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EFFECTS OF THE TYPE OF DIETARY FAT AT TWO LEVELS OF VITAMIN E IN WISTAR MALE RATS DURING DEVELOPMENT AND AGING.
I. LIFE SPAN, SERUM BIOCHEMICAL PARAMETERS AND PATHOLOGICAL CHANGES

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SUMMARY

This experiment was designed to study in rats the implications of the dietary type of fat at two levels of vitamin E on the life span as well as on several biochemical and anatomopathological age-related changes. For this purpose, six different isoenergetic diets containing 15% coconut oil (SFD), safflower oil (UFD) or a combination of both (CFD) with 2 or 200 mg% of *dl*-α-tocopherol were offered *ad libitum* to outbred Wistar male rats from weaning to senescence. The results indicated that up to 9–12 months the body weights of rats consuming the CFD or the UFD increased generally faster than those fed the SFD, and that all rats developed moderate degrees of obesity. Age-dependent changes in organ weights (kidneys, testes, spleen, brain, liver and heart) were unaffected by diet. Serum levels of vitamin E generally reflected the corresponding dietary levels, but were also influenced by the type of dietary fat. Serum cholesterol levels were not significantly affected by the type of diet or by age. Only transient hypotriglyceridemic and hypophospholipidemic effects of the UFD were observed and, while the levels of triglycerides decreased with age up to the 18th month followed by an increase at 24 months, the levels of serum phospholipids remained unchanged. Neither diet nor age modified the serum albumin/globulin ratios. While no differences in maximum life span were found between dietary groups, the 50% survival time of rats fed the UFD at high level of vitamin E was significantly longer than in all the other groups. This beneficial effect was related to postponement of the onset and reduction of incidence of malignant neoplasms, but was apparently not related to any particular influence on the incidence or severity of chronic nephropathy which practically developed in all rats. Various neoplastic, degenerative and inflammatory diseases encountered in rats dying during the course of the experiment were tabulated and compared with similar findings reported by others in different strains of rats. Pituitary and adrenocortical adenomas as well as adrenocortical and renal carcinomas were the most frequent tumors found in this study. All the pathological changes provided useful baseline information for the evaluation of data presented in this and subsequent communications of this series of studies.

INTRODUCTION

Although the type of dietary fat has been considered of importance in relation to the development and to the life span of experimental animals [1–3], the results in this area of research are still inconclusive. While in female C3H mice Harman [2] found that increasing the degree of unsaturation of fat in diets largely supplemented with vitamin E decreased the mean life span without affecting the maximum life span, no significant effects were observed when the same dietary regimens were offered to male CD rats and to male Swiss mice. Furthermore, in experiments conducted by Morin [4] to explore the effect of dietary saturated (coconut oil) *versus* unsaturated fat (safflower oil) on the life span of male C3H and LAF_1 mice, no significant differences were found between the groups.

While the conflicting results may have been due in part to differences in animal species, strains and sex, the large levels of vitamin E and synthetic antioxidants used in these experiments may have prevented a clear differential effect. It has been shown in this line that several antioxidants increased the mean life span of mice and rats without affecting maximum life span [5–11]. On these bases, Kohn [7] has suggested that even if antioxidants do not inhibit the processes which determine maximum life span, they at least may inhibit some harmful environmental or nutritional factors. The observed beneficial effect of antioxidants would therefore lend some support to the free radical theory of aging originated by Harman [12], who proposed that the degradative (*i.e.* lipoperoxidative) changes with age may be due in part to the deleterious action of free radicals on cellular macromolecules.

The possible mode of action of antioxidants and that of the type of dietary fat may be perhaps related to an effect on the development of malignant neoplasms or other age-related diseases which determine the mean life span.

Since aging can be defined as the progressive structural and compositional changes and loss of functional capacity and adaptability of an organism, all of which result in a decreased survival capacity [13, 14], any attempts to determine the effect of diet on aging should evaluate the influence exerted not only on the mean and maximum life span of the individuals but also on their innate morphofunctional changes of aging as well as on the incidence of age-related diseases. While recognizing that this goal is difficult if not impossible to achieve in a single experiment, we have attempted to explore some of these aspects in rats fed saturated and unsaturated fat diets at two extreme levels of vitamin E.

The present report is the first of a series of four, and deals with the results obtained on the changes in body and organ weights, on the serum levels of vitamin E, lipid fractions and albumin/globulin ratios, on the anatomopathological findings of rats dying during the course of the experiment and on the life span parameters. Subsequent reports will deal with the lipoperoxidative phenomenon *in vitro* and *in vivo*, certain tissue biochemical parameters and some stereological determinations on tissue structure as well as ultrastructural variations of subcellular organelles and aging pigment in brain, liver and heart.

MATERIALS AND METHODS

Animals

A total of 376 weanling male rats (46.00 ± 0.60 g initial body weight) of a Wistar-originated outbred stock (obtained from Simonsen Lab., Gilroy, CA, U.S.A.) were distributed into six different dietary groups (A–F) of 63 or 62 rats each on the basis of uniformity in body weight. All animals were housed individually in suspended wire-bottomed cages and were randomly assigned to positions on the racks. The animal rooms were air-conditioned with the temperature generally maintained around 24 °C (except when occasional failures in the system caused temperatures to rise to about 27–28 °C for periods of 1 or 2 days) and the relative humidity around 50%. Hours of lightness and darkness were maintained on a 12:12 hour cycle. Common cleaning practices and sanitary precautions were routinely taken in the animal quarters to minimize significant infectious diseases. Rats presenting signs of sickness (*i.e.* nasal mucous–bloody discharge, marked snuffling, persistent diarrhea, marked reduction of food intake, major weight loss, *etc.*) were isolated in other rooms destined for this purpose until death or improvement occurred. Body weight changes of each rat were recorded at weekly intervals until the animals were killed or died. A number of "clinically healthy" rats from each group were used for biochemical and morphological determinations at the end of 3, 6, 12, 18 and 24 months after the initiation of the experiment. The remainder of animals were used to determine life span parameters, possible causes of death and incidence of several common diseases. Autopsies were generally performed within 10 hours after death. In these autopsied rats, the weights of brain, heart, lungs, kidneys, liver, spleen, pancreas and testes were recorded. Samples from these organs and eventually from others presenting gross evidence of abnormalities (*i.e.* tumors, inflammatory or necrotic processes, *etc.*) were collected, fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections for histological examinations were always stained with hematoxylin and eosin, but occasionally also with other selective staining methods.

Diets

The composition of the six different diets (A–F) is shown in Table I. All diets were isoenergetic providing approximately 435 kcal per 100 g. Linoleic acid was incorporated at the expense of sucrose into the saturated fat diets (SFD) offered to groups A and B to prevent any deficiency of essential fatty acids [15]. The extreme low and high amounts of *dl*-α-tocopherol (2 mg per 100 g of diet for groups A, C, and D, and 200 mg per 100 g of diet for groups B, D, and F) were expressly chosen to facilitate or to prevent possible development of changes. The lowest amount is, however, sufficient to promote normal growth and to maintain normal tissue concentrations of vitamin E in weanling rats [16], while the highest amount is higher than that reportedly associated with certain lipid changes in rats [17]. The contents of vitamin E in coconut and safflower oils were determined and, therefore, the total levels in the different diets (in mg per 100 g of diet) were: A, 2.07; B, 200.07; C, 2.52; D, 200.52; E, 2.34; and F, 200.34. Vitamin E was the only antioxidant present in all these diets. The amount of fat used in all diets was 15 g

TABLE I

COMPOSITION OF THE DIETS

The values given are expressed as g per 100 g of diet.

Ingredients[1]	*Dietary groups*					
	A	*B*	*C*	*D*	*E*	*F*
Vitamin-free casein	20.000	20.000	20.000	20.000	20.000	20.000
Coconut oil	15.000	15.000	—	—	6.000	6.000
Safflower oil	—	—	15.000	15.000	9.000	9.000
Linoleic acid	0.200	0.200	—	—	—	—
Sucrose	34.798	34.600	34.998	34.800	34.998	34.800
Dextrine	20.000	20.000	20.000	20.000	20.000	20.000
Non-nutritive bulk[2]	4.000	4.000	4.000	4.000	4.000	4.000
Salt mixture[3]	4.000	4.000	4.000	4.000	4.000	4.000
Vitamin mixture[4]	2.000	2.000	2.000	2.000	2.000	2.000
dl-α-Tocopherol[5]	0.002	0.200	0.002	0.200	0.002	0.200

[1] All ingredients were obtained from United States Biochemical Corp., Cleveland, OH, U.S.A.
[2] Cellufil.
[3] Hubbel, Mendel and Wakeman, *J. Nutr., 14* (1937) 273.
[4] The vitamin mixture provides (in mg per 100 g of diet): ascorbic acid, 90; choline chloride, 150; calcium pantothenate, 6; inositol, 10; menadione, 4.5; niacin, 9; *p*-aminobenzoic acid, 10; pyridoxine·HCl, 2; riboflavine, 2; thiamine·HCl, 2; ergocalciferol, 4; retynil acetate, 3.6; biotin, 0.04; folic acid, 0.18; vitamin B_{12}, 0.0027.
[5] 500 IU/g. (See text for further details.)

per 100 g (or 31 kcal per 100 kcal) because it has been shown that at this dietary level there was an almost 90% reduction in hepatic lipogenesis of rats, regardless of the type of dietary fat [18], and that higher amounts may decrease the life span of rats [19]. Diets containing unsaturated fat are abbreviated UFD, and those with a combination of saturated and unsaturated fat, CFD. The oils used in the diets were analyzed for the presence of conjugated dienes [20] as well as for their capability to produce malonaldehyde upon incubation [21]. As expected, no diene conjugates were detected in the samples of coconut oil but they were present in the safflower oil as indicated by a typical peak absorption (233–235 nm) in the difference spectrum between these two oils. The malonaldehyde production was 8.28 nmoles/g of coconut oil and 176.63 nmoles/g of safflower oil. The safflower oil used in our studies contained 70–77% linoleic acid, 0–0.3% linolenic acid, 15–22% oleic acid, and 6–7% saturated fatty acids (mainly myristic and palmitic). The coconut oil contained 44–50% lauric acid, 18–21% myristic, 8.5–11% palmitic, 3–10% stearic, 5.5–8.5% capric, 5–7 caprylic, and 3.5–7.5% unsaturated fatty acids (mainly oleic and with no more than 1.5% of linoleic acid).

