΑΤ	ED TISSUES :- IRREGULAR OESTROUS CYCLING PREMATURE CESSATION OF REPRODUCTIVE CAPABILITY INCREASED HYPERPLASIA INCREASED NEOPLASIA
Non-endoci	RINE TISSUES	:-	INCREASED NEOPLASIA

Premature Death

TABLE 3

1

EFFECT OF TIME-RESTRICTED ACCESS TO FOOD ON BODY AND ORGAN WEIGHTS AND BONE LENGTHS (MALE WISTAR RATS AGED 19 MONTHS)

					(FROM	Conybeare 198	6)	
		W	EIGHTS	(G)		LENGTHS (MM)		
	Body	LIVER	Kidney	Heart	Brain	MANDIBLE	Femur	
24-hr/day fed	493	18.1	3.85	1.54	2•37	28.4	39.7	
6-HR/DAY FED	426	12•6	3.06	1.38	2•38	28•5	39•7	
		LIVER: WT		Kidney: wt		Heart:Brain wt		
24-hr/day 6-hr/day		7.6 5.2		1.6 1.2	29	0.65 0.58		
		LIVER: WT		Kidney: WT				
24-hr/day 6-hr/day		0.0 0.0		0.00 0.00				

Diet restriction does not imply undernutrition

It is our view that all toxicity and carcinogenicity tests in animals should be conducted under conditions of controlled feeding. The easiest way to reduce food intake is to restrict access to food to about 6 hours per day. This is equivalent to cutting caloric intake to about 80% of that of 24-hour/day ad libitum-fed animals. Under these conditions, animals are not stunted. Their bodies weigh less but their bone lengths and brain weights are the same as those of 24-hour/day-fed animals. They exhibit much less adipose tissue and smaller livers and kidneys (see TABLE 3). The right way to look at these data is to regard the figures for 6-hour/day as closer to physiological values than those for the 24-hour/day-fed animals. When one does this for the 19-month-old male Wistar rats considered in TABLE 3, one at once sees that the 24-hour/day-fed animals have enlarged livers and kidneys compared with the 6-hour/day-fed animals. This is true in terms of absolute weights, liver or kidney:brain weight ratios, or even liver or kidney:body weight ratios. If now one compares phenobarbitone-sleeping times in 6-hour/day-fed and 24-hour/day-fed rats, one finds that the latter behave as if they are already in a microsomal enzyme-induced state. Thus, their sleeping times are shorter.

Misleading results from carcinogenicity studies conducted in unphysiological animals

There are, at present, relatively few data from carcinogenicity studies conducted under conditions in which animals remain in normal physiological status. In the case of known potent genotoxic carcinogens, positive results are obtained irrespective of the physiological status of animals. It is, however, in relation to non-genotoxic carcinogenicity that the use of unphysiological animals is a serious source of misleading data. Under conditions of overfeeding, the administration of diets or agents which increase body weight also increase tumour incidence (e.g. 20% sucrose) increases the incidence of liver tumours in mice (8). Bv contrast, agents which decrease body weight gain non-specifically usually decrease but occasionally increase the incidences of tumours (9). Similarly, test drugs which reduce body weight gain tend, non-specifically, to reduce the incidence of a wide spectrum of age-related degenerative diseases. In these cases we are faced with the paradoxical situation that with increasing toxicity in terms of reduced weight gain, there is a dose-related benefit in terms of longevity and the incidence of various degenerative diseases.

It seems that rats, as a species and particularly if they are overfed, have difficulty in coping with diets containing unnecessarily high levels of phosphate and/or calcium. They readily develop various forms of nephrocalcinosis under such conditions. Overfeeding which predisposes to chronic progressive nephropathy in rats magnifies the problem and so does any factor which increases the absorption of calcium from the gut. Dietary lactose has this latter effect and so do some drugs. Recently, we found a relationship between increased calcium absorption and increased incidence of adrenal medullary hyperplasia and neoplasia in rats (10). The data for lactose are shown in TABLE 4. Since nephropathy compromises the capacity of the rat to excrete excess calcium, it is perhaps not surprising that we have recently found there to be a highly significant correlation between severity of nephropathy, severity of adrenal medullary proliferation and the incidence of phaeochromocytoma. Thus, in a carcinogenicity study on a prospective drug conducted in overfed rats, most of the males in all groups including the controls developed slight to severe nephropathy. Treatment was associated

