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The importance of dietary control during toxicological studies

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ABSTRACT

When pathogen free rats and mice are fed ad-libitum they become obese, die early, exhibit major endocrine abnormalities and very high incidences of a variety of tumours. Such animals are not suitable models for the safety evaluation of chemicals for man. All toxicity and carcinogenicity tests should be conducted under conditions of controlled feeding. This may be achieved simply by limiting the proportion of each day during which animals have access to food. It is not necessary to weigh out rations of food for each animal each day.

KEY WORDS

Carcinogenesis, diet-restriction, endocrine status, obesity, overfeeding, safety evaluation, tumour incidence.

It goes without saying that the protocols for animal studies in the field of toxicology must include control groups which are comparable in every way to treated groups except in relation to exposure to the test material. The main point I shall seek to make in this paper is that even in properly controlled experiments, overfeeding and other abnormal aspects of the laboratory environment, can render the interpretation of studies difficult or impossible.

It will be easier for me to explain what I mean if I put the matter into a historical perspective. My main interest for over 30 years has been in the field of carcinogenesis. However, the matters to which I now seek to draw attention are not only relevant to the interpretation of carcinogenicity tests. They are also relevant to the interpretation of chronic toxicity tests and possibly shorter-term tests too. In the 1950's and 1960's my studies in the field of cancer research mainly involved the exposure of animals to known potent carcinogens, in response to which they developed tumours in the matter of a few months. Even so, many experiments were cut short by outbreaks of fatal disease. Many is the time our mouse colony was dessimated by ectromelia, while it was a regular feature of rats that they suffered from debilitating and eventually fatal chronic respiratory disease and/or chronic progressive nephropathy. Such was the prevalence of disease that we dared not plan a study to last for much more than 18 months.

When in the 1960's the main thrust of my research changed from studies on known carcinogens to attempts to test hitherto uninvestigated substances for carcinogenicity, it was clearly essential to build facilities in which animals could be kept free of disease. And so, along with others, we designed and built a Specified Pathogen Free (SPF) animal unit. In this barriered unit we built up strains of rats and mice from Caesarian-derived, hand-reared stock. In this way we solved the problem of early death from infectious disease, but created a new problem, or maybe just exaccerbated an existing problem of which we were up to then unaware.

It was necessary to feed our SPF animals on diets that were free of pathogenic microorganisms and parasites. But if we autoclaved the feed, the pellets broke up unless we increased the fat content. Thus it was that we came to feed our animals a relatively high fat diet. On this, successive generations grew more and more obese. But that was not all, we began to notice in rats, ever increasing incidences of endocrinological abnormalities and neoplasms of endocrine glands and of hormone-response tissues (e.g. mammary gland). Thus it would be quite usual for untreated control rats at the end of a 2-year test to exhibit a 50-100% incidence of pituitary tumours and close to a 100% incidence of mammary tumours. At the same time the incidence and severity of chronic progressive nephropathy were, if anything, increased compared with disease-ridden conventional rats. Our SPF mice were also giving us troubles. They too suffered from gross obesity and high tumour incidence. The only difference was that the tumour sites mainly affected were the liver, the lung and the lympho-reticular system (i.e. malignant lymphoma).

So we asked ourselves, what were we doing wrong? Part of the answer came with Mary Tucker's observations in the early 1970's, when she observed that obesity and high tumour incidences could be abolished by simple diet restriction (Roe and Tucker, 1973; Tucker, 1979; Conybeare, 1980). Another part of the answer came with Morton Gellatly's clear demonstration of the influence of dietary fat content on the incidence of liver tumours in mice (Gellatly, 1975). It is sometimes said that 'there is nothing new under the sun'. So it is quite easy to find buried in the literature, many earlier papers which illustrate the association between amount and composition of diet and tumour risk. However, what was new was the recognition that diet restriction, in rats at least, reduced tumour incidence by restoring animals to an endocrine status that was less abnormal than that which is characteristic of ad libitum-fed animals (Roe, 1981)

By restricting the food intake of rats or mice to between 75% and 85% of the amount that ad libitum-fed animals eat, the animals can be rendered slimmer, healthier, with better coats, more lively, and longer-lived. Moreover, the diet-restricted rats had far less chronic progressive nephropathy than their ad libitum fed counterparts. Recent evidence suggests that this beneficial effect on the kidneys is also hormone-mediated. One of the features of ad libitum-fed rats of both sexes is that they exhibit very high levels of prolactin in the blood. Diet-restriction reduces the incidence of hyperplasia and neoplasia of prolactin-producing cells in the pituitary. Bromocriptine, which inhibits prolactin secretion, dramatically reduces the incidence of chronic progressive nephropathy of rats. It is thus tempting to suspect that diet restriction reduces nephropathy by restoring circulating prolactin levels to normal.