All diets were prepared fresh at least twice weekly and kept before using in tightly stoppered containers at 4 °C in order to minimize lipoperoxidation and destruction of vitamin E. Diets and tap water were offered *ad libitum*. Daily food intakes were periodically recorded in all rats but the averages of daily food-intake gross energy (kcal) and

daily food efficiency (g of body weight gained per g of food consumed X 100) per rat in the different dietary groups were calculated from the values obtained only in "clinically healthy" rats during the first week of the experiment when the rats were one month old and when they were 3, 6 and 9 months old. Beyond 9 months of age the data lose significance due to the variable occurrence of disease [19] and therefore these determinations were discontinued.

Killing and sampling of tissues

For the biochemical determinations at the established periods a number of randomly selected "healthy" rats from each group were fasted for a period of 8–10 hours and then weighed and anesthetized by peritoneal injection of sodium pentobarbital (Nembutal; Abbot Laboratories, North Chicago, IL, U.S.A.) at the dosage of 50–60 mg/kg of body weight. The abdominal cavity was opened and blood was drawn from the inferior vena cava with heparinized needles and syringes and plasma was immediately separated and frozen. After blood extraction, liver, heart and brain were removed and weighed, immersed in ice-cold saline, cleaned and frozen. Kidneys, testes and spleen were also removed and weighed, but were stored in 10% neutral buffered formalin.

Other "healthy" animals were also randomly selected and killed at 3, 6, 12, 18 and 24 months for light- and electron-microscopic studies on brain, liver and heart, but the methods of vascular fixation, tissue sampling and processing, stereological analysis and the results obtained will be reported in subsequent publications.

Biochemical analysis

Plasma albumin/globulin ratios were determined using Rodkey's procedure [22], serum triglycerides by the method of Van Handel and Zilversmit [23], phospholipids by the method of Hurst [24] and cholesterol by the method of Zack *et al.* [25]. The colorimetric micromethod of Quaife *et al.* [26] was used for the assay of serum vitamin E (total tocopherols). Biochemical determinations of vitamin E, lipid fractions, protein, RNA, DNA, and connective tissue as well as the *in vitro* and *in vivo* tissue lipoperoxidation were also performed in brain, liver and heart, but the procedures and results will be published separately.

General statistical analysis

Conventional statistical methods were used for calculations of means, S.E.M. and correlation coefficients of the data on body and organ weights, food intakes, biochemical parameters and autopsy findings. Significance of differences between mean values was tested using Student's *t*-test (two-tailed test) or by means of analysis of variance. The differences and regression coefficients were considered statistically significant for values of $p < 0.05$.

Determination and analysis of life span parameters

The mean life span, the 50% survival time and the 95% maximum life span were estimated for each of the six different groups. The starting point from which these age

parameters were estimated was in fact the weaning period, although 22 days were added in order to move this point to the birth of the rats, disregarding the possible postnatal mortality, because the effect of the different dietary treatments was tested from the weaning period.

The mean life span was simply derived from the median value of each cohort. The 50% survival time (and the construction of the survivors curves presented in Fig. 2) was calculated from data compiled in a survivorship table [27], which included the rats actually dying at sequential time points, as well as those withdrawn and killed at 3, 6, 12, 18 and 24 months for biochemical and morphological studies. The testing for differences among median survival times of the various groups was carried out by analysis of variance using the single variable classification, model I, as described by Dixon and Massey [28]. The 95% maximum life span for each group was estimated on the basis of graphs of cumulative percentage of deaths (transformed into probits) *vs.* log (survival days) [29], and the determination of the best fitting line by regression analysis. The estimated variance for the analysis of the regression data was obtained by the method described by Goldstein [30]. A 95% confidence level was used for the estimation of differences.

RESULTS

Changes in body weights

The body weights of the rats in all groups rapidly increased from weaning to the period of 3 months after the initiation of dietary treatment (age 3 months and 21–22 days) and they continued increasing, although at a less rapid rate, until 15 months ("increasing phase"). No substantial changes were then observed at 18 and 21 months ("stabilizing phase"), but thereafter (at 24 and 27 months) the body weights decreased in all groups ("decreasing phase") (Fig. 1).

No significant differences in body weights were found throughout the entire experiment between groups fed diets with the same type of fat at different levels of vitamin E (groups A and B, C and D, or E and F). On the other hand, the type of dietary fat did influence in most instances the body weights during the "increasing phase" (*i.e.* from weaning to 15 months). Thus, at 3 months the body weights of rats fed SFD (groups A and B) were significantly lower than those fed the UFD (groups C and D) or CFD (groups E and F). While at 6 months the weights of groups A and B were not significantly different from those of groups C and D, they were still significantly lower than those of groups E and F. Furthermore, although at this period the average weight of group C was not significantly lower than those of groups E and F, the weight of group D was lower than those of groups E and F. At 9 months the weights of groups A and B were again not significantly different from those of groups C and D, but the weight of group A was still significantly lower than those of groups E and F, and the weight of group B was significantly lower than that of group F. In addition, the weight of group D was at 9 months significantly lower than that of group F. At 12 months after initiation of

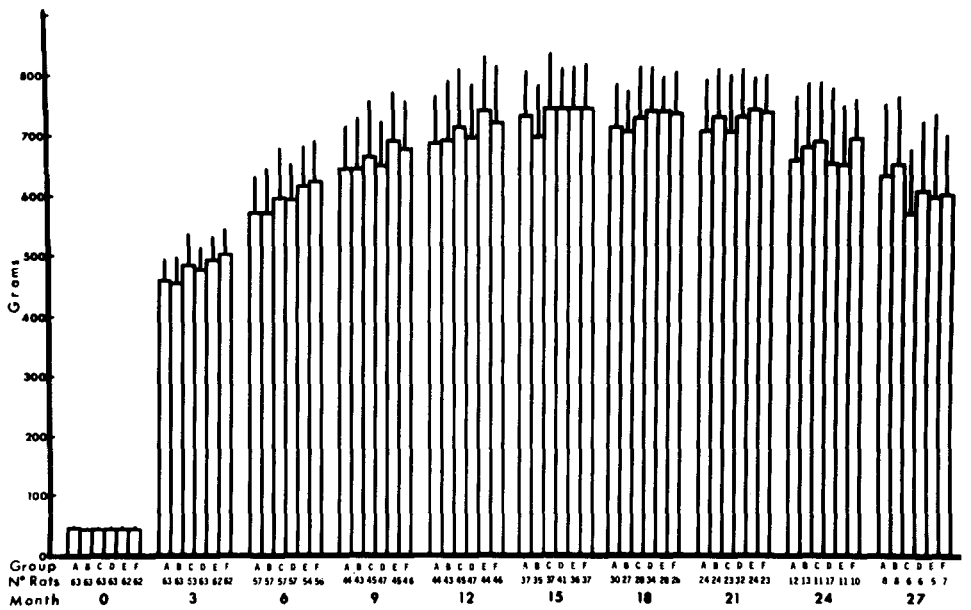

Fig. 1. Changes in body weights ± S.D. in the different groups.

treatment the weights of groups A and D were lower than that of group E, while at 15 months the weight of group B was significantly lower than those of groups C, D, E and F. Subsequently, during the "stabilizing phase" (18–21 months) and the "decreasing phase" (after 21 months) no significant differences were found between groups.

Organ weights

The relative weights (in g per 100 g body weight) of both kidneys, both testes and spleen of rats killed at 3, 6, 12, 18 and 24 months after the initiation of the experiment are presented in Table II, and those of brain, liver and heart in Table III. No significant differences in the relative weights of kidneys, testes and spleen were found between groups at each of these periods. In the brain the only difference was found at 24 months when the relative weight of group C was significantly lower than those of groups A, B and D. In the liver, at 6 months the relative weights of group E and F were significantly lower than that of group D, and at 24 months the relative weight of group E was significantly lower than that of group A. In the heart, at 18 months the relative weight of group D was significantly lower than that of group A.

While the absolute weights of kidneys, spleen, brain, liver and heart (not shown) progressively increased with age at a rate practically similar in the different groups, the absolute weight of testes remained almost stationary. On a global basis (irrespective of dietary treatments) the percentages of organ weight increments from 3 to 24 months were as follows: kidneys, 40%; spleen, 108%; brain, 43%; liver, 22%; and heart, 58%.

TABLE II

RELATIVE WEIGHTS OF KIDNEYS, SPLEEN AND TESTES

Weights are expressed in g per 100 g body weight, mean ± S.E.M. of 4 rats per group. The differences between means at each of the periods studied were not statistically significant.

Group	*3 Months*	*6 Months*	*12 Months*	*18 Months*	*24 Months*
Both kidneys					
A	0.70 ± 0.08	0.64 ± 0.02	0.53 ± 0.04	0.53 ± 0.01	0.71 ± 0.05
B	0.72 ± 0.04	0.54 ± 0.03	0.51 ± 0.02	0.52 ± 0.02	0.71 ± 0.07
C	0.72 ± 0.05	0.53 ± 0.04	0.49 ± 0.02	0.49 ± 0.01	0.62 ± 0.06
D	0.67 ± 0.05	0.53 ± 0.02	0.51 ± 0.01	0.46 ± 0.01	0.60 ± 0.02
E	0.70 ± 0.03	0.52 ± 0.03	0.53 ± 0.05	0.49 ± 0.01	0.62 ± 0.03
F	0.63 ± 0.03	0.52 ± 0.01	0.62 ± 0.08	0.49 ± 0.02	0.69 ± 0.02
Spleen					
A	0.17 ± 0.01	0.17 ± 0.02	0.16 ± 0.01	0.15 ± 0.01	0.23 ± 0.03
B	0.17 ± 0.02	0.16 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.25 ± 0.03
C	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.03	0.16 ± 0.01	0.19 ± 0.02
D	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.02	0.17 ± 0.01	0.28 ± 0.03
E	0.16 ± 0.01	0.17 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.23 ± 0.02
F	0.18 ± 0.01	0.18 ± 0.01	0.23 ± 0.08	0.14 ± 0.01	0.27 ± 0.01
Both testes					
A	0.73 ± 0.11	0.66 ± 0.02	0.47 ± 0.04	0.45 ± 0.03	0.57 ± 0.03
B	0.91 ± 0.13	0.62 ± 0.07	0.48 ± 0.04	0.40 ± 0.02	0.58 ± 0.02
C	0.73 ± 0.08	0.52 ± 0.06	0.43 ± 0.03	0.44 ± 0.01	0.49 ± 0.02
D	0.72 ± 0.05	0.61 ± 0.05	0.48 ± 0.02	0.39 ± 0.02	0.53 ± 0.02
E	0.71 ± 0.02	0.47 ± 0.04	0.43 ± 0.02	0.42 ± 0.03	0.48 ± 0.03
F	0.66 ± 0.01	0.61 ± 0.06	0.47 ± 0.04	0.44 ± 0.01	0.57 ± 0.02

TABLE III

RELATIVE WEIGHTS OF BRAIN, LIVER AND HEART

Weights are expressed in g per 100 g body weight, mean ± S.E.M. of 4 rats per group.

