The inter-relation between nutrition and pathology in rodents

Talk to be presented by Dr. Francis J.C. Roe at Labsure Conference '82 at the Albany Suite, Albany Hotel, Smallbrook, Queensway, Birmingham on Tuesday 19th October, 1982

Introduction

I have spent all too much of my brief life trying to evaluate the results of long-term dietary studies in laboratory rodents and I often wonder whether we do not expect too much from the control groups included in our experimental designs.

In a 2-year rat study which I am presently evaluating, 67% of the male controls have moderately severe to very severe glomerulonephritis. A similar percentage have some degree of hyperplasia of the parathyroid glands, 34% show calcification of the aorta, 32% exhibit adrenal medullary hyperplasia and/or neoplasia, 20% have adrenal medullary tumours and 83% have some degree of chronic myocarditis with focal fibrosis.

Slide 1

Now there is nothing terribly unusual about these findings particularly as far as the incidence of neoplasms is concerned. Kociba (1979) reported high incidences of a variety of tumours particularly of endocrine glands and hormone-influenced tissues in untreated rats and so, more recently did Ross et al (1982)

Slide 2

Slide 3

There is no population of humans that exhibits any one of these neoplastic or non-neoplastic changes in such high incidence. Are then the rats used in these studies, proper and suitable models for assessing the possible toxicity of chemicals or other agents for man?

At this meeting we are primarily concerned with diet and our hosts are clearly especially concerned with the quality of diet. However, to my mind important though that is, quality of diet is at best much less than half the problem. The most perfect diet in the world will not protect against the dire consequences of gluttony or obsessive eating. Similarly, by choosing the best claret or the finest malt whisky, the alcoholic cannot avoid the risk of developing cirrhosis.

In the 1600's Thomas Adams said of certain humans "They have digged their grave with their teeth". In the 1980's he could have said as much for the untreated control mice and rats in most long-term studies, irrespective of the strain.

Slide 4

There will be those here who have heard me speak previously on the beneficial effects that diet restriction has on the well-being, longevity and tumour

incidence of rats and mice. I make no apology for saying the same again and for showing the same slides again.

Slide 5 6 7 I believe these facts are astounding and until those who design animal experiments pay heed to them, I propose to continue to draw attention to them over and over again.

However, in one particular I am, from now on, going to change my tune. Hitherto I have said I was comparing ad libitum-fed animals with diet-restricted animals. This suggests I regard ad libitum-feeding as 'normal' and 'right' and diet-restriction is 'abnornal' and 'experimental'. In future I propose to apply the term "controlled feeding" to experiments in which animals are given an amount of food judged, on the basis of research, to be appropriate and optimal for their age and sex and the term "uncontrolled feeding" to what is now called ad libitum-feeding. In this way I hope to persuade not only experimentalists but also feed suppliers and animal breeders that controlled feeding is right and should become the norm and that ad libitum-feeding, although presently the usual procedure, is wrong, unnatural and unscientific.

As an independent pathologist I become involved in numerous carcinogenicity studies on drugs. Hitherto all such studies have been conducted on ad libitum-fed animals because that is what ignorant Regulatory Authorities think they want and in any case that is the way in which such test have been traditionally performed. In a majority of such studies there is one or more than one problem in relation to tumour incidence. Most of these problems consist of increases in the incidence of tumours of endocrine glands or of hormonally-influenced tissues - that is to say - increased incidences of tumours of the same kinds as are influenced by overfeeding.

I give you as an example an important and interesting drug. I will refer to it as drug X. Two carcinogenicity studies in Wistar rats showed enhancement by treatment of neoplasia, or various endocrine and hormonally-influenced tissues. Slide 8 In one study in Wistar rats the incidences of 5 kinds of tumour were affected and in the other that of only 3 kinds of tumour. In both cases tumours of all the types affected by treatment were conspicuous in the corresponding controls.

