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CHAPTER 26

1200-Rat Biosure Study: Design and Overview of Results

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Introduction

It is clear from many earlier studies that restriction of caloric intake is associated with improved survival and reduced incidence of ageing-associated non-neoplastic and neoplastic diseases both in mice (Conybeare 1980) and rats (Berg and Simms 1960; Tucker 1979). In the case of rats, an earlier small-scale study (Salmon et al. 1990) suggested that calorie restriction reduces the age-standardized incidence of fatal and/or potentially fatal cancers of many kinds. This latter observation needed to be checked in a larger-scale study and a number of other questions merited careful investigation. In the light of these needs scientists in several organizations agreed to undertake a large-scale study giving their own time and services for minimum fees or without any charge. The individuals and companies involved are listed in Table 26.1 and the main aims of the study in Table 26.2.

At the time of the preparation of this preliminary report the histopathological evaluation was virtually complete for 10 of the 12 groups of rats in the study with only a very few additional tissue sections awaited. Most of the in-life data for individual animals have been entered into the computer but no attempt has yet been made to analyze the data for individual animals in relation to the last aim listed in Table 26.2.

A full and detailed report on the study will be prepared for publication in due course.

Materials and Methods

Twelve groups consisting of 50 male and 50 female SK&F Wistar weanling rats aged 3 weeks were constructed using a random allocation procedure. They were numbered and housed five to a cage in grid-bottomed cages. Sick animals were

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Table 26.1. Collaborating scientists and sponsors.

Scientist	Role	Sponsor
David Kelly	Protocol design and organization	Biosure
Geoffrey Conybeare	In-life observations and necropsies	Smith Kline and French
Graham Tobin	Nutritional aspects	Biosure
Peter Donatsch	Hormonal assays	Sandoz
David Prentice, Bernhard Matter, and Peter Stirnimann	Histopathological processing and second opinion on assessment	Sandoz
Francis Roe	Histopathological assessment	Biosure and self
Peter Lee	Data processing and statistical analysis	Sandoz and self[a]

[a]Further sponsors are needed.

isolated to avoid cannibalism. Euthanasia was by carbon dioxide asphyxia. Animals were checked twice daily for general health and clinically examined and weighed once weekly.

Table 26.3 lists the data that were collected during life, the observations made at necropsy, and the tissues examined microscopically.

Study Design

Table 26.4 lists the diets given to the 12 groups of rats. Included in the different dietary formulations in varying proportions were barley, maize meal, oats, oat feed, wheat, wheat feed, dried skimmed milk, single cell (yeast) protein, soya protein, white fish meal, yeast, minerals, vitamins, and essential amino acids. (Although the actual formulae of the diets are commercially sensitive, further information will not be withheld in the event of enquiries from interested scientists.)

The composition of the diets in terms of nutrients and certain minerals, by analysis, is summarized in Table 26.5.

Table 26.2. Main aims of 1200-rat Biosure study.

1. To see whether dietary restriction (80% of ad libitum) reduces the age-standardized incidence of fatal or potentially fatal neoplasia before the age of 30 months
2. To see whether the beneficial effects of diet restriction can be achieved by (a) limiting the daily period of access to food to 6 h, or by (b) limiting the energy value of the diet
3. To see whether reduced calorie intake between weaning and age 4 months influences survival and/or incidence of non-neoplastic and neoplastic diseases
4. To compare effects of food consumption, energy intake and protein intake on survival and disease
5. To study the relationships between body weight at different ages with eventual survival and disease incidence
6. To provide a data base for studying relationships between various in-life measurements and eventual survival and disease incidence in individual animals

Table 26.3. Data collected in life and at necropsy and stored.

In-life	
Measure	Time interval
Body weight	Weekly
Food consumption	During 7 days weekly at first, then monthly
Water consumption	During 7 days during week 4, then 12-weekly
Clinical chemistry	20 rats/sex per group 6-monthly
Haematology	20 rats/sex per group 6-monthly
Urinalysis	During 4 h 20 rats/sex per group 6-monthly
Terminal plasma	Samples in store at −18°C
At necropsy	
Measure	Tissue
Macroscopic examination	Complete except spinal cord
Weight	Liver, kidneys, heart, adrenals, testes, prostate, seminal vesicles, ovaries, pituitary, and brain
Microscopic examination	Liver (×3), kidneys (×2), heart, spleen, lungs (×2), pancreas, adrenal cortex (×2), adrenal medulla (×2), thyroid (×2), parathyroid (×2), testes (×2), epididymides (×2), ovaries (×2), uterine horns (×2), skin, mammary gland, and pituitary. *Plus* all tissues thought to be abnormal at necropsy.

N.B. All tissues in formalin fixative.
Terminal blood plasma samples at −18°C
Carcasses stored in formalin.

Table 26.4. Study design.