Group	*3 Months*	*6 Months*	*12 Months*	*18 Months*	*24 Months*
Brain					
A	0.34 ± 0.02	0.34 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.37 ± 0.02
B	0.36 ± 0.03	0.37 ± 0.03	0.27 ± 0.03	0.28 ± 0.01	0.37 ± 0.03
C	0.34 ± 0.02	0.34 ± 0.03	0.25 ± 0.01	0.26 ± 0.01	0.27 ± 0.01[1]
D	0.38 ± 0.01	0.38 ± 0.02	0.27 ± 0.04	0.24 ± 0.01	0.35 ± 0.02
E	0.39 ± 0.01	0.33 ± 0.03	0.28 ± 0.02	0.26 ± 0.01	0.34 ± 0.03
F	0.37 ± 0.02	0.33 ± 0.02	0.28 ± 0.03	0.26 ± 0.01	0.38 ± 0.05
Liver					
A	2.90 ± 0.09	2.77 ± 0.13	2.39 ± 0.08	2.16 ± 0.07	2.41 ± 0.07
B	2.84 ± 0.10	2.98 ± 0.28	2.30 ± 0.07	2.19 ± 0.10	2.72 ± 0.41
C	2.89 ± 0.14	2.34 ± 0.15	2.27 ± 0.14	2.06 ± 0.06	2.30 ± 0.07
D	2.94 ± 0.15	2.89 ± 0.20[2]	2.18 ± 0.09	2.12 ± 0.06	2.68 ± 0.22
E	2.68 ± 0.12	2.33 ± 0.09	2.28 ± 0.14	1.88 ± 0.09	2.03 ± 0.07[3]
F	3.03 ± 0.07	2.40 ± 0.05	2.21 ± 0.09	2.05 ± 0.13	2.48 ± 0.35

(Continued on facing page)

TABLE III *(continued)*

Group	*3 Months*	*6 Months*	*12 Months*	*18 Months*	*24 Months*
Heart					
A	0.27 ± 0.02	0.26 ± 0.02	0.22 ± 0.02	0.26 ± 0.01	0.34 ± 0.05
B	0.31 ± 0.02	0.26 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.35 ± 0.03
C	0.26 ± 0.02	0.20 ± 0.03	0.21 ± 0.01	0.21 ± 0.02	0.25 ± 0.04
D	0.27 ± 0.01	0.23 ± 0.01	0.22 ± 0.05	0.20 ± 0.01[3]	0.31 ± 0.01
E	0.29 ± 0.02	0.22 ± 0.01	0.21 ± 0.02	0.23 ± 0.01	0.27 ± 0.03
F	0.30 ± 0.01	0.25 ± 0.02	0.24 ± 0.03	0.22 ± 0.02	0.34 ± 0.02

[1]Significantly different ($p < 0.05$) from groups A, B, and D.
[2]Significantly different from groups E and F.
[3]Significantly different from group A.

TABLE IV

FOOD-INTAKE GROSS ENERGY AND FOOD EFFICIENCY

Group	*1 Month*	*3 Months*	*6 Months*	*9 Months*
Caloric intake[1]				
A	44.37 ± 0.74(60)[2]	90.48 ± 1.35(60)	83.52 ± 1.13(45)	85.69 ± 2.04(40)
B	43.50 ± 0.61(63)	89.61 ± 1.39(63)	81.34 ± 1.43(49)	83.52 ± 1.70(33)
C	39.15 ± 0.83(55)[3]	91.35 ± 1.57(51)	81.34 ± 1.61(39)	87.00 ± 1.26(39)
D	41.76 ± 0.61(59)	84.82 ± 1.35(51)[5]	76.56 ± 1.09(42)[5]	81.34 ± 1.78(32)[6]
E	44.80 ± 0.56(61)[4]	88.74 ± 1.30(64)	80.04 ± 1.35(48)	86.56 ± 1.48(40)
F	42.63 ± 0.70(60)	88.74 ± 1.10(58)	81.34 ± 1.17(50)	83.95 ± 1.61(33)
Food efficiency[7]				
A	64.30 ± 1.15(60)[2]	16.60 ± 0.50(60)	5.42 ± 0.36(45)	9.05 ± 0.53(40)[10]
B	66.10 ± 2.19(63)	15.10 ± 0.54(63)	7.42 ± 0.47(49)[10]	5.80 ± 0.48(33)
C	65.90 ± 0.95(55)	18.10 ± 0.43(51)[9]	5.38 ± 0.28(39)	5.15 ± 0.37(39)
D	66.10 ± 0.84(59)	16.00 ± 0.31(51)	5.54 ± 0.33(42)	4.19 ± 0.34(32)
E	63.90 ± 0.70(61)	15.60 ± 0.31(64)	5.48 ± 0.28(48)	7.31 ± 0.44(40)[11]
F	67.80 ± 0.89(60)[8]	15.40 ± 0.31(58)	5.78 ± 0.37(50)	5.22 ± 0.47(33)

[1]Expressed in kcal per day per rat.
[2]Mean ± S.E.M. (number of rats in parentheses).
[3]Lower than in all other groups ($p < 0.05$).
[4]Lower than F.
[5]Lower than A and D.
[6]Lower than C.
[7]Expressed in g of body weight gained per day per g of food consumed per day per rat × 100.
[8]Higher than E.
[9]Higher than B, D, E and F.
[10]Higher than in all other groups.
[11]Higher than C, D and F.

Food-intake gross energy and food efficiency

The data are presented in Table IV. In 1-month-old rats (first week of the experiment) the average daily food-intake gross energy per rat in group C was significantly

lower than in all the other groups. At 3 and 6 months the intake of group D was significantly lower than those of groups A, B and C, while at 9 months the intake of group D was only significantly lower than that of group C.

The results on food efficiency were quite variable in relation to diet and time. At 1 month, for example, the average daily food efficiency of rats from group F was significantly higher than in group E, but at 3 months the food efficiency of group C was significantly higher than in groups B, D, E and F. At 6 months the efficiency of group B was significantly higher than in all other groups, while at 9 months the food efficiency in group A was significantly higher than in all other groups and the food efficiency of group E was significantly higher than in groups C, D and F.

Life span parameters

The results obtained are presented in Table V. The statistical analysis showed no significant differences in the mean and maximum life span between different groups. However, the 50% survival time of group D was significantly longer than those of all the other groups and that of group E was significantly longer than those of groups A and C.

TABLE V

LIFE SPAN PARAMETERS

Values (in days) are expressed as mean ± S.E.M.

Group	*Mean life span*	*50% Survival time*	*95% Maximum life span*
A	724.66 ± 29.34	600.13 ± 30.57	1013 ± 50.03
B	739.47 ± 34.05	594.66 ± 35.59	1037 ± 63.07
C	713.45 ± 31.22	578.73 ± 33.29	1027 ± 65.06
D	796.00 ± 18.99	719.27 ± 13.29[1]	950 ± 181.01
E	731.11 ± 22.62	646.13 ± 26.08[2]	1030 ± 41.19
F	710.55 ± 29.28	602.73 ± 34.61	1050 ± 62.99

[1] Significantly ($p < 0.05$) higher than all other groups.
[2] Significantly higher than A and C.

The survivorship curves (Fig. 2) showed that mortality was negligible in all groups until about 12 months after the initiation of the experiment, and that no mortality occurred in group D before the 19th month.

Serum vitamin E

At all the periods studied the serum levels of vitamin E (total tocopherols) generally reflected those of the diets (Table VI). Among rats fed the SFD, the levels of group A (low vitamin E) were always significantly lower than those of group B (high vitamin E), while among rats fed the UFD the levels of group C were significantly lower than those of group D at 3, 6, 12 and 18 months, but not at 24 months. However, among rats fed the CFD a significant difference between groups E and F was only observed at 3 months.

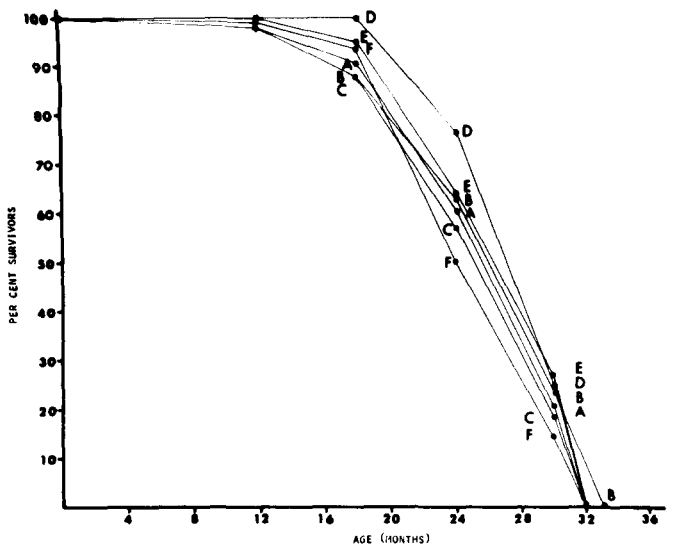

Fig. 2. Survivorship curves of the different groups.

TABLE VI

SERUM LEVELS OF VITAMIN E

Values are expressed in mg/dl of serum, mean ± S.E.M., number of rats in parentheses. Means in vertical columns not sharing a common superscript letter are significantly different at $p < 0.05$.