in a dose-related manner with increases in both the severity of the nephropathy and in the incidence and severity of proliferative changes in the adrenal medulla (TABLE 5). After correction for the grade of nephropathy, there was no effect of treatment on the adrenal medulla. Almost certainly this problem would never have arisen had physiologically normal (i.e. not overfed) rats been used for the study.

A GLANCE INTO THE FUTURE

ļ

It is, I hope, obvious from what I have said, that the science underlying the present approach to the testing of proposed new drugs for carcinogenicity is in a state of turmoil. Firstly, the epidemiologists are telling us that probably only 1% or less of human cancers are attributable to drugs compared with 30% due to smoking and 35% to diet and general life-style factors. Secondly, Bruce Ames is telling us that DNA-damaging substances (i.e. mutagens) abound in Nature and in the food we eat and that such substances are even produced within our own bodies during normal life processes. Thirdly, the results of carcinogenicity tests in animals are chaotically confused not only because rats and mice, like humans are prone to develop cancers spontaneously, but also because virtually all such tests are conducted on unphysiologically maintained animals. Fourthly, Regulatory Authorities tend to demand that carcinogenicity tests on drugs are carried out at maximum tolerated doses irrespective of the relationship between such doses and those used clinically. However, the coup de grace has been the tendency for Authorities to accept an increase in the incidence of one or other form of tumour in an animal study as evidence of carcinogenicity irrespective of the circumstances or any consideration of the mechanisms involved and irrespective of the fact that the incidence of tumours of other kinds is reduced. For these various reasons we have reached a

TABLE 4

(50/GROUP)		
	Control	20% lactose
% OF RATS WITH ADRENAL MEDULLARY HYPERPLASIA OR NEOPLASIA	36	68 ⁺
🖁 WITH PHAEOCHROMOCYTOMA	20	40 +
% WITH MALIGNANT PHAEOCHROMOCYTOMA	6	18
% with Leydig-cell tumour	4	24 +
% WITH PANCREATIC ISLET-CELL TUMOUR	14	2 -
% WITH PITUITARY TUMOUR	24	14

INCIDENCES OF CERTAIN ENDOCRINE TUMOURS IN MALE RATS EXPOSED TO A DIET CONTAINING 20% LACTOSE

+ = SIGNIFICANTLY HIGHER THAN EXPECTED (P<0.05) - = SIGNIFICANTLY LOWER THAN EXPECTED (P<0.05)

TABLE 5

ASSOCIATION BETWEEN GRADE OF NEPHROPATHY AND ADRENAL MEDULLARY HYPERPLASIA/NEOPLASIA IN 196 MALE RATS AGED 26 MONTHS

	NEPHROPATHY		
Adrenal medulla	<u>0-2</u>	<u>3-5</u>	
Hyperplasia grade 0-2	106	48	
Hyperplasia grade 3-5 and/or phaeochromocytoma	12	30	
$\chi^2 = 20.8$			

P<0.0001

low ebb in the rate at which new drugs are being licensed. Are there grounds for hoping for better in the future?

Undoubtedly, the most important change that is needed is in the interpretation of data. Regulators must consider mechanisms and must recognise that there are many non-genotoxic carcinogenic mechanisms by which tumour incidences may be increased or reduced in animals maintained in an unphysiological state or when animals are exposed to unrealistically high and unnecessarily toxic doses of prospective drugs.

In this day and age, it is unusual for a genotoxic agent to be developed as a drug except, possibly, for the treatment of a life-threatening condition. Consequently, when positive results arise in carcinogenicity studies on prospective drugs they usually come as a shock. Nevertheless, such shocks are all too common. Most of them are examples of non-genotoxic carcinogenicity and many of these only arise because unphysiologically-maintained animals are used or animals are exposed to unrealistically high doses which disturb their physiological status.