Group (50 males + 50 females)	During first week after weaning	From 1 week after weaning for 12 weeks	13 weeks–30 months
1	SB24	SB24	SM24
2	SB24	SB24	LM24
3	SB24	SB24	SMR
4	SB24	SB24	SM6
5	SB24	SBR	SMR
6	SB24	SBR	LM24
7	SB24	SBR	SM24
8	SB24	SB6	SM6
9	LB24	LB24	SM24
10	LB24	LB24	LM24
11	LM24	LM24	LM24
12	PRD24	PRD24	PRD24

SB, standard breeding diet; SM, standard maintenance diet; LB, low nutrient breeding diet; LM, low nutrient maintenance diet; PRD, Porton Research diet; 24, ad libitum for 24 h/day; 6, ad libitum for 6 h/day; R, restricted to 80% of ad libitum

Table 26.5. Percent composition of diets in terms of nutrients and certain minerals by analysis.

Nutrients	Diet				
	SB	LB	SM	LM	PRD
Crude oil	3.4	3.2	3.2	3.0	3.0
Crude protein	19.7	16.0	14.4	13.4	19.8
Crude fibre	2.3	9.2	6.4	11.6	5.4
Carbohydrate	55	53	57	53	53
Starch	49	32	42	26	31
Calcium	0.71	0.73	0.71	0.85	0.65
Phosphorus	0.74	0.70	0.69	0.77	0.68
Magnesium	0.17	0.24	0.2	0.28	0.17
Ca:P ratio	0.98	1.03	1.02	1.1	0.95
Lignosulphonates	0	1.0	0 up to 1.5	0 up to 1.8	0

SB, standard breeding diet; LB, low nutrient breeding diet; SM, standard maintenance diet; LM, low nutrient maintenance diet; PRD, Porton Research diet

Statistical Analysis

All data were entered onto ROELEE 84, a computer-based system for recording, processing, reporting, and statistically analyzing pathological and other toxicological data. Analyses of data recorded after 13 weeks on test were complicated by the change in dietary regimen so that in assessing the possible effects of different diets prior to changeover, comparison had to be made between pairs of groups fed differently before 13 weeks, but the same after. Thus, standard breeding diet ad libitum for 24 h/day (SB24) and standard breeding diet restricted to 80% of ad libitum (SBR) could be compared based on the pairs of groups 1 and 7 (both given standard maintenance diet ad libitum for 24 h/day, SM24, after week 13), 2 and 6 low-nutrient maintenance diet ad libitum for 24 h/day (LM24), and 3 and 5 standard maintenance diet restricted to 80% of ad libitum (SMR). These three sets of comparisons can then be combined for a more powerful test, "stratified" for diet after changeover. Initial comparisons of diets after changeover were stratified for diet before, but as it became clear that for many endpoints diet before week 13 had little or no effect, many of the analyses presented here are unstratified, thus allowing inclusion of data from all relevant groups and a somewhat more powerful test.

In the case of in-life measurements, the analysis of data at a given time point involved a combination of presentation of distributions, means, and standard errors. Rank tests were used to test statistical significance, stratification being carried out as described by Fry and Lee (1988).

Histopathological data were analyzed by the method of Peto et al. (1980), which compares the observed and expected incidences of a lesion after adjustment for time of death, taking account of whether the condition was classified as having caused or heavily contributed to death (fatal) or having not done so (incidental). The method allows stratification.

Comparisons were normally made with the SB24 or SM24 diets or with group 1, with tests carried out for males, females, and, as appropriate, for sexes com-

Table 26.6. Mean food consumption (g/day), energy intake, (kJ/day), and protein (g/day) during weeks 0–52 as percent of group 1.

	Males			Females		
Group	Food consumption (%)	Energy intake (%)	Protein intake (%)	Food consumption (%)	Energy intake (%)	Protein intake (%)
1	100	100	100	100	100	100
2	110	112	104	108	110	102
3	84	84	85	84	84	85
4	107	107	106	103	103	102
5	80	79	79	80	80	79
6	110	111	102	105	107	98
7	91	91	90	95	95	94
8	100	99	98	94	94	93
10	117	118	105	116	117	101
12	117	119	146	108	111	136

bined (stratified for sex). Both the rank tests and the method of Peto et al. (1980) result in chi-squared statistics from which two-tailed p values were calculated. P values are usually presented as $***p<0.001$, $**p<0.01$, $*p<0.05$, or $(*)\,p<0.1$ for positive differences, minus signs similarly being used for negative differences, and NS (not significant) indicating $p \geq 0.1$.

Results

Food Consumption and Energy and Protein Intake. Table 26.6 summarizes the data for food consumption, energy intake, and protein intake. The mean values for rats in group 1 (SB24 for 13 weeks then SM24) were taken to be 100%. The food consumption and other values for the two groups rationed to 80% of ad libitum from week 13 (groups 3 and 5) approximated to 80% of group 1 as planned. The values for rats fed continuously on the Porton Research diet (PRD) (group 12) were considerably higher than those for group 1 in terms of all three intake parameters. In this study, although not in a previously reported study in rats of the same strain, restricted access to food to a period of 6 h per day throughout life (groups 4 and 8) had little effect on their intakes of food, energy, or protein. Rats fed ad libitum on LM24 from week 13 (groups 2, 6, and 10), by increasing their food consumption, increased their mean energy intake to above that of group 1 and managed to equal group 1 in mean protein intake.