Group	*3 Months*	*6 Months*	*12 Months*	*18 Months*	*24 Months*
A	$0.23 \pm 0.04(4)^{a}$	$0.33 \pm 0.01(3)^{a}$	$0.32 \pm 0.01(4)^{a}$	$0.32 \pm 0.10(4)^{a}$	$0.63 \pm 0.09(3)^{a}$
B	$2.20 \pm 0.21(4)^{b}$	$1.66 \pm 0.32(3)^{b}$	$1.50 \pm 0.19(4)^{bf}$	$0.99 \pm 0.10(4)^{bd}$	$1.75 \pm 0.34(4)^{bd}$
C	$1.03 \pm 0.07(4)^{c}$	$0.50 \pm 0.05(3)^{c}$	$0.56 \pm 0.06(4)^{c}$	$0.56 \pm 0.06(4)^{ac}$	$1.48 \pm 0.28(4)^{bd}$
D	$2.34 \pm 0.07(4)^{bd}$	$1.19 \pm 0.07(3)^{bd}$	$1.16 \pm 0.10(4)^{bd}$	$1.25 \pm 0.10(4)^{b}$	$1.76 \pm 0.36(4)^{b}$
E	$0.93 \pm 0.08(4)^{ce}$	$0.49 \pm 0.01(3)^{ce}$	$0.90 \pm 0.08(4)^{de}$	$0.80 \pm 0.15(4)^{cd}$	$1.13 \pm 0.19(4)^{abd}$
F	$1.22 \pm 0.01(4)^{f}$	$1.24 \pm 0.12(3)^{bf}$	$1.23 \pm 0.16(4)^{df}$	$1.04 \pm 0.13(4)^{bd}$	$1.18 \pm 0.15(4)^{cd}$

Among rats fed the diets low in vitamin E (groups A, C and E) the level of group A (SFD) was significantly lower than those of C (UFD) and E (CFD) at 3, 6 and 12 months, but at 18 months the level of A was only significantly lower than that of E, and at 24 months only significantly lower than that of C. In addition the level of group C was significantly lower than that of group E at 12 and 18 months. On the other hand, among rats fed the diets high in vitamin E (groups B, D and F), the only significant differences were found at 3 months when the level of group F was lower than those of B and D.

The results also showed that the levels of group B progressively declined with time up to 18 months when the level of this group was significantly lower than at 3 months. The levels of groups C and D significantly decreased from 3 to 6 months and remained

almost stationary up to 18 months, but the levels of groups A and F did not show any significant changes with time throughout the experimental period. Finally, although the level of each of the different groups was higher at 24 months than at 18 months, this late increase was only statistically significant in the case of group C.

Serum lipids (Table VII)

Serum cholesterol. At 12 months the level of serum cholesterol in group F (CFD, high vitamin E) was significantly lower than that in group E (CFD, low vitamin E). No other significant difference between groups was found at any of the periods studied, and no significant changes with age were observed in this study.

Serum triglycerides. No significant differences in serum triglyceride levels were found between groups at 3 and 18 months, and the only difference detected at 12 months was between groups B (SFD, high vitamin E) and F (CFD, high vitamin E). At 6 months the level of group B was significantly higher than those of groups C and D, while at 24 months the level of group A was significantly higher than those of groups C, D and F. Although changes of serum triglycerides with time varied in each group, on a global basis there was a decreasing trend from 3 to 18 months and a subsequent rebound at 24 months.

Serum phospholipids. As in the case of triglycerides, no significant differences in the levels of serum phospholipids were found between groups at 3 and 18 months. At 6 months the levels of groups C and D were significantly lower than those of B and E, while the level of the latter was also lower than that of group F. At 12 months the levels of groups D and F were lower than that of group E, while at 24 months the level of group D was significantly lower than that of group E.

Serum albumin/globulin ratios. No significant differences in these ratios were found between groups at each of the periods studied (not shown) and no significant changes with age were observed.

Lesions encountered in dead rats

A wide variety of neoplastic, inflammatory and degenerative lesions were found with increasing frequency in rats of the different groups during the course of the experiment. All rats had generally more than one lesion and the combination of the pathologic changes in individual rats also increased with age.

Neoplasms

The group distribution and percentage incidence of benign and malignant neoplasms is presented in Table VIII. The percentage of rats with neoplasms (benign or malignant) ranged from 40% in group F to 60% in group A. Pituitary adenomas were by far the most frequent neoplasms encountered in rats of all groups (Table IX). The overall incidence ranged from 20% in group C to 33.3% in group A. Although a pituitary adenoma was found in a rat of group B dying 17 months after the initiation of the experiment, in this as well as in all the other groups these tumors were encountered in animals dying after the 18th month. In 25% of the total cases from all groups combined, the

TABLE VII

LEVELS OF SERUM CHOLESTEROL, TRIGLYCERIDES AND PHOSPHOLIPIDS

Values are expressed in mg/dl, mean ± S.E.M., number of rats in parentheses. Means in the vertical columns not sharing a common superscript letter are significantly different at $p < 0.05$.

Group	*3 Months*	*6 Months*	*12 Months*	*18 Months*	*24 Months*
Cholesterol					
A	82.05 ± 7.91(4)^{a}	56.46 ± 3.30(3)^{a}	80.29 ± 11.78(4)^{ab}	68.59 ± 14.25(3)^{a}	77.86 ± 11.84(6)^{a}
B	63.67 ± 9.13(4)^{a}	75.09 ± 11.01(3)^{a}	78.96 ± 11.70(4)^{ab}	57.99 ± 10.45(5)^{a}	75.40 ± 21.08(5)^{a}
C	83.08 ± 12.52(4)^{a}	63.91 ± 5.66(3)^{a}	86.46 ± 7.60(4)^{ab}	72.93 ± 14.15(4)^{a}	51.92 ± 10.94(4)^{a}
D	80.73 ± 9.24(4)^{a}	62.34 ± 3.30(4)^{a}	76.46 ± 14.17(4)^{ab}	89.00 ± 7.44(4)^{a}	63.40 ± 13.61(5)^{a}
E	77.20 ± 12.43(4)^{a}	74.70 ± 12.61(3)^{a}	103.38 ± 9.83(4)^{a}	95.78 ± 16.22(6)^{a}	79.72 ± 16.78(4)^{a}
F	71.31 ± 9.20(5)^{a}	66.87 ± 3.15(3)^{a}	66.46 ± 5.80(4)^{b}	67.77 ± 5.34(4)^{a}	62.34 ± 6.21(6)^{a}
Triglycerides					
A	133.44 ± 54.01(3)^{a}	154.90 ± 62.22(2)^{ab}	115.55 ± 23.33(4)^{ab}	48.00 ± 20.25(4)^{a}	109.47 ± 20.41(5)^{a}
B	93.45 ± 3.08(3)^{a}	82.62 ± 2.46(2)^{a}	65.45 ± 2.06(4)^{a}	44.97 ± 4.18(4)^{a}	80.64 ± 14.77(4)^{ab}
C	71.08 ± 30.60(3)^{a}	40.36 ± 10.93(3)^{b}	76.81 ± 12.50(4)^{ab}	58.12 ± 7.19(4)^{a}	46.70 ± 9.73(6)^{ab}
D	93.04 ± 11.89(3)^{a}	51.63 ± 7.29(3)^{bc}	71.58 ± 12.13(4)^{ab}	47.42 ± 7.22(5)^{a}	52.92 ± 15.01(4)^{bc}
E	87.10 ± 27.33(3)^{a}	98.00 ± 19.79(2)^{ab}	90.17 ± 11.45(4)^{ab}	40.79 ± 8.67(5)^{a}	70.69 ± 19.42(5)^{ab}
F	80.11 ± 42.83(2)^{a}	77.41 ± 22.43(2)^{ab}	105.98 ± 11.90(4)^{b}	35.43 ± 7.93(4)^{a}	44.48 ± 5.56(6)^{b}
Phospholipids					
A	217.51 ± 33.50(4)^{a}	173.57 ± 30.49(3)^{abc}	198.00 ± 19.58(4)^{ab}	241.15 ± 78.20(3)^{a}	219.38 ± 16.15(6)^{ab}
B	185.40 ± 11.75(3)^{a}	195.12 ± 9.61(2)^{ab}	179.65 ± 27.00(4)^{ab}	182.18 ± 21.44(6)^{a}	207.05 ± 22.63(5)^{ab}
C	209.59 ± 16.21(3)^{a}	137.52 ± 10.77(3)^{c}	178.28 ± 22.71(4)^{ab}	172.87 ± 19.79(5)^{a}	235.75 ± 21.81(6)^{ab}
D	201.89 ± 10.42(4)^{a}	140.00 ± 6.77(3)^{c}	174.12 ± 15.16(4)^{a}	149.07 ± 13.19(5)^{a}	211.35 ± 9.85(5)^{a}
E	211.37 ± 12.75(4)^{a}	212.05 ± 4.25(2)^{a}	223.85 ± 9.94(4)^{b}	166.80 ± 12.39(5)^{a}	256.79 ± 12.09(6)^{b}
F	218.15 ± 13.96(3)^{a}	136.05 ± 13.02(2)^{bc}	190.56 ± 3.90(4)^{a}	166.60 ± 19.31(4)^{a}	229.67 ± 12.74(6)^{ab}

TABLE VIII

DISTRIBUTION AND INCIDENCE OF NEOPLASMS

	Group					
	A	*B*	*C*	*D*	*E*	*F*
No. rats	30	30	30	30	29	27
Total no. rats with neoplasms	18(60)[1]	20(66.6)	16(53.3)	17(56.6)	12(41.4)	10(40.7)
Total no. neoplasms	20	22	16	17	12	11
Total no. malignant neoplasms	6(20)[2]	9(30)	5(16.6)	2(6.6)	2(6.8)	2(7.4)
Total no. benign neoplasms	14(46.6)[3]	13(43.3)	11(36.6)	15(50)	10(34.4)	9(33.3)

[1] Percentage of rats with neoplasms in parentheses.
[2] Percentage of rats with malignant neoplasms in parentheses.
[3] Percentage of rats with benign neoplasms in parentheses.

pituitary tumors were associated with other benign or malignant neoplasms, prominently with adrenocortical adenomas (8.5% of cases). Others were associated with carcinomas of adrenal glands, kidneys and lungs, reticulum cell sarcoma or with benign neoplasms such as islet cell adenoma of the pancreas, renal lipoma and subcutaneous fibroma. Fifty-five per cent of the rats with pituitary adenomas had emaciation at the moment of death (*i.e.* > 30% weight loss as compared with the average weights attained by normal rats of the same group at similar periods). The pituitary tumors were generally spherical and circumscribed, brownish red, soft and friable. They measured on the average 0.5 cm in their largest diameter and ranged from a minimum of 0.1 cm to a maximum of 1.5 cm. Practically all tumors protruded from the sella turcica and produced varying degrees of brain compression. In one case, the rupture of a tumor measuring 0.7 cm across had produced a massive cerebral hemorrhage that was the most probable cause of death. Histologically, all these tumors were chromophobic, sparsely granulated acidophilic adenomas with slight architectural variations conferred by the degree of capillary dilation (Fig. 3). Most were totally or partially angiomatous and compressed the remnant normal hypophyseal tissue. Two of the tumors had frankly malignant histologic features but had no signs of invasion or metastasis. In one tumor examined by electron microscopy the cells had in general consistently uniform ultrastructural features characterized by concentric whorls of rough endoplasmic reticulum occupying relatively large portions of the cytoplasm and surrounding spherical secretory granules (Fig. 4a). The latter were either small and uniformly dense or were large and had a dense core and a lighter shell (Fig. 4b and c). Other whorls only contained empty or less-opaque vacuoles. The Golgi apparatus was generally prominent and the mitochondria were moderate in number, spherical or rod-shaped and had regularly spaced cristae. Nuclei were large, spherical or slightly polyhedral, rich in chromatin and with one or occasionally two nucleoli.