Regulatory Authorities are bound to exercise extreme caution, nevertheless they are gradually becoming more responsive to persuasive data based on good science. When I am asked to assess the status of a drug in relation to carcinogenic risk for man, I ask to see all the available general toxicological, metabolic, pharmacokinetic and human data and not just the results of carcinogenicity and mutagenicity tests. Given all this information, I feel that I am much less likely to conclude either that a truly carcinogenic drug is safe for man or that a truly safe drug poses a cancer risk for man. REFERENCES

- Doll R, Peto R (1981) J Natl Cancer Inst 66:1191-1308
- 2. Cairns J (1975) Nature 255:197.
- Reitz RH, Watanabe PG (1985) In: Hoel et al (eds) Banbury Report 19: Risk Quantitation and Regulatory Policy. Cold Spring Harbor Laboratory, p 241.
- 4. Ames BN (1985) Science 221:1256-1263.
- Sugimura T, Sato S, Ohgaki H, Takayama S, Nagao M, Wakabayashi K (1986) In: Knudsen I (ed) Genetic Toxicology of the Diet. Alan R Liss Inc, New York, pp 85-107.
- 6. Sugimura T (1985) Mutation Res 150:33-41
- Conybeare G (1986) Interdisciplinary discussion group on carcinogenicity studies, June 23-26, International Life Sciences Institute Nutrition Foundation, Chapel Hill, N. Carolina.
- Hunter B, Graham C, Heywood R, Prenctice DE, Roe FJC, Noakes DN (1978) Huntingdon Research Centre Report No. HLR/25/77774
- 9. Haseman JK (1983) Fund & App Tox 3:1.
- 10. Roe FJC, Baer A (1985) Human Toxicology 4:27-52.

Discussion - Drugs and chemicals : carcinogens, procarcinogens and promoters.

G.J. Mulder

I think that you have quite clearly shown that we overfeed our rats and shorten their lifetime, but I have two questions. What do the diet restricted animals die of, and what is the incidence of tumors in these animals when they die?

F.J.C. Roe

Late in life restricted animals develop some of the age related diseases that are seen earlier and in higher incidence in overfed animals. The pattern of disease often is not extremely changed but this varies from study to study. In many studies the overall incidence of tumors, both benign and malignant, is highly significantly less, even though the animals live longer.

L.F. Prescott

Presumably both in overfed animals and in those with restricted access to food the diet is artificial and very different from the normal diet for a rat.

F.J.C. Roe

I am not sure how to answer that. It is usually a regular laboratory chow. Whether that is a "natural" diet is really another question. It probably is not. I feel that animal nutritionists have done us great disservice over the years since they have based their philosophy for designing laboratory diets on the same assumptions as for farm animals. The quicker the animals get fat for the least cost, the better. It is as though we were going to eat our rats and mice at the age of about ten weeks!

P.G. Watanabe

While numerous governmental regulatory bodies have instituted classification categories for carcinogenic activity of chemicals, it appears that implementation of these classification schemes leaves little flexibility for scientific interpretation. There is little debate concerning those materials that have demonstrated human carcinogenic potential by well conducted, replicated, epidemiologic studies in conjunction with evidence in animals. However, considerable controversy concerning potential human risk exists for those materials that show tumorigenic activity at very high doses in animal studies. Frequently these studies demonstrate an increase in tumors at sites with a considerable spontaneous background and only after lifetime exposure. The animal results often do not coincide with epidemiologic evidence or historical experience in humans creating a dilemma of potential human However, if the animal bioassays are interpreted relevance. properly they may be totally consistent with lack of epidemiologic evidence due to pharmacokinetic and/or pharmacodynamic properties operating differently in high dose animal studies when compared to much lower exposure frequently in the human environment. Moreover, as discussed in this presentation, the practice of administering an unrestricted diet to rodents as well as specific or nonspecific stimulation of endocrine or other pharmacologic/toxicologic effects by ultra high doses, may confound results in animals having little relevance to humans at lower doses. The only scientifically acceptable alternative is to examine, in a comprehensive manner, all data pertaining to a material's potential for carcinogenic activity at relevant exposure levels for humans. Such an evaluation would include review of pharmacology, toxicology, molecular mechanisms, genetics and also the entirety of animal bioassays both negative and positive. Science and society would be better served by such an approach.