Water Consumption. Compared with rats fed ad libitum on SM24 (group 1), rats restricted to 80% of ad libitum (groups 3 and 5–SMR) drank significantly less water on all the occasions water consumption was measured, up to and including week 108. Not unexpectedly water consumption by rats in groups 4 and 8 (SM6) which managed to eat as much food in 6 h as group 1 ate in 24 h was similar to

Table 26.7. Survival.

Group	Diet to week 13	Diet after week 13	Males: Deaths before week 52	Males: Deaths before week 104	Males: Deaths before week 130	Females: Deaths before week 52	Females: Deaths before week 104	Females: Deaths before week 130
1	SB24	SM24	0	15	29	1	9	30
2	SB24	LM24	0	12	26	1	5	20
3	SB24	SMR	0	7	15	2	5	12
4	SB24	SM6	0	6	19	1	19	27
5	SBR	SMR	0	6	16	0	6	12
6	SBR	LM24	1	8	23	1	8	20
7	SBR	SM24	1	13	30	3	14	36
8	SB6	SM6	0	8	18	3	13	28
9	LB24	SM24	1	12	30	0	17	31
10	LB24	LM24	1	13	28	0	8	20
11	LM24	LM24	2	13	26	3	9	24
12	PRD24	PRD24	1	18	36	1	12	29

Diet abbreviations as in Table 26.4.

that of group 1 rats. By contrast, rats on the LM diet or the PRD diet drank significantly more water than rats on the SM diet.

Survival. Survival data are summarized in Table 26.7. Compared with rats on the SM diet from week 13 (groups 1, 7, and 9–SM24), rats in both sexes in the two restricted groups (groups 3 and 5–SMR) showed very much improved survival ($p<0.001$). Survival was also significantly improved ($p<0.001$) in

Table 26.8. Body weights at start, 1 week, 13 weeks, 12 months, and 18 months.

			Body weight (g)									
			Males					Females				
Group	Diet to week 13	Diet from week 13	Start	Week 1	Week 13	Month 12	Month 18	Start	Week 1	Week 13	Month 12	Month 18
1	SB24	SM24	81	125	459	545	554	71	104	249	293	337
2	SB24	LM24	82	121	416	460	471	72	105	243	256	268
3	SB24	SMR	84	124	420	410	405	70	103	243	251	256
4	SB24	SM6	82	123	423	484	489	70	102	239	255	273
5	SBR[a]	SMR	83	126	353	391	389	71	103	218	245	254
6	SBR[a]	LM24	83	125	359	459	477	72	104	221	253	269
7	SBR[a]	SM24	81	121	344	505	528	71	103	222	291	323
8	SB6[a]	SM6	82	125	364	460	470	70	102	217	250	272
9	LB24	SM24	83	120	385	525	543	69	97	224	282	316
10	LB24	LM24	80	119	381	453	468	70	99	222	251	266
11	LM24	LM24	82	115	340	457	471	70	96	212	251	268
12	PRD24	PRD24	82	125	419	542	546	70	103	237	299	327

Diet abbreviations same as in Table 26.4.

[a]During the 1st week after weaning, rats in group 5–8 were fed on SB24. Diet restriction began thereafter.

Table 26.9. Liver and kidney weight relative to body weight in terminally killed rats.

			Males				Females			
Group	Diet to 13 weeks	Diet after week 13	BW	BW as % of group 1	LW/BW (%)	KW/BW (%)	BW	BW as % of group 1	LW/BW (%)	KW/BW (%)
1	SB24	SM24	456	100	3.6	0.79	318	100	4.3	0.91
2	SB24	LM24	392	86	4.0	0.78	268	84	4.6	0.92
3	SB24	SMR	405	89	3.3	0.72	258	81	3.8	0.85
4	SB24	SM6	418	92	3.4	0.77	276	87	3.8	0.91
5	SBR	SMR	386	85	3.3	0.72	251	79	3.4	0.84
6	SBR	LM24	371	81	4.0	0.81	264	83	4.5	0.89
7	SBR	SM24	452	99	3.6	0.75	301	95	4.1	0.88
8	SB6	SM6	404	89	3.4	0.78	273	86	3.9	0.86
9	LB24	SM24	433	95	3.9	0.85	291	91	4.3	0.88
10	LB24	LM24	374	82	3.8	0.81	262	82	4.4	0.91
11	LM24	LM24	383	84	3.8	0.81	259	81	4.7	0.94
12	PRD24	PRD24	411	90	4.2	0.98	311	98	4.7	0.89

Diet abbreviations as in Table 26.4.
BW, body weight; LW, liver weight; KW, kidney weight

males in the interrupted groups (groups 4 and 8—SM6) and in females on the LM diet (groups 2, 6, 10, and 11—LM24). Survival of rats fed PRD24 throughout the study (group 12) was similar to that of rats fed SM24 from week 13.