Adrenocortical adenomas followed the pituitary adenomas in frequency. Their rather uniform incidence between groups ranged from 6.7% in group C to 11% in group F and were never found in rats dying before the 18th month (Table IX). These tumors were

TABLE IX

THE FOUR MOST FREQUENT NEOPLASMS

Group		Age (months)					Total no. cases	Percentage overall incidence	Total no. rats examined
		<12	12–18	18–24	24–30	>30			
A	No. rats examined	1	3	12	12	2			30
	Pituitary adenoma	–	–	5	5	–	10	33.3	
	Adrenocortical adenoma	–	–	–	1	2	3	10.0	
	Adrenocortical carcinoma	–	–	–	1	–	1	3.3	
	Renal cell carcinoma	–	–	1	2	–	3	10.0	
B	No. rats examined	1	5	10	10	4			30
	Pituitary adenoma	–	1	3	3	1	8	26.6	
	Adrenocortical adenoma	–	–	–	2	1	3	10.0	
	Adrenocortical carcinoma	–	–	1	1	–	2	6.6	
	Renal cell carcinoma	–	–	–	–	–	–	–	
C	No. rats examined	1	5	12	10	2			30
	Pituitary adenoma	–	–	1	4	1	6	20.0	
	Adrenocortical adenoma	–	–	2	–	–	2	6.7	
	Adrenocortical carcinoma	–	–	–	–	–	–	–	
	Renal cell carcinoma	–	–	–	–	–	–	–	
D	No. rats examined	–	–	10	16	4			30
	Pituitary adenoma	–	–	1	6	1	8	26.6	
	Adrenocortical adenoma	–	–	–	1	2	3	10.0	
	Adrenocortical carcinoma	–	–	–	1	–	1	3.3	
	Renal cell carcinoma	–	–	–	–	–	–	–	
E	No. rats examined	–	2	13	14	–			29
	Pituitary adenoma	–	–	4[1]	4	–	8	27.6	
	Adrenocortical adenoma	–	–	–	2	–	2	6.9	
	Adrenocortical carcinoma	–	–	1	–	–	1	3.4	
	Renal cell carcinoma	–	–	–	–	–	–	–	
F	No. rats examined	–	2	15	9	1			27
	Pituitary adenoma	–	–	6[1]	1	–	7	26.0	
	Adrenocortical adenoma	–	–	1	1	1	3	11.1	
	Adrenocortical carcinoma	–	–	–	–	–	–	–	
	Renal cell carcinoma	–	–	1	–	–	1	3.7	

[1] One of these pituitary tumors was a carcinoma.

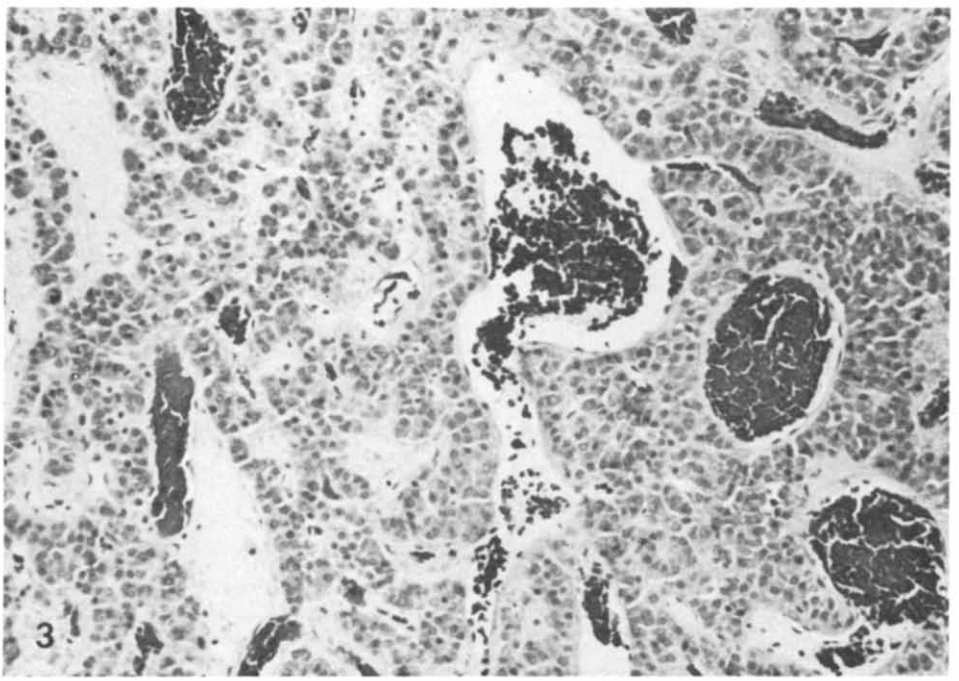

Fig. 3. Angiomatous pituitary adenoma; haematoxylin–eosin, ×200.

generally spherical, greyish and moderately firm. Their varying sizes ranged from 0.3 to 1.5 cm and, with one exception, were all bilateral. Histologically, they were generally composed of cells displaying homogeneous, finely granulated or conspicuously vacuolated cytoplasm (Fig. 5). Their usually spherical nuclei had moderate amounts of peripheral and nucleoplasmic chromatin and one or two nucleoli. These cells tended to arrange in fascicles or clusters in association with capillaries that occasionally adopted angiomatous configurations.

Five primary adrenocortical carcinomas were also found, one in group A, two in group B, one in D and one in E (Table IX). All these cancers had invaded the capsule of the gland and in three cases there were multiple metastases to the lung. In three cases they were bilateral. The size of these carcinomas ranged from 0.5 to 2 cm in the major diameter and histologically they had variable patterns from poorly differentiated to more trabecular forms.

Of the four renal cell carcinomas, three were found in group A and one in group F, all in rats dying after the 18th month (Table IX). The tumor of group F measured approximately 0.8 cm in its major diameter, was histologically predominantly of papillary type and although signs of active invasion to the capsule and perirenal fat presented, no metastases were seen in other organs. Conversely, one of the tumors in group A measured 1.2 cm in diameter, was well circumscribed, had a glandular type and had metastasized to regional lymph nodes (Fig. 6). The other two renal carcinomas in group A measured

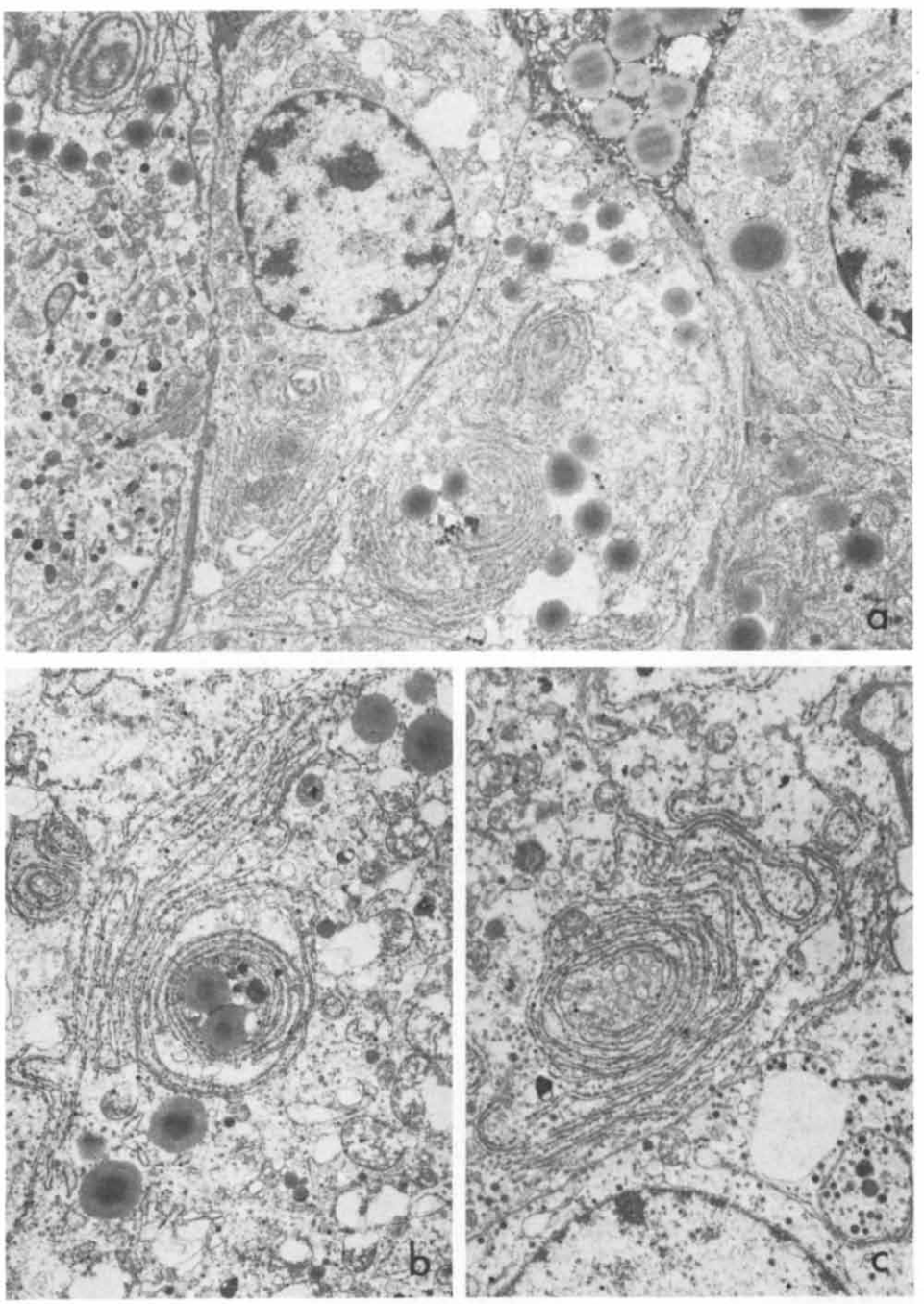

Fig. 4. Ultrastructural features of cells from a pituitary adenoma. (a) Uranyl acetate and lead citrate stain, ×5200. (b) Concentric whorls of rough endoplasmic reticulum surrounding small dense and larger granules with dense cores and lighter shells, ×9000. (c) Concentric whorls surrounding empty vacuoles, ×9000.