The imposition of rigid classification schemes, some compartmentalized to consider only one species at a time, does little to encourage sound scientific interpretation and instead encourages a "lowest common denominator" mentality which results in a default to a decision based on political economic factors. While it is acknowledged that sociopolitical factors are involved in such decisions, the scientific input should be made by scientists with expertise in the interpretation of technical data.

O. Pelkonen

I completely agree with Dr Roe that all of us who are interested in cancer and carcinogens should every now and then think about the definitions we use in the field of chemical carcinogenesis, but I do not think that it is fair or even useful to discard the concept of tumor promotion.

F.J.C. Roe

Certainly, I do not think that we can quite discard with the term "tumor promoter" yet. It is too much built into the way many people think and talk, but I would warn against its over free and careless use. At present the term means different things to different people. It would be much better if we restricted ourselves to precisely defined terms, based on an understanding of mechanisms.

G.L. Plaa

Of course, the difficulty with classifications is not knowing the mechanisms, but from a toxicological standpoint and from a regulatory standpoint, one still needs to classify these compounds. In an admitedly simplistic manner I make a differentiation between a compound that can produce a new tumor that does not normally appear in an animal, and one that accelerates the appearance of spontaneous tumors. For instance, aflatoxins can produce a tumor in animals after only a few weeks or a couple of months of exposure, and this is quite different from the case of a substance that after a life-time treatment produces simply an increased number of spontaneous tumors. Would you comment on these cases?

F.J.C. Roe

I sympathize with what you are saying, but the trouble is that there are all points in between those two extremes. There are clearly genotoxic compounds which in one system can fall into one of your categories and in another system can fall in the other. It is even possible that the same compound, tested in the same system, falls in one category or in the other depending on the dosage.

H. Vainio

A couple of years ago, the possibility that chemical carcinogens could be classified according to their mechanism of action was discussed in Lyon to a very great extent, and at that time the consensus was that we do not have methods that permit such a classification. On the other hand, I am afraid that the general impression that we have received after this presentation concerning the value of bioassays in the prediction of chemical carcinogenesis, is quite a pessimistic one.

F.J.C. Roe

I know it is IARC's stance and other people's stance that we do not really know anything about mechanisms, but this is really nonsense because we know a lot about mechanisms. The problem is that there are people who are really unwilling to look at what we know, and the reason they are unwilling is that the minute one starts looking at mechanisms it becomes highly complex. People are looking for nice, neat solutions. They want to have a test for promoters and when people like myself get up and say that there are a thousand and one different forms of promotion they say forget it, we still want a nice simple test for promotion and since we do not know the mechanism anything will do. However, we do know a lot about mechanisms and we would know even more, particularly in the whole animal, if we bothered to look at such factors, such as I was talking about, that affect the incidence of tumors in untreated animals. Now as to pessimism about to bioassays, I am pessimistic only about the ways studies are still done. I think that we could do them much better. The trouble is that the regulatory system is a long way behind in its thinking and in still believing that nice simple formulae exist.

N.I. Redmond

What are then your recommendations concerning bioassays in carcinogenesis and their interpretation?

F.J.C. Roe

As Dr. Watanabe said, the really important thing is that interpretation should be based on all the data which exist. In a carcinogenesis bioassay the final result is not simply the number of tumors that one sees at the end of one study. Whether or not one is dealing with a carcinogen depends on all the information which one has, particularly pharmacokinetic information, metabolism, pharmacological effects, the results of short and long-term toxicity studies, and of mutagnicity tests. As for the future, I think we will end up with doing better assays. Diet restriction and perhaps monitoring of animals for levels of circulating hormones at regular intervals may play a role in this regard.