Body Weight. Initial mean body weights and mean body weights at 1 week, 13 weeks, 12 months, and 18 months are shown in Table 26.8. The feeding of LB24 or LM24 instead of SB24 during the first 13 weeks of the study reduced body weight gain up to week 13 by 20%–22%. Thereafter the rats of these groups tended to catch up with other groups fed similarly after week 13. Sustained reduction in body weight was seen in the two groups whose food intake was restricted to 80% of ad libitum from 13 weeks (groups 3 and 5). Males of these groups weighed on average 26% less than group 1 males at 12 months and 28% less at 18 months. The corresponding reductions for females were 15% and 14%, respectively. The feeding of LM24 instead of SM24 after week 13 reduced weight gain in males by about 14% and in females by about 20%.

Liver and Kidney Weight Relative to Body Weight in Terminally Killed Rats. In both sexes kidney weight relative to body weight in terminally killed rats was significantly lower in the restricted groups (groups 3 and 5) than in any of the other groups. Relative liver weights in the same two groups were significantly lower than in the group fed SM24 or PRD24 after week 13. They were also low in the SM6 groups. The data are summarized in Table 26.9.

Urinalysis Data. Urine samples were collected over 4 h between 1600 and 2000 hours. Since ad libitum-fed animals both eat and drink mainly during the dark (1800 to 0600 hours) animals in the 24-h per day-fed groups would have taken in little food or water during the 10 h prior to being put in metabolism cages without access to either food or water. By contrast animals in the restricted groups (SBR,

SMR, and SM6) which were provided with food at 1000 hours each day would have been eating and drinking up to, perhaps, 2 h before being put in metabolism cages. Not surprisingly, therefore, the volumes of urine samples collected from restricted animals significantly exceeded those for 24-h per day-fed animals.

The pH of urine collected from SM24 and PRD24 groups ranged from 5 to 7, that from LM24 groups from 5 to 9, that from SM6 groups from 6 to 8, and that from SMR groups from 7 to 9. Throughout the study urine samples from SMR, SM6, and LM24 groups (both sexes) contained less protein than SM24 or PRD24 groups. After 18 months females of the SMR, SM6 and LM24 groups had higher levels of ketones than the SM24 or PRD24 groups. Also after 18 months, urine samples from males in SM24 and PRD24 groups had more blood than males from SMR, SM6, or LM24 groups.

Clinical Chemistry Data. SMR, SM6, and LM24 were associated with lower total serum protein levels and lower serum albumin levels during the first half of the study than SM24. However, a difference in the opposite direction was evident at 30 months. Blood glucose was consistently lower in SMR groups than in SM24 groups.

Haematological Data. White blood cell counts (WBC), red blood cell counts (RBC), and haemoglobin (Hb) were significantly lower in the SMR groups than in the SM24 groups during the first 18 months of the study. The same is true for SM6 in respect of WBC. After 18 months WBC, RBC, and Hb tended to be lower in LM24 groups than SM24 groups.

Tail Necrosis. Tail necrosis was seen significantly more frequently in the two restricted groups (groups 3 and 5). This may be due to the fact that animals in these groups were more active but drank less than animals in other groups during long stretches of the day when their food baskets were empty.

Histopathological Findings: General. At the time of preparing this report the data for groups 9 and 11 are not available.

Corticomedullary and Pelvic Nephrocalcinosis. As shown in Table 26.10, diet restriction (SBR or SB6 prior to week 13, groups 5 to 8) irrespective of diet after 13 weeks was associated with significantly higher incidence of corticomedullary nephrocalcinosis in males and a significantly lower incidence of the same change in females than SB24 prior to week 13 (group 1). LB24 followed by LM24 (group 10) was also associated with less corticomedullary nephrocalcinosis in females. PRD24 gave rise to significantly less corticomedullary nephrocalcinosis (females) and significantly more pelvic nephrocalcinosis (both sexes) than was seen in any other group. It is noteworthy that analysis of the diets indicated that only in the cases of SB and PRD was the calcium-to-phosphorus ratio less than unity (see Table 26.5).

Nephropathy, Myocarditis, Polyarteritis, and Prostatitis. The findings in respect of nephropathy, myocarditis, polyarteritis, and prostatitis are summarized in

Table 26.10. Percent incidences of corticomedullary and pelvic nephrocalcinosis.

Males	Group 1	2	3	4	5	6	7	8	10	12
Diet to week 13	SB24	SB24	SB24	SB24	SBR	SBR	SBR	SB6	LB24	PRD24
Diet from week 13	SM24	LM24	SMR	SM6	SMR	LM24	SM24	SM6	LM24	PRD24
Corticomedullary nephrocalcinosis										
Any	0	0	0	0	24**	24***	30***	14*	0	0
Moderate/severe	0	0	0	0	4	6	6	2	0	0
Severe	0	0	0	0	0	0	2	0	0	0
Pelvic nephrocalcinosis										
Any	8	6	8	8	6	10	10	6	2	24*
Moderate/severe	0	0	2	0	2	0	0	0	2	10(*)
Females										
Corticomedullary nephrocalcinosis										
Any	100	96(*)	98	96	86	74***	68***	86*	54***	32***
Moderate/severe	80	64(*)	76	56	38**	20***	26***	36***	10***	2***
Severe	16	24	18	22	12	4	4	6	0*	0*
Pelvic nephrocalcinosis										
Any	0	0	0	2	0	0	2	4	0	28***
Moderate/severe	0	0	0	0	0	0	0	0	0	4

Diet abbreviations same as in Table 26.4.
Significance values relate to comparisons with group 1.
(*)$p<0.1$; *$p<0.05$; **$p<0.01$; ***$p<0.001$

Table 26.11. Compared with SM24, PRD24 was associated with higher incidences of all these changes except prostatitis, while SMR, SM6, and LM24 were associated with lower incidences of all these changes.