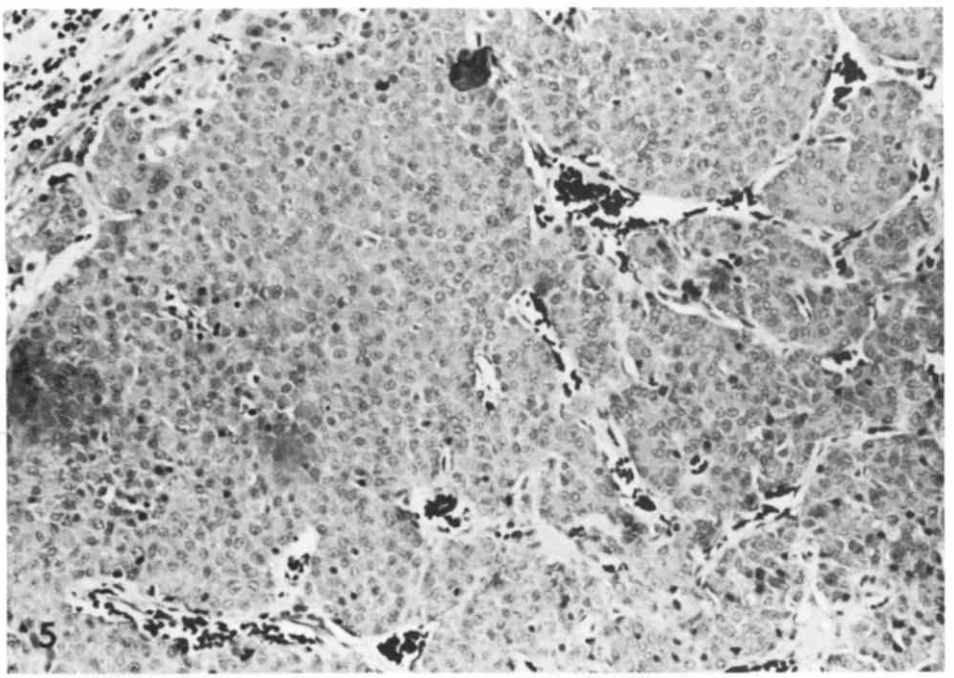

Fig. 5. Adrenocortical adenoma; haematoxylin–eosin, ×200.

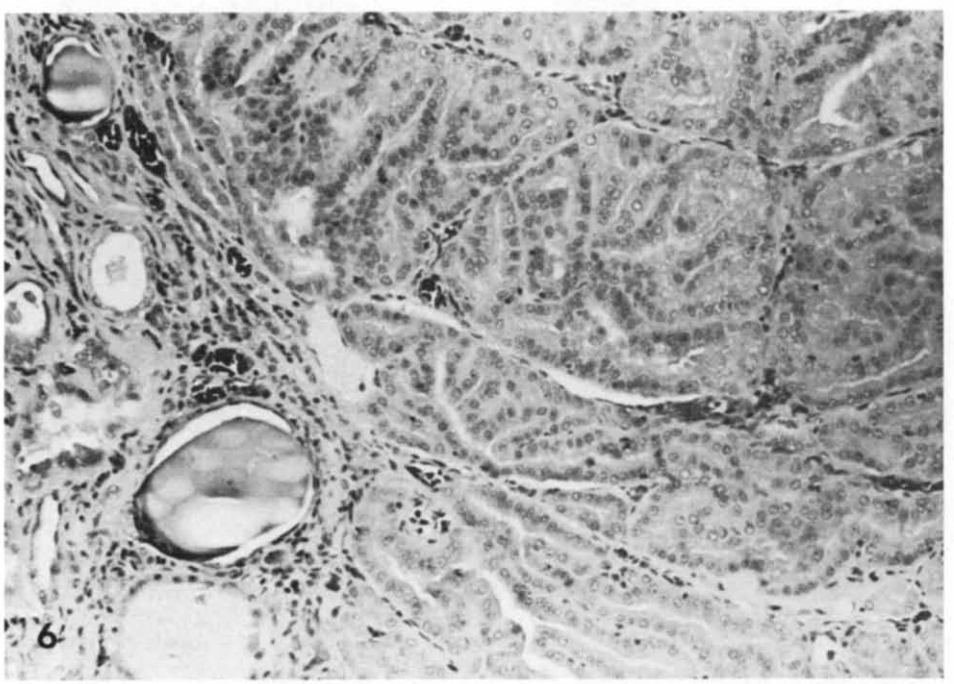

Fig. 6. Renal cell carcinoma, glandular type, and a portion of kidney with chronic nephropathy; haem atoxylin–eosin, ×200.

7–8 cm in diameter and weighed up to 34 g; they were of glandular type although both also had areas of more solid anaplastic type. In one of these cases the tumor involved both kidneys and had no detectable metastases, while in the other metastases were found in regional lymph nodes, pancreas and spleen. In no case were metastases found grossly or in sections of the lungs examined microscopically. Less-frequently encountered benign and malignant neoplasms are presented in Table X. The earliest finding was a monocytic leukemia occurring at 13 months in a rat of group F that presented splenomegaly (9.3 g) with profuse infiltration of mononuclear cells in the splenic red pulp and in the portal spaces of liver. This rat had in addition marked dilatation of the urinary bladder with bloody urine retention, and during the 3–4 months prior to death had paralysis of the hindlegs, probably due to radiculitis (not examined histologically).

TABLE X

LESS-FREQUENT NEOPLASMS

Group A	*Group B*	*Group C*	*Group D*	*Group E*	*Group F*
Prostatic carcinoma (20)[1]	Liposarcoma, retroperitoneal (19)	Colonic carcinoma (17)	Hepatic hemangioma (23)	Subcutaneous osteoma (19)	Monocytic leukemia (13)
Osteoma, parietal bone (28)	Adenocarcinoma, unknown origin (19)	Gastric carcinoma (19)	Islet cell adenoma, pancreas (24)		
Fibrosarcoma, hind-leg (30)	Reticulum cell sarcoma (21)	Transitional cell carcinoma, urinary bladder (19)	Leiomyosarcoma, neck (25)		
	Hepatocellular carcinoma (23)	Reticulum cell sarcoma (23)	Splenic hemangioma (26)		
	Bronchiolar carcinoma (26)	Mucoepidermoid carcinoma, salivary gland (25)	Subcutaneous fibroma (28)		
	Myxosarcoma, neck (29)	Renal lipoma (25)			
	Prostatic carcinoma (29)	Hepatic hemangioma (31)			
	Rhabdomyofibroma, hind-leg (30)	Islet cell adenoma, pancreas (32)			
	Gingival leiomyofibroma (32)				

[1] Age at death in months.

The prostatic carcinomas found in groups A and B appeared to arise from the ventral prostate and were presented as lobulated masses of approximately 2–2.5 cm across emerging from the lumbopelvic area. In both instances they were associated with bilateral hydronephrosis and with involvement of abdominal lymph nodes. Histologically, one of these tumors was a poorly differentiated scirrhous adenocarcinoma (Fig. 7), and the other a less-differentiated adenocarcinoma but also with abundant collagenous stroma.

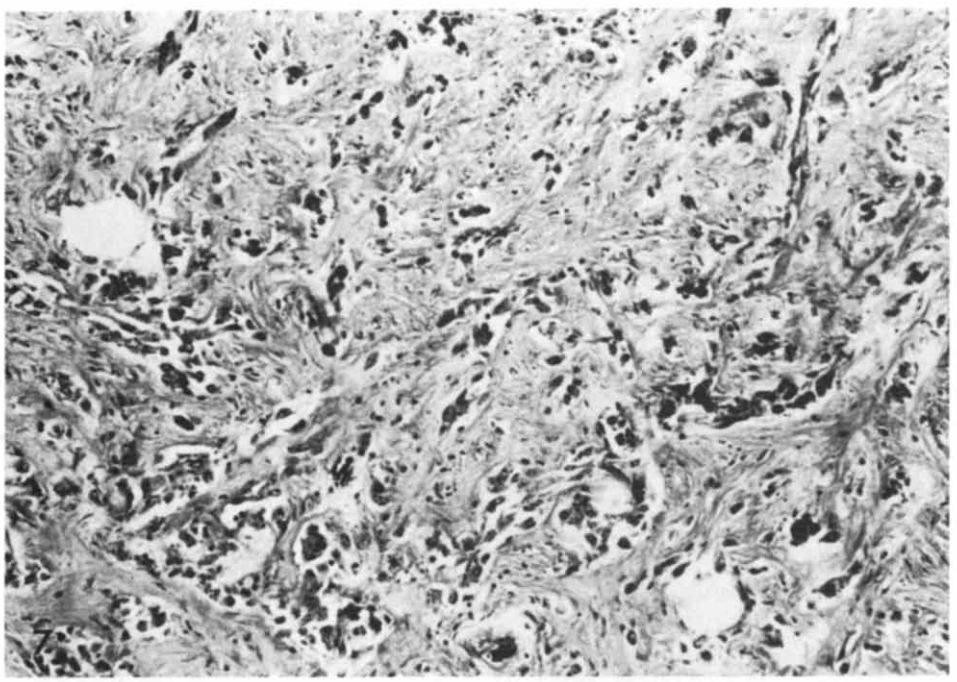

Fig. 7. Prostatic carcinoma with abundant collagenous stroma; haematoxylin–eosin, ×200.