While you contemplate these figures let me tell you something about Drug X. It is one of a group of drugs all of which have been found in similar tests to

enhance the incidence of one or more of the same kinds of tumour as occur in higher incidence in ad libitum fed animals than in diet-restricted animals. Like many other drugs, Drug X is a prolactin-release agent. Drug X is remarkably non-toxic in short term studies. It has been tested for teratogenicity in 3 species with unequivocally negative results. It has been exhaustively tested in vitro and in vivo for mutagenicity and clastogenicity with completely negative results. In the carcinogenicity tests to which I refer it had no effect on the incidence of those kinds of tumour which most commonly arise in response to true carcinogens (for example:- hepatocellular carcinomas, ear duct carcinomas in rats).

And so I put it to you, now much value do you attach to findings like these? Do you think that animals with these high incidences of tumours in the controls are an appropriate model for man? Considering that virtually all the pituitary tumours in both sexes are prolactinomas, do you think it sensible to have spent perhaps a £¼ m. testing a prolactin-release agent for toxicity/safety in animals
Slide 9 that are totally abnormal with respect to circulating prolactin levels?

In normal non-pregnant women serum prolactin levels remain below 20 ng/ml. In the untreated control rats as well as in the rats exposed to drug X, serum prolactin levels rose to over 1000 ng/ml - and the higher the level found during life, the greater the chance of finding a pituitary tumour at death.

Now if there is anyone of a mind to think that this high incidence of pituitary tumours in control rats is attributable to bad choice of rat strain - that is to say - to bad genes, let me make it quite clear that this is not so. These high spontaneous tumour incidences can be seen in virtually any strain of rats if they are maintained under sufficiently unnatural environmental conditions.

The figures I showed you from Ross et al were for Charles River rats. Those from Kociba et al were for Sprague Dawley rats. The studies in Drug X were in
Slide 2 Wistar rats. Goodman et al (1979) reported high incidences of endocrine tumours in ad libitum fed Fischer 344 rats killed at the age of 110 weeks.
Slide 10

I conclude that ridiculously high incidences of endocrine and hormone-influenced tumours can be a feature of virtually any strain of rat kept under environmental conditions that favour endocrine disturbance.

Slide 11 Slide 11 lists unnatural aspects of a control rats life which influence its risk of developing manifestations of endocrine disturbance and the various

tumours I have been discussing.

Excessively nutritious diets

In the case of mice, Gellatly's work illustrated how doubling the fat content from 5 to 10% could increase the risk of development of liver tumours by more than 5-fold.

Slide 12

There is abundant evidence that high fat diets enhance mammary tumours in both rats and mice. Also, the recent paper of Ross, Lustbader and Bras (1982) to which I referred earlier, points to several other aspects of diet and feeding habits which affect tumour risk. In this study rats were able to select any of 3 semi-synthetic diets which differed in protein:calorie ratio. All the diets contained 13.5% corn oil and the same levels of minerals.

Slide 13 Multifactorial statistical analysis enabled the authors to identify variables which maximised overall tumour risk

Effect of dietary minerals

I am not here to talk solely about the effects of quantity and quality of diet on tumour incidence. There has been a raging epidemic of nephrocalcinosis in laboratory rats which has its origins in the mistaken belief that one's only obligation is to provide animals with adequate amounts of calcium, phosphorus and vitamin D. The fact is that many current problems stem from the over-provision of calcium and phosphorus and an inadequate provision of magnesium. Yesterday evening, our host, Mr. Turnbull, told me that Labsure has solved this problem with a new diet formula, so hopefully I am talking about an obsolescent problem.

Slide 14 Slides 14 - 22 illustrate various forms of nephrocalcinosis

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I became involved in the problem of nephrocalcinosis in rats in relation to toxicological studies on certain chemically modified starches and certain polyols.

The key to understanding what was going on came from studies on lactose

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24
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26

Three polyols, mannitol, sorbitol and xylitol also cause caecal enlargement, and increased calcium absorption. In addition, however, in high dietary
Slide 27 concentrations they increase the incidence of adrenal medullary hyperplasia and neoplasia.