Mammary Acinar Hyperplasia and Secretory Activity. Diet restriction (SMR–groups 3 and 5) was associated with significantly less mammary hyperplasia and secretory activity in females and less secretory activity in males than SM24. The same is true for mammary hyperplasia and secretory activity in females in LM24 groups (groups 2, 6, and 10).

Neoplasms of Endocrine Glands and the Exocrine Pancreas. As shown in Table 26.12, SMR and LM24 from 13 weeks were associated with significant ($p<0.01$ or $p<0.001$) reductions in incidence of neoplasms of the anterior and intermediate lobes of the pituitary and islet cell tumours of the pancreas compared with SM24. Adenomas of the exocrine pancreas were encountered less frequently ($p<0.05$) in SMR and SM6 groups. PRD24 was associated with a significantly higher incidence of tumours ($p<0.05$) of the adrenal medulla. Leydig cell tumours were seen in significantly higher ($p<0.05$) incidence in rats fed LM24

Table 26.11. Nephropathy, myocarditis, polyarteritis, and prostatitis.

		Diet from week 13				
	Gender	SM24	SMR	SM6	LM24	PRD24
Group		1,7	3,5	4,8	2,6,10	12
Kidney						
Nephropathy						
Any	M	95	42***	86***	77***	98***
Severe/very severe	M	21	0***	5***	1.3***	46***
Any	F	93	42***	66***	59***	100
Severe/very severe	F	15	0***	3**	0***	16
Heart						
Chronic myocarditis						
Any	M	34	38	45	29.3	66***
Any	F	46	25***	19***	11.3***	58
Fibrosis						
Any	M	23	6***	14(*)	11.3(*)	44*
Any	F	19	1***	3***	2.7***	28
Polyarteritis						
Pancreatic artery	M	8	4	2*	8.7	28***
Pancreatic artery	F	10	0***	3(*)	2.7**	20
Mesenteric artery	M	1	1	1	2.7	18***
Mesenteric artery	F	1	0	0	0.7	2
Prostatitis						
Acute						
Any		19	4***	9*	9.3*	16
Moderate/severe		16	2***	7	2***	6
Chronic						
Any		10	3*	8	14.7	12
Moderate/severe		5	1(*)	5	4.7	2

*$p<0.05$; **$p<0.001$; ***$p<0.001$

after week 13. No clear differences between groups were seen in tumours of the adrenal cortex, ovary, parathyroid, thyroid follicular cells, or thyroid C cells.

Neoplasms of Epidermis, Adnexa, Jaw, Subcutaneous Tissue, and Mammary Gland. The incidence of neoplasms of epidermis, adnexa, jaw, subcutaneous tissue, and mammary gland is summarized in Table 26.13. Compared with rats on SM24 from week 13, the two SMR groups (groups 3 and 5) developed significantly fewer epidermal adnexal and jaw tumours (both sexes, $p<0.05$), significantly fewer subcutaneous tumours ($p<0.05$) and highly significantly fewer mammary tumours (females, $p<0.001$). In the SM6-fed and LM24-fed rats (groups 2, 4, 6, 8, and 10), mammary tumour incidence was significantly lower ($p<0.01$) than in group 1. In PRD24 rats (group 12) the incidence of subcutaneous tumours was significantly higher in females ($p<0.05$) than in group 1.

Neoplasms of the Lungs. As shown in Table 26.14, diet restriction (SMR– groups 3 and 5) significantly reduced the risk of developing an adenoma or primary adenocarcinoma of the lungs.

Table 26.12. Neoplasms of endocrine glands.

	Diet from week 13				
	SM24	SMR	SM6	LM24	PRD24
Group	1,7	3,5	4,8	2,6,10	12
Adrenal cortex					
B or M (males)	0	1	1	0.7	2
B or M (females)	2	3	2	0.7	2
Adrenal medulla					
B or M (males)	3	4	7	6.7	8
B or M (females)	3	1	4	0.7	10
Ovary					
B or M	4	3	0	3.3	0
Endocrine pancreas (islet cell)					
B or M (males)	10	1**	3(*)	2.7*	4
B or M (females)	2	1	0	0.7	2
Exocrine pancreas (males only)					
B	5	0*	0*	1.3	4
Parathyroid					
B (males)	5	1	2	4	4
B (females)	2	1	4	4	2
Pituitary: anterior lobe					
B or M (males)	30	14***	29	21.3	24
B or M (females)	62	46**	47	45.3***	52
Pituitary: intermediate lobe					
B or M (males)	13	9	8(*)	4.7**	8
B or M (females)	6	0**	5	0.7**	10
Testis/Leydig cell					
B	23	30	29	38.7*	36
Thyroid/follicular					
B or M (males)	1	1	1	1.3	4
B or M (females)	2	0	2	0.7	4
Thyroid/C cell					
B or M (males)	2	2	8	6(*)	2
B or M (females)	8	4	2(*)	2.7*	0(*)

