MICROBIOLOGICAL STANDARDISATION OF LABORATORY ANIMALS

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The Chairman's summing-up

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Broadly, I feel that we nowadays do have the knowledge and the means to produce essentially disease-free animals for research. Admittedly not all animal suppliers maintain the same high standards of our hosts today, and those who normally achieve high standards occasionally fall temporarily from grace. Nevertheless, we have during the past ten to fifteen years moved from the position where scientists engaged in biologically precise work were trying to persuade and educate animal suppliers to provide clean animals, to a position where educated animal suppliers are trying to persuade recalcitrant conservative animal users that the quality of their research would be greatly improved if they not only started their experiments with clean animals but also invested in facilities that enabled them to maintain animals under clean conditions in which they remain in good health. Personally, I believe that the use, in 1981, of randomly diseased and parasitised animals for any form of research is scientifically indefensible.

On the other hand, I have reservations about the advantages of inbred over random-bred animals for some kinds of experiment. If the animal is being used as a substitute for a test tube in order to measure some kind of biological activity for which there is presently no chemical or physical test, then obviously, inbred animals offer advantages: fewer are needed, variation in response is less, and confidence limits are narrower. But where the results of animal studies are to be extrapolated to man, inbred animals may give wholly misleading results. Some of the characteristics of inbred strains that have been deliberately or accidentally bred into them

have little or no general human counterpart. Furthermore, I am suspicious that certain viral diseases have flourished because of inbreeding. I believe that Dr Rutty shares some of my doubts in this regard.

With the cleaning-up of laboratory animals, we have been able to see clearly for the first time problems that have probably been there all the time. In addition, as Mr Sebesteny pointed out, we have, during the cleaning-up process, introduced new problems. In this connection he mentioned the examples of vitamin K deficiency and vitamin E deficiency which occurred because these vitamins were destroyed during the pasteurisation of diets. I would add to this list the problems that we are now seeing because too much fat is added to diets. Without any thought of the nutritional consequences, the proportion of fat has been increased because a high fat content helps to prevent a pelleted diet from crumbling on exposure to steam used for sterilising purposes. Table 1, taken from Gellatly (1975), illustrates one disastrous effect of doubling the concentration of fat in a semisynthetic (SS) diet.

Table 1 – Incidence of liver tumours in C_{57}BL female mice fed on a semisynthetic diet (SS) containing 5% or 10% ground nut oil (GNO).

	Mice with liver tumours (%)	
	Benign or malignant	Malginant
SS diet with 5% GNO	8	1
SS diet with 10% GNO	43	9

From Gellatly (1975).

Dr Turnbull emphasised that he wants to know from animal suppliers precisely what animals have been fed on before they arrive at his laboratory. He is quite right to want to know this because nutrition during the early days of life can have profound effects on the subsequent growth and health of animals. Dr Rutty said a few things about the effects of dietary restriction on tumour incidence. I will, if I may, extend what he said.

Nutritionists are in general obsessed by the spectre of deficiency disease, but give much less thought to the consequences of over-nutrition. Their concept of the best diet is one that achieves the most rapid growth most efficiently during the first period of an animal's

life. By comparison, they have given little thought to the diseases of overnutrition, and no one has warned the average experimentalist involved in long-term studies that if he goes on feeding unlimited amounts of unsuitably rich diets to animals throughout their lives they will unnecessarily develop a whole galaxy of diseases which will interfere with the results of experiments and render their interpretation difficult.

When animal colonies were cleaned-up many sources of stress were removed from the environment of the animal laboratory. I think that this stress, as undesirable as it was in other ways, served to protect animals from the effects of overnutrition. Our pathogen-free animals today lack exercise, lack sexual fulfilment (despite sitimulation by the smell of the opposite sex often housed in the same room), are provided *ad libitum* with unsuitably rich diets, and lack any form of stress at all. In addition they now live long enough for us to see in clear relief the evil effects of the artificiality of the conditions in which we keep them.

It is my strongly held view that there is an urgent need for us to reconsider the nutritional and social requirements of small laboratory animals particularly during long-term experimentation.

Table 2 summarises tumour incidence data for untreated control Sprague Dawley rats as reported by Kociba *et al.* (1979). Is it reasonable to accept, for example, a 63% incidence of pituitary tumours

Table 2 – Hormone-associated neoplasms (%) in *ad libitum* fed untreated control Sprague Dawley rats observed for up to 26 months (86 rats of each sex).