Could these changes in the adrenal medulla be secondary to enhanced calcium intake and hypercalcaemia. This possibility was consistent with findings
Slide 28 reported by Brion and Dupuis (1980). I was therefore stimulated to see whether lactose causes adrenal medullary proliferation. To my delight, I found a 25% incidence of adrenal medullary hyperplasia in 20 female rats fed on 30% lactose diet for 9 months.

Slide 29

Slide 29 shows florid adrenal medullary hyperplasia in one of these rats.

This then brings me back to the basic diet. Pathologists are finding high incidences of:

(i) nephrocalcinosis
(ii) adrenal medullary changes

in untreated control rats.

Analysis has shown that most rat diets contain from 0.6 to 1.0% phosphorus and 0.7 to 1.2% calcium. I suggest that these levels are far too high, especially those of phosphorus and that excessive provision of Ca and P may be one of the factors responsible for high incidences of adrenal medullary changes in untreated control rats.

Conclusion

I leave you then with 3 take-home messages.

Slide 1

Pathology in control S-D male rats

	%
Moderate to severe glomeronephritis	67
Parathryoid hyperplasia	67
Calcification of aorta	34
Adrenal medullary - hyperplasia/neoplasia	32
- neoplasia	20
Chronic fibrosing myocarditis	83
	etc

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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 gland	- fibroadenoma		76
	adenoma	5	12
	other		29

(from Kociba et al, 1979)

Slide 3

Tumours in 119 ad libitum fed male rats

Endocrine

Pituitary	42
Islet cell pancreas	15*
Adrenal medulla	7
Thymus	3

Hormone-influenced

Mammary	1
Fibroma and other C.T.	8
Exocrine pancreas	9
Testis	2

* Appeared to protect against soft tissue tumours

Ross, Lustbader and Bras,
Nutrition and Cancer, 1982

Slide 4

They have digged their grave
with their teeth

Thomas Adams, 1612-1653

Slide 5

Effect of simple dietary restriction on tumour incidence in mice†

no. of mice which developed tumours at any time during the study. There were 160 mice of each sex in each group

	Males		Females	
Feeding regimen ...	*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**

*$P<0.05$, **$P<0.01$, ***$P<0.001$.
†Conybeare, 1980.

Slide 6

Effect of dietary restriction on 'spontaneous' tumour incidence in rats†

	Males		Females‡	
Feeding regimen ...	*Ad lib.*	Restricted	*Ad lib.*	Restricted
Food consumption (g/d)	20	15	15*	15
Survival for 2 years (%)	72	90	68	88
Tumour-bearing animals before or at 2 years (%)	66	24***	82	56*
Mean number of tumours/rat	0·94	0·27***	1·18	0·76**

*$P<0.05$, **$P<0.01$, ***$P<0.001$.
†Tucker, 1979.
‡The *ad lib.* fed females ate less than anticipated and in fact consumed only the same amount daily as the restricted animals. The difference was that the *ad lib.* fed animals were never faced with an empty food basket.

Tucker, 1979

Slide 7

Effect of dietary restriction on incidence of pituitary and mammary tumours in rats†

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

**$P<0.01$, ***$P<0.001$.

Tucker, 1979

Slide 8

Effects of Drug X on % tumour incidence in Wistar rats

	Males		Females	
	Control	Test	Control	Test
Pituitary	21	67	73	82
Islet-cell	3	42	3	28
Adrenal medulla	15	72	19	30
Thymus	6	46	20	48
Mammary	0	13	52	82

Slide 9

Data from untreated control rats and rats exposed to Drug X showing:-

(a) very high serum prolactin levels* and

(b) the relationship between these levels and pituitary tumour incidence

Serum prolactin (ng/ml)	% with pituitary adenomas
⩽200	31
201 - 500	55
501 - 1000	78
1000 +	94

*N.B. The normal prolactin level in a non-pregnant woman is less than 20 ng

Slide 10

Incidences of various tumours in untreated Fischer 344 rats (Goodman et al, 1979)

	%	
	Male	Female
Lymphoma/leukaemia	12	9
Pituitary	11	30
Adrenal medullary tumour	9	3
C-cell tumours of thyroid	6	6
Pancreatic islets	4	1
Mammary gland	2	18
Testis	81	-
Mesothelioma	2	0.2

Slide 11

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.