Diet abbreviations same as in Table 26.4.
B, benign; M, malignant
(*)$p<0.1$; *$p<0.05$; **$p<0.01$; ***$p<0.001$

Haemangiomas and Angiosarcomas of the Mesenteric Lymph Node. A particularly surprising finding in the study was a significantly higher incidence of haemangiomas and angiosarcomas of the mesenteric lymph node ($p<0.01$ for the sexes combined) in the three groups fed the LM24 diet from week 13 (groups 2, 6, and 10) than in the groups fed SM24 from week 13 (groups 1 and 7). By contrast, SMR significantly reduced the incidence of tumours ($p<0.05$ for the sexes combined) of these kinds. The data are summarized in Table 26.15.

Neoplasms of the Uterus. Table 26.16 summarizes the data for neoplasms of the uterus. At this site diet restriction was without significant effect. However, rats fed LM24 after week 13 (groups 2, 6, and 10) developed significantly more

Table 26.13. Neoplasms of epidermis, adnexa, subcutaneous tissue, and mammary gland.

Diet from week 13	SM24	SMR	SM6	LM24	PRD24
Group	1,7	3,5	4,8	2,6,10	12
Males					
Epidermis/adnexa					
B or M	14	7*	10	8	10
M	7	3	7	5.3	8
Subcutaneous tissue					
B or M	18	6*	10	10.7	18
M	6	4	5	7.3	16
Mammary gland					
B or M	0	0	1	0	2
M	0	0	0	0	0
Females					
Epidermis/adnexa					
B or M	5	0*	2	2.7	6
M	5	0*	2	2	6
Subcutaneous tissue					
B or M	1	2	7	3.3	10*
M	1	2	6	3.7	8(*)
Mammary gland					
B or M	37	9***	19**	9.3***	22
M	5	1(*)	5	2	4

Diet abbreviations same as in Table 26.4.
B, benign; M, malignant
(*)$p<0.1$; *$p<0.05$; **$p<0.01$; ***$p<0.001$

uterine tumours ($p<0.01$), most of which were adenomatous polyps, adenocarcinomas, or anaplastic carcinomas.

Neoplasms of All Sites. As shown in Table 26.17, SMR (groups 3 and 5) significantly reduced the incidence of rats developing one or more benign or malignant tumours ($p<0.001$) before the termination of the study as compared with SM24.

Table 26.14. Neoplasms of the lungs.

Diet from week 13	SM24	SMR	SM6	LM24	PRD24
Group	1,7	3,5	4,8	2,6,10	12
Primary tumours					
B + M (males)	6	0*	2	4.7	2
M (males)	2	0	0	0.7	0
B + M (females)	4	0*	0(*)	1.3	2
M (females)	2	0	0	0.7	0
B + M (males and females)	5	0***	1*	3	2
M (males and females)	2	0*	0(*)	0.7	0

Diet abbreviations same as in Table 26.4.
B, benign; M, malignant
(*)$p<0.1$; *$p<0.05$; ***$p<0.001$

Table 26.15. Haemangiomas and angiosarcomas of the mesenteric lymph node.

Diet from week 13	SM24	SMR	SM6	LM24	PRD24
Group	1,7	3,5	4,8	2,6,10	12
Mesenteric lymph node					
B or M (males)	15	7(*)	11	34**	12
M (males)	6	0**	1*	15.3(*)	0
B or M (females)	8	2	2	14.7	0
M (females)	1	0	0	5.3	0
B or M (males and females)	11.5	4.5*	6.5(*)	24.3**	6
M (males and females)	3.5	0**	0.5*	10.3*	0

Diet abbreviations same as in Table 26.4.
B, benign; M, malignant
(*)$p<0.1$; *$p<0.05$; **$p<0.01$

This was true for both sexes and for malignant neoplasms. The incidence of rats bearing more than one tumour was also significantly reduced ($p<0.001$) in both sexes, and in males the diet restriction significantly reduced the risk of an animal developing two malignant tumours of different kinds ($p<0.01$). PRD24 was associated with a significantly higher incidence ($p<0.05$) of male rats bearing one or more tumours while significantly fewer female rats ($p<0.001$) fed on LM24 developed one or more benign or malignant neoplasms or malignant neoplasms of more than one site.

Table 26.16. Neoplasms of the uterus.