My personal interest in the value of diet-restriction stemmed from the fact that I found it difficult or impossible to interpret the results of carcinogenicity tests in which close to 100% of the untreated controls had one or multiple neoplasms. I simply could not believe that such animals were appropriate models for man. However, I should have been just as concerned if I had been trying to interpret chronic toxicity tests on, say, new drugs. How could I expect to pick up a

chronic toxic effect on the kidney in an experiment in which 100% of the controls were suffering from chronic progressive nephropathy?

Personally, I am now convinced that the ad libitum feeding of laboratory rodents, particularly in long-term tests, is fundamentally unphysiological and wrong, and that all such experiments should be conducted under conditions of controlled feeding. The results of a recent study indicate that restriction need not be by the laborious method of providing animals with a weighed daily ration. Instead it may be more simply achieved by limiting the period of each 24-hour day during which animals have access to food, for example, to just 6 hours.

Not surprisingly, Regulatory Agencies and testing laboratories are reluctant to modify the way in which they carry out tests, because change would devalue their stores of background data. Nevertheless, I am sure that change must come and that one day it will become a requirement that for tests to be valid, they must be carried out in animals which are manifestly normal from the viewpoint of nutritional and endocrinological status.

However, I must end on a note of caution. Diet-restriction does not solve all the problems that are evident with present day laboratory animals. In some studies even diet-restricted animals exhibit evidence of abnormal endocrine status and incidences of pituitary and other tumours that are still higher than seen in man. Thus, there may be other aspects of the laboratory environment that require attention. In this context, clearly further research on the composition of laboratory animal diets merits high priority. The diets we use today were, for the main-part, formulated by nutritionists whose concept of a good diet was one that gave rise to maximum growth rate during the period from weaning to early adulthood. Yet maximum growth at this time is associated with enhanced cancer risk (Ross et al, 1982). What we need is a diet, or a series of diets suitable for animals of different ages, designed to sustain animals in good health and normal endocrine status until they are old. Such animals would be a much more appropriate model than the obese, tumour-ridden, endocrinological cripples that fill our laboratories today.

Conybeare, G. (1980): Effect of quality and quantity of diet on survival and tumour incidence in outbred swiss mice. Fd. Cosmet. Toxicol., 18, 65-75.

Gellatly, J.B.M. (1975): The natural history of hepatic parenchyman nodule formation in a colony of C57BL mice with reference to the effect of diet. Chapter in "Mouse Hepatic Neoplasia", Edited by W.H. Butler and P.M. Newberne, Elsevier: Amsterday, pp. 77-109.

Roe, F.J.C. (1981): Are nutritionists worried about the epidemic of tumours in laboratory animals? Proc. Nutr. Soc., 40, 57-65.

Roe, F.J.C. and Tucker, M.J. (1973): Recent developments in the design of carcinogenicity tests on laboratory animals. In Experimental model systems in Toxicology and their significance in man. Proceedings of the European Society for the Study of Drug Toxicity, 15, 171-177.

Ross, M.H., Lustbader, E.D. and Bras, G. (1982): Dietary practices of early life and spontaneous tumors of the rat. Nutrition and Cancer, 3, 150-167.

Tucker, M.J. (1979): The effect of long-term food restriction on tumours in rodents. Int. J. Cancer, 23, 803-807.

Quand les rats et les souris exempts d'organismes pathogènes sont nourris ad-libitum, ils deviennent obèses, meurent jeunes, présentent de graves anomalies endocriniennes et une incidence très importante de toute une variété de tumeurs. De tels animaux ne sont pas des modèles convenables pour l'évaluation de la sécurité des produits chimiques destinés aux hommes. Toutes les études de toxicité et de carcinogénèse devraient être réalisées dans des conditions contrôlées de nourriture. Cela peut être réalisé simplement en limitant la durée durant laquelle les animaux ont chaque jour accès à la nourriture. Il n'est pas nécessaire de peser quotidiennement les rations de nourriture pour chaque animal.