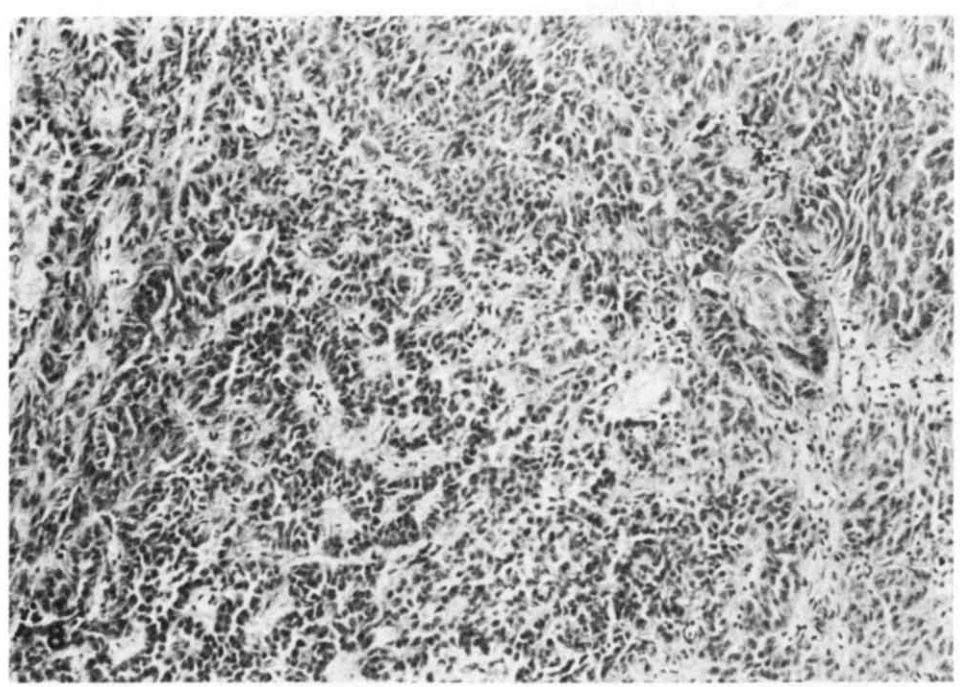

Fig. 8. Transitional cell carcinoma of urinary bladder; haematoxylin–eosin, ×170.

The transitional cell carcinoma of the urinary bladder (Fig. 8) encountered in a rat of group C was presented as a polypoid mass (~ 1 cm across) attached to the posterior wall of this greatly dilated viscus and infiltrating the lumbopelvic surrounding tissues.

The gastric carcinoma of group C appeared as a fungiform and infiltrating mass in the wall of the glandular stomach which also had a deep erosion in the anterior wall and adhesions to epiplon and liver. This tumor was histologically a poorly differentiated adenocarcinoma and had profusely metastasized to abdominal lymph nodes, liver, lungs and thymus (Fig. 9).

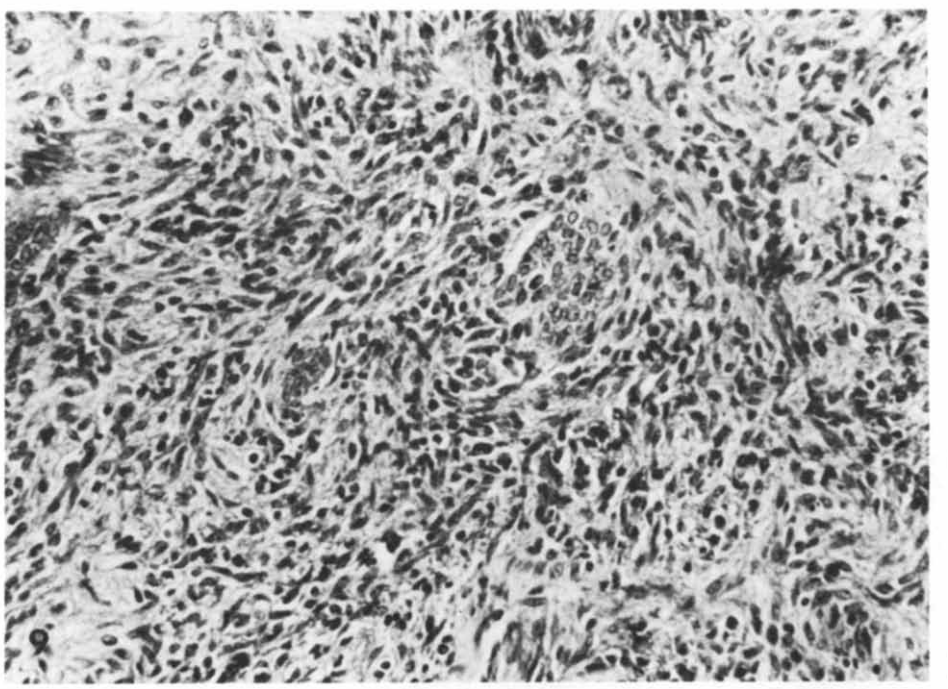

Fig. 9. Poorly differentiated gastric adenocarcinoma; haematoxylin–eosin, ×270.

The colon carcinoma of group C was found as a large pear-shaped mass of approximately 5 cm in its major diameter and weighing 37.5 g. This tumor was fixed to the wall of the descending colon and histologically had the features of a poorly differentiated but mucosecreting carcinoma. Although this tumor did not involve the regional lymph nodes or other organs of the abdominal cavity, metastases to the lungs were profuse.

The hepatocellular carcinoma of group B was a well-circumscribed nodule which grossly appeared simply to be an adenoma. However, histologically, there were areas of frank malignancy and, rather surprisingly, it had metastasized to the spleen but not to the lungs. Of the two reticulum cell sarcomas, the one in group C involved only the spleen and produced moderate enlargement of this organ (4.5 g), while the other in group B produced marked splenomegaly (28 g) and involved the liver, lungs and the left cardiac appendage.

The mucoepidermoid carcinoma of the salivary gland appeared to arise from the submaxillary glands and was presented as a lobulated mass (~ 3 cm across) in the left anterior side of the neck. Although the mucoepidermoid components predominated in this tumor, there were areas resembling the malignant form of the pleomorphic variant. This tumor was highly invasive and metastases were found in several subcutaneous nodes of the upper chest as well as in the lungs and thymus.

The islet cell adenomas of the pancreas found in groups C and D were both almost spherical and measured about 0.5 cm in diameter. Histologically they had a benign aspect and consisted of clusters and cords of cells resembling normal beta cells interspaced by a capillary net (Fig. 10). The cytoplasm of these cells was in general moderately granulated and showed discrete vacuolations. The tumor in group C was associated with pituitary adenoma.

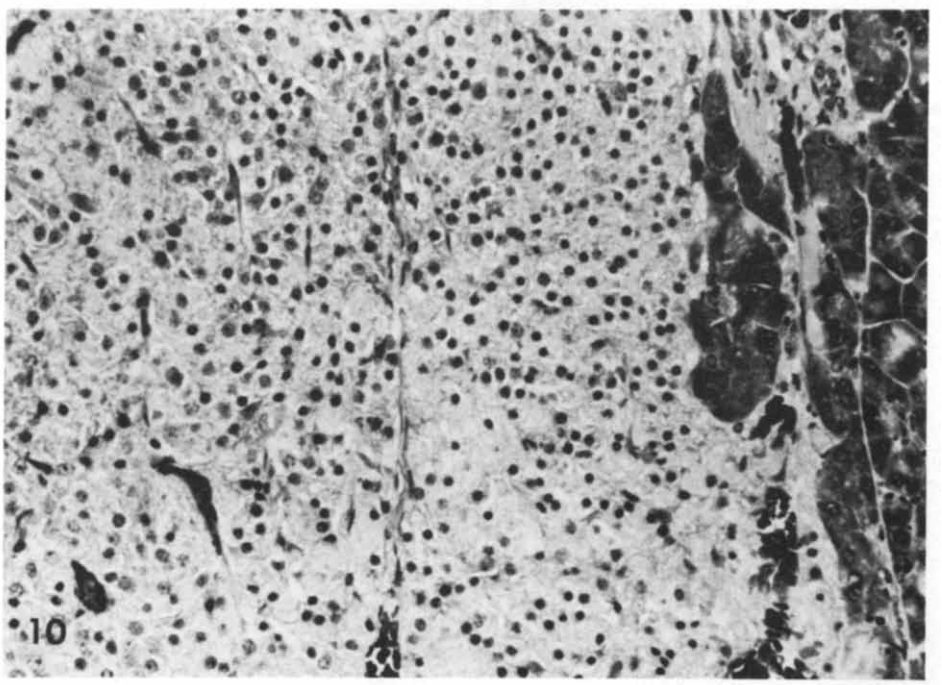

Fig. 10. Islet cell adenoma of pancreas; haematoxylin–eosin, X270.

The bronchiolar (alveolar) carcinoma of group B was grossly seen as a rounded whitish mass about 0.7 cm across, located in the lower lobe of the left lung. Histologically it showed the typical features of these tumors and appeared to have a low degree of malignancy (Fig. 11). This tumor was also associated with a pituitary adenoma.

The fibrosarcoma found in the left hindleg of a rat from group A measured about 7 cm across and apparently had not metastasized to other organs.