		♂	♀
Pituitary		31	63
Adrenal	– cortex	2	7
	medulla	51	8
Thyroid	– C-cell	8	8
Parathyroid		0	1
Pancreas	– exocrine	33	0
	endocrine	16	9
Testis		7	–
Ovary		–	5
Mammary	– fibroadenoma		76
gland	adenoma	5	12
	other		29

From Kociba *et al.* (1979).

and a more than 80% incidence of different kinds of mammary tumours in females, or a 51% incidence of adrenal medullary tumours and a 33% incidence of exocrine tumours of the pancreas in males as a suitable background for conducting a meaningful carcinogenicity study?

It is notable that most of these high-incidence tumours are of endocrine glands or sex hormone-controlled tissues. This, along with the fact that elderly control animals exhibit high incidences of non-neoplastic changes in the same tissues indicates that they are in severe disarray with regard to hormonal status.

Elsewhere (Roe 1981) I have pointed out that part of this disarray, but only part of it, can be rectified by simple and non-severe dietary restriction. By comparison with *ad libitum*-fed animals, diet-restricted animals are less obese, more active, and sleeker. Furthermore they live longer, and despite this their lifelong expectation of tumour development is significantly reduced. Surprisingly this reduction is not confined to tumours of kinds that are clearly linked to hormonal status. Table 3, taken from Tucker (1979), illustrates the dramatic effects of diet restriction in relation to pituitary and mammary tumours in rats, and Table 4, taken from Conybeare (1980), does so for a variety of tumours including liver, lung, and lymphoreticular neoplasms in mice.

There is growing evidence that the effects of dietary restriction are not solely attributable to reduction in calorie intake. The mere fact of an animal being faced for a part of each day by an empty food basket seems to be important. In the absence of a visible source of food, plasma cortico-steroid levels rise and it is perhaps the regular occurrence of this that protects animals from the ravages of progressive hormonal disarray. One marker of such increasing disarray in rats is the pattern of pathologically high and rising serum prolactin levels with age from about the sixth month of life onwards (Table 5).

I suggest, then, that one of the biggest challenges for the future is to devise ways of maintaining untreated laboratory animals in normal hormonal status throughout their lives. Until we do this it is unreasonable to regard them as models for the study of age-related disease in humankind, or as suitable for the testing of chemical agents for carcinogenicity. I hope that this can be an area in which the researchminded animal breeder as well as the nutritionist can play a big role, but the real breakthrough may come only when we have available methods for monitoring the hormonal status of individual living rats and mice – that is to say micro-methods that entail the analysis of only very small samples of blood. When we have these methods, then it will be possible to investigate the influence of the

Table 3 – Effect of dietary restriction on incidence of pituitary and mammary tumours in rats

Feeding regimen	Males		Females	
	Ad lib.	Restricted	*Ad lib.*	Restricted
Rats with pituitary tumours (%)	32	0***	66	39**
Rats with mammary tumours (%)	0	0	34	6***

$^{**}P < 0.01$; $^{***}P < 0.001$.
From Tucker (1979).

Table 4 – Effect of simple dietary restriction on tumour incidence in mice. Number of mice which developed tumours at any time during the study. There were 160 mice of each sex in each group.

Feeding regimen	Males		Females	
	Ad lib.	Restricted to 75% of *ad lib.*	*Ad lib.*	Restricted to 75% of *ad lib.*
Type of tumour				
Lung	30	19*	24	8**
Liver	47	12***	7	1*
Lymphoma	4	1	11	4*
Other	8	4	12	4*
Any tumour at any site	71	36***	50	17**
Any malignant tumour	17	7*	23	7**

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
From Conybeare (1980).

Table 5 – Serum prolactin levels in *ad libitum*-fed Sprague-Dawley rats.

Age (months)	ng/ml	
	0	0
2	26	21
3	27	37
4	28	34
7	35	74
13	128	214
19	119	345

N.B. Level in non-pregnant women = 20 – 40 ng/ml.

unnatural aspects of the life of caged laboratory animals listed in Table 6.

Table 6 – Unnatural aspects of the life of a control rat.

1. Food available 24 hours per day
2. Excessively nutritious diet
3. No need to forage
4. Doesn't have to avoid predators
5. Enforced celibacy despite sexual stimulation
6. General boredom

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