Diet from week 13	SM24	SMR	SM6	LM24	PRD24
Groups	1,7	3,5	4,8	2,6,10	12
Total no. of rats	100	100	100	150	50
Benign tumours					
Papillary adenoma	0	1	0	0	0
Adenomatous polyp	2	2	5	8	1
Squamous polyp	0	0	0	2	0
Fibromyoma	0	0	0	1	0
Total(%)	2(2)	3(3)	5(5)	11(7.3)	1(2)
Malignant tumours					
Adenocarcinoma	3	6	7	20	0
Anaplastic carcinoma	1	1	0	6	0
Squamous carcinoma	0	0	0	1	0
Sarcoma	1	1	6	4	1
Haemangiosarcoma	0	0	0	1	0
Total (%)	5(5)	8(8)	13(13)	32**(21.3)	1(2)
Total tumours (%)	7(7)	11(11)	18(18)	43**(28.7)	2(4)

Diet abbreviations as in Table 26.4
**$p<0.01$

Table 26.17. Neoplasms of all sites.

Diet from week 13	SM24	SMR	SM6	LM24	PRD24
Group	1,7	3,5	4,8	2,6,10	12
One or more sites					
B or M (males)	78	69***	83	84.7	88*
B or M (females)	86	66***	76	78.7***	80
M (males)	30	13***	33	41.3	44(*)
M (females)	34	18***	33	34	40
More than one site					
B or M (males)	52	26***	44*	57.3	46
B or M (females)	55	16***	38*	30***	52
M (males)	7	0**	2(*)	6	0
M (females)	4	1	4	4	6

Diet abbreviations same as in Table 26.4.
B, benign; M, malignant
(*)$p<0.1$; *$p<0.05$; **$p<0.01$; ***$p<0.001$

Discussion

Although some of the results of this study are not yet available, several important conclusions may be confidently drawn in relation to the main aims of the study whilst the associations between the LM24 diet and increased risks of mesenteric node and uterine carcinomas merit further research.

Effect of Diet Restriction (80% of Ad Libitum) on the Incidence of Malignant Neoplasia of All Sites. The finding reported earlier that restriction of calorie intake to about 80% of ad libitum reduces the risk of premature death from malignant neoplasm has been confirmed (see Table 26.17). This beneficial effect appeared to apply to all kinds of malignant neoplasms for which there were sufficient data for comparisons to be made. There was no example of a significantly higher incidence of malignant neoplasia at any particular site in the SMR groups as compared with the SM24 groups.

Effect of Limiting Daily Access to Food to a 6-h Period. Although in the study reported earlier (Salmon et al. 1990), diet restriction to about 80% of ad libitum was achieved by limiting the access of rats to food to 6.5 h per day, this stratagem did not work in the present study. For reasons that are not clear, in the present experiment rats given access to food for only 6 h per day (between 1000 hours and 1600 hours) managed to consume almost as much food as rats given access to food throughout the 24 h of each day. It is not surprising therefore that relatively few differences were seen between rats fed SM6 and SM24 after 13 weeks. Among the differences recorded, however, were significantly lower incidences of nephropathy and myocarditis (Table 26.11), mammary hyperplasia, and mammary tumours (see Table 26.13), and mesenteric lymph node tumours (see Table 26.15). By contrast a significantly higher incidence of subcutaneous neoplasms was seen in SM6 females than in SM24 females (see Table 26.13).

Effects of Limiting the Energy Value of the Diet. Reduction of the energy values of diets fed from weaning to week 13 or from week 13 to the termination of the study led to increased consumption of food and water and to increased energy intake (see Table 26.6). The intake of protein of rats on LM24 was similar to that of rats on SM24. However, like diet-restricted rats (SMR), rats on LM24 had lower serum protein levels and lower urinary protein levels than SM24 rats. The incidences of nephropathy, myocarditis, polyarteritis, acute prostatitis, mammary hyperplasia, and secretory activity were also markedly lower in LM24 rats than in SM24 rats (see Table 26.11). Beneficial effects of LM24 as compared with SM24 were seen in the cases of tumours of the anterior and intermediate lobes of the pituitary gland and islet cell tumours of the pancreas (see Table 26.12) and mammary tumours (see Table 26.13). However, as discussed below, three kinds of tumour—Leydig cell tumours, tumours of the mesenteric lymph node, and uterine tumours—arose significantly more frequently in LM24-fed rats than in SM24- or PRD24-fed rats. Overall benign and malignant tumour incidence was lower in females fed LM24 than in females fed SM24 or PRD24 (see Table 26.17).

Effect of Diet Restriction During the 13 Weeks After Weaning. The effects of diet restriction during the first 13 weeks of the study could be assessed by comparing the findings in groups 5, 6, and 7 with those in groups 3, 2, and 1, respectively. These comparisons revealed very few differences. The most obvious of these was the enhancement of corticomedullary nephrocalcinosis in males and its reduction in females (see Table 26.10). Diet restriction confined to the first 13 weeks of the study had little or no effect on survival, on the incidences of ageing-associated non-neoplastic diseases, or on the incidence of neoplasms of any kind.