Slide 12

Dietary fat and liver tumours in C57BL female mice•

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

GNO, groundnut oil.
•Gellatly, 1975.

Slide 13

Variables associated with enhanced tumour risk, by Ross, Lustbader and Bras (1982)

1. High protein intake shortly after weaning.
2. High efficiency in converting consumed food to body mass at the time of puberty.
3. High protein intake relative to body weight during early adult life.
4. High level of food intake
5. Rapid growth rate during early post natal life.

Slide 14 Diagram of Rat kidney

Slide 15 Diagram showing various sites at which mineral deposits may be found in rat kidney

Slide 16 Photomicrograph depicting C-MN

Slide 17 Diagram showing different forms of PN

Slides 18-20 Photomicrographs depicting different forms of PN

Slide 21 Photomicrograph depicting deposition of calcium in basement membranes or cortical tubules in glomeruli in a rat with parathyroid hyperplasia.

Slide 22 Diagram depicting acute tubular atrophy.

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EFFECTS OF INCREASING DIETARY LACTOSE up to 60% IN RATS

1. Increasing caecal enlargement
2. Increasing calcium absorption from gut
3. Increasing Ca levels in renal tissue
4. Increasing urinary calcium
5. Increasingly severe manifestations of nephrocalcinosis

C-MN ⟶ PN ⟶ Urinary calculi ⟶ Acute tubular nephropathy

Slide 24

Comparison of control and lactose-fed rats after 26 weeks (Experiments I and II)

		Control	30% Lactose
Urinary Ca (μ mol/d)	I	41	178***
	II	30	93***
Urinary Mg (m mol/l)	I	7	9
	II	7	8
Urinary P (m mol/l)	I	35	32
	II	46	43

*** $p < 0.001$

I - 1 month at start
II - 9 months at start

(Hodgkinson et al, 1982)

Slide 25

Calcium content of kidney by chemical analysis

		Control	30% Lactose
Ca (μ mol/g dry weight	I	9	23***
	II	25	59

*** $p < 0.001$

(Hodgkinson et al, 1982)

Slide 26

Effect on caecal weight as a % of body weight

		Control	Lactose
Caecum + contents	I	1.2	2.5***
	II	1.4	2.6***
Empty Caecum	I	0.36	0.61***
	II	0.41	0.66***

*** $p < 0.001$

(Hodgkinson et al, 1982)

Slide 27

Effects of feeding 20% sorbitol or xylitol on adrenal medulla

	♂(%)	♀(%)
Sorbitol		
Hyperplasia	28	14
Tumour	7	7
Xylitol		
Hyperplasia	29	24
Tumour	16	9
Control		
Hyperplasia	6	3
Tumour	9	2

Slide 28

Brion and Dupuis Canad. J. Physiol. Pharmacol., 58, 1431, 1980

1. Correction of hypocalcaemia in vitamin D-deficient rats restores adrenal medullary hypofunction to normal.

2. Hypocalcaemia corrected by 20% lactose in diet.

Slide 29 Florid adrenal medullary hyperplasia in a rat fed on a diet containing 30% lactose for nine months.

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OPTIMAL DIETARY MINERAL LEVELS FOR MATURE RATS

	Minimum recommended by US NRC	Optimum suggested by experience
Ca	0.5%	0.5 - 0.6%
P	0.4%	0.4%
Mg	0.06%	0.2%

Slide 30 1. Optimise and standardise mineral levels in diets.

2. Move from ad libitum feeding to controlled feeding.

3. Undertake the basic research in animal husbandry needed to identify conditions in which laboratory rats and mice can be maintained into old age in essentially normal hormonal status.

Until we are masters of the background, we can never really understand the foreground of what we are doing.

Collection and storage of data on the incidences of tumours or other pathology in untreated animals will remain a farce until we control the environment in which we keep animals and thereby prevent the variation from experiment to experiment.

Feed compounders have an important role to play - but they cannot achieve very much until the effects of all aspects of animal husbandry including especially, the amount of diets fed, are controlled.

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