Prediction of Risk of Tumour Development from Food, Energy, or Protein Intake During the First 12 Months of the Study. Groups 3 and 5 (SMR from week 13) consumed less food, less energy and less protein than any of the other groups. The same two groups experienced the best survival and the lowest incidences of nonneoplastic and neoplastic diseases. Group 12 (PRD24 throughout) had the highest consumption of food energy and protein and exhibited the highest incidences of all the groups of nephropathy, myocarditis, polyarteritis and of one or more tumours of all sites. The data from the study do not enable one to distinguish between the predictive values of the three forms of intake.

Prediction of Risk of Tumour Development (All Sites) from Body Weight Gain Earlier in the Study. Tumour incidences were highest in the two groups which put on most weight during the first 18 months of the study (groups 1 and 12) and lowest in the two groups which put on least weight during the same period (groups 3 and 5). Restricting access to food to 6 h per day (SM6) reduced body weight gain, nephropathy and myocarditis but had little or no effect on tumour incidence. Survival was improved by SM6 in males but not in females. These results, which confirm and extend those of Turnbull et al. (1985), are consistent with there being a moderately good correlation between body weight gain and tumour risk.

Relationships Between In-Life Measurements, Survival, and Necropsy Findings in Individual Animals. The accumulating data base derived from the study will permit such relationships to be studied. However, no analyses of this kind have yet been undertaken, partly because further data are awaited and partly because further funding is needed.

Adrenal Medullary Tumours and Pelvic Nephrocalcinosis. Earlier we pointed to there being associations between adrenal medullary proliferative disease, enhanced calcium absorption from the gut, and pelvic nephrocalcinosis in rats (Roe and Baer 1985). In the present study, PRD24 gave rise both to the highest incidence of pelvic nephrocalcinosis and the highest incidence of adrenal medullary tumours (see Tables 26.10 and 26.12). The difference in incidence between PRD24 (group 12) and SM24 (groups 1 and 7) was significant ($p<0.05$), and a similar difference was seen in the incidence of adrenal medullary hyperplasia between the same groups.

The Association Between the LM24 Diet and Tumours of the Mesenteric Lymph Node. The finding of an association between LM24 diet and tumours of the mesenteric lymph node is, as far as we know, an entirely new one and, as such, was wholly unexpected. The mechanism remains to be elucidated.

In the present study high incidences of mesenteric lymph node tumours were seen in all groups, the incidence being higher in males than in females. Thus 89 out of the 500 males (18%) and 34 out of the 500 females (7%) for which histopathological evaluation is complete had haemangiomatous tumours of the mesenteric lymph node. This suggests that the Wistar strain of rats used for the study may have been genetically prone to develop such tumours. Nevertheless an explanation is needed of why, in animals fed on LM24 from week 13, the incidences of these tumours reached 34% in males and 14.7% in females compared with 15% in males and 8% in females fed on SM24 (groups 1 and 7).

Samples of the LM diet preserved in cold storage will now be analyzed for known carcinogens.

The Association Between the LM Diet and Carcinomata of the Uterus. The significantly higher incidence ($p<0.01$) of uterine tumours in groups 2, 6 and 10 (21.3%) than in groups 1 and 7 (5%) poses the same questions as the higher incidence of mesenteric lymph node tumours in these same groups. The fact that benign uterine tumours – particularly adenomatous and squamous polyps – also occurred in highest incidence in the same groups (7.3% as compared with 2% in groups 1 and 7) reinforces the need to seek answers to the questions posed.

Conclusions

It has long been recognized that genetic and environmental factors interact in the determination of incidence of most diseases. In the past there has been a tendency for experimentalists to assume that the pattern of diseases seen in control groups

is determined mainly, or solely, by the genetic constitution of the strain of animals used. The results of the present study illustrate the extent to which diet and food consumption may influence survival and the incidence of degenerative and neoplastic diseases in rats. Both the composition of diets and the amounts consumed are important determinants of disease incidence. However, diet restriction by rationing animals to 80% of what 24 h/day ad libitum-fed animals eat from the age of 4 months seems to be more effective in reducing degenerative disease and neoplasia than any of the other stratagems tried in this study.

The beneficial effects of calorie restriction on the incidence of pituitary tumours, mammary tumours, nephropathy, myocarditis, and polyarteritis are well known. Less well known, although previously reported, is the effect of calorie restriction in reducing the incidence of prostatitis. We believe that the present study provides the first evidence for an effect of calorie restriction on lung tumour incidence in rats, although such an effect has previously been described in mice (Conybeare 1980).

The confirmation provided by the present study that calorie restriction nonspecifically reduces the risk of fatal or potentially fatal malignant neoplasia of seemingly all sites provides substantial support for the view (Gensler and Bernstein 1981; Yu 1989; Roe 1989) that endogenously generated electrophiles (e.g., during lipid peroxidation) may be important determinants both of ageing-associated degenerative diseases and of neoplasia. The underlying theory is that higher calorie intake is associated with higher rates of generation of electrophiles as a consequence of normal metabolic processes and that these electrophiles increase the risks both of ageing-related diseases and cancer.

The high incidences of mesenteric lymph node tumours and uterine carcinomas in rats fed on a low nutrient, high-fibre diet in the present study demonstrates a need for further research.

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