The retroperitoneal liposarcoma of group B was seen as a large mass of about 10 cm in its major diameter displacing anteriorly the left kidney and the rest of the abdominal

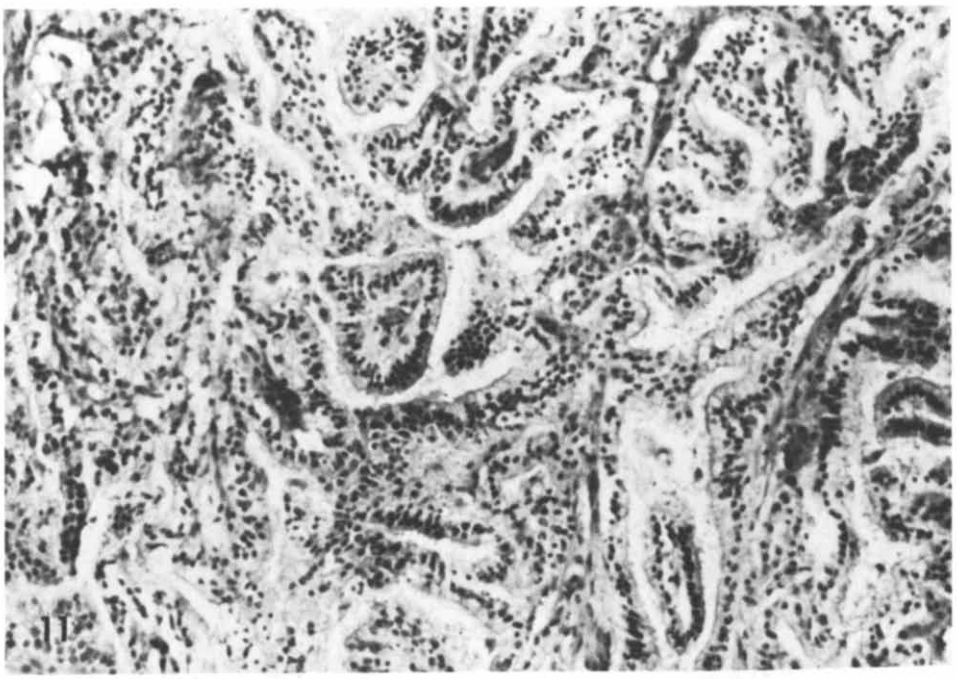

Fig. 11. Bronchiolar (alveolar) carcinoma; haematoxylin–eosin, ×200.

viscera to the right. The left kidney of this animal showed moderate hydronephrosis and had a 2 mm depressed cortical infarct, but no metastases were found in this or other organs.

The myxosarcoma found in the subcutaneous tissue of the anterior aspect of the neck in a rat from group B was a lobulated gelatinous mass approximately 7 cm across. This tumor was associated with another firmer mass about 2 cm across in the left axilla. Histologically both tumoral masses had almost similar features although the one in the axilla had more collagenous stroma.

The leiomyosarcoma found in the subcutaneous tissue of the anterior middle aspect of the neck of a rat from group D measured 3 cm in its major diameter and apparently had not metastasized.

The rhabdomyofibroma in the left hindleg of a rat from group B presented as a solid and rather well-circumscribed large mass, 8 cm in its major diameter and weighing 77 g. Histologically it showed vast areas of hyalinization and necrosis.

The gingival leiomyoma in group B was found as a round nodule adjacent to the incisors and measuring 0.4 cm across.

The renal lipoma measured 1 cm in its major diameter and appeared to be more a fat ingrowth than a benign neoplasm because the contours were imprecise; it was not surrounded by a capsule and did not compress the remaining renal tissue. However, this formation bulged from the surface of the kidney and the adipose cells were of extremely variable sizes. The hepatic hemangioma in group C was a very small (~ 0.1 cm) sub-

capsular tumor but the other in group D was a large multicentric cavernous formation. A large cavernous hemangioma was also found in the spleen of another rat from group D.

The subcutaneous fibroma in a rat from group D was a conglomerate of nodules of various dimensions (1–5 cm across) found in the lower abdominal wall.

The subcutaneous osteoma found in the posterior aspect of the neck in a rat from group E was a well-encapsulated spherical tumor that measured 3 cm in diameter and weighed 23.5 g. The other osteoma found in a rat from group E arose from the left parietal bone, measured 0.8 cm in its major diameter and grew internally, markedly displacing the brain to the right.

Major non-neoplastic lesions

Urogenital system

The vast majority of rats dying during the experiment had the well-defined spectrum of renal lesions characteristic of the so-called "chronic nephropathy". These lesions involve all segments of the nephron and consist of various degrees of glomerulosclerosis, degenerations and atrophy of the tubular epithelium, tubular dilatation, hyalin casts, thickening of the arteries and arterioles as well as interstitial inflammation and fibrosis (Fig. 12).

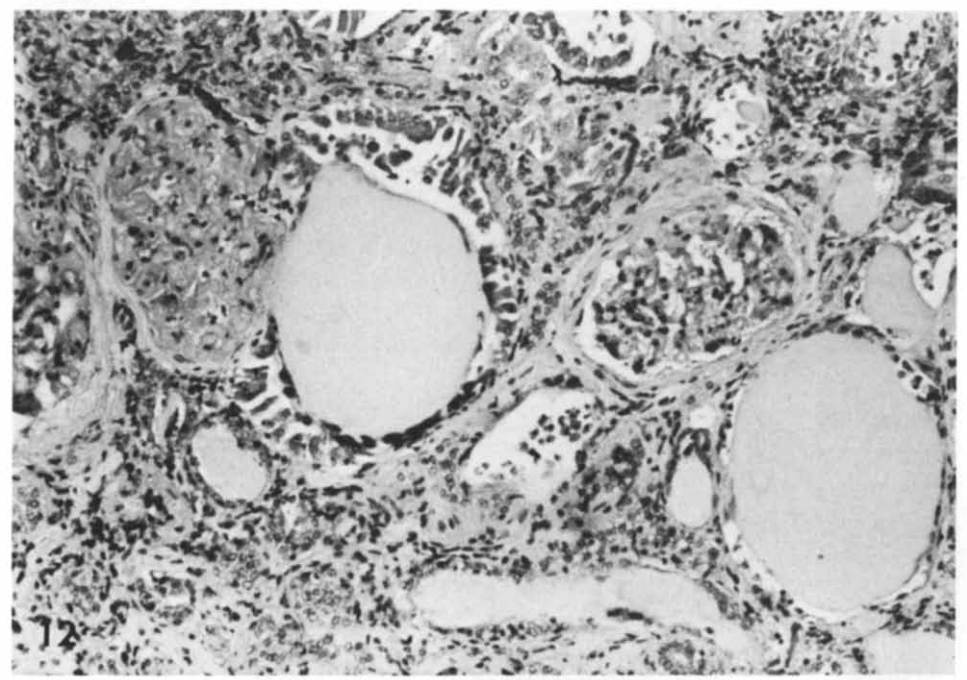

Fig. 12. Cortical area of a kidney with advanced chronic nephropathy; haematoxylin–eosin, X 200.

The index criteria for the degree of severity of this renal disease was based on the semiquantitative estimation of the magnitude of each of the different lesions in a given kidney with particular emphasis on the glomerular lesions. Since there was a significant

correlation between the increment in the relative weight of the kidneys and the increment of severity of the lesions, the renal weights were also used for indexing criteria. The results of these evaluations in the different groups are presented in Table XI. Correlation coefficients based on the sample size (not shown) varied in the different groups but all showed a significant relationship of increasing age with increasing severity of chronic nephropathy. Despite obvious differences in the distribution of the various degrees of severity between groups, we were unable to obtain a statistical expression denoting significant differences that could be attributed to the influence of the diet. A significant relationship was also found (not shown) between the weights of the affected kidneys

TABLE XI

DISTRIBUTION AND DEGREES OF CHRONIC NEPHROPATHY

Group		*Age (months)*					*Total no. lesions*	*Percentage overall incidence*	*Total no. rats*
		<12	*12–18*	*18–24*	*24–30*	*>30*			
A	No. rats	1	3	12	12	2			30
	Grade 1	–	1(33.3)[1]	5(41.6)	3(25)	1(50)	10	33.3	
	Grade 2	–	2(66.6)	3(25)	3(25)	–	8	26.6	
	Grade 3	–	–	3(25)	4(33.3)	–	7	23.3	
	Grade 4	–	–	1(8.3)	2(50)	–	4	13 3	
B	No. rats	1	5	10	10	4			30
	Grade 1	1(100)	5(100)	2(20)	1(10)	–	9	30.0	
	Grade 2	–	–	1(10)	1(10)	1(25)	3	10.0	
	Grade 3	–	–	5(50)	5(50)	3(75)	13	43.3	
	Grade 4	–	–	2(20)	3(30)	–	5	16.6	
C	No. rats	1	5	12	10	2			30
	Grade 1	–	2(40)	1(8.3)	–	–	3	10.0	
	Grade 2	–	2(40)	6(50)	4(40)	–	12	40.0	
	Grade 3	–	1(20)	1(8.3)	5(50)	1(50)	8	26.6	
	Grade 4	–	–	4(33.3)	1(10)	1(50)	6	20.0	
D	No. rats	–	–	10	16	4			30
	Grade 1	–	–	–	2(12.5)	–	2	6.7	
	Grade 2	–	–	4(40)	3(18.7)	–	7	23.3	
	Grade 3	–	–	2(20)	2(12.5)	2(50)	6	20.0	
	Grade 4	–	–	4(40)	9(56.2)	2(50)	15	50.0	
E	No. rats	–	2	13	14	–			29
	Grade 1	–	2(100)	5(38.4)	2(14.3)	–	9	31.0	
	Grade 2	–	–	4(30.7)	2(14.3)	–	6	20.7	
	Grade 3	–	–	2(15.3)	2(14.3)	–	4	13 8	
	Grade 4	–	–	2(15.3)	8(57.1)	–	10	34.5	
F	No. rats	–	2	15	9	1			27
	Grade 1	–	1(50)	3(20)	–	–	4	14.8	
	Grade 2	–	–	5(33.3)	3(33.3)	–	8	29.6	
	Grade 3	–	–	1(6.6)	3(33.3)	1(100)	5	18.5	
	Grade 4	–	–	6(40)	3(33.3)	–	9	33.3	

[1] Figures in parentheses represent the percentage incidence of rats within the age group with a given degree of lesion (for index criteria see text).

and the weight of the heart, lungs and liver. Rats with severe nephropathy almost invariably showed hypertrophy of the heart and passive congestion of the liver, as well as congestion and edema of the lungs. In addition, a large number of rats dying with the most advanced degrees of chronic nephropathy also showed accumulation of serosanguinous fluid in the abdominal and thoracic cavities and sometimes even in the pericardial sack.

Another lesion found in kidneys was the already mentioned hydronephrosis secondary to tumor compression.

Urinary bladder lesions consisted of one case of hemorrhagic cystitis (group A), one case of purulent cystitis in a rat from group C with abscessed pneumonia, and three cases of marked dilatation of this viscus, one in a rat of group B with prostatic carcinoma and two in rats of groups E and F that had paralysis of the hindlegs and therefore probably had neurogenic atony. The rat of group F had in addition a 0.3 cm periurethral abscess.

Testicular atrophy was common in all rats and its incidence increased with age, particularly in rats dying after the 18th month. This testicular atrophy was found equally in all groups and could not be correlated with any particular disease or cause of death other than with aging *per se*. The only other testicular lesion observed was the massive coagulative necrosis of the left testis due to venous infarction in a rat from group A dying with an invasive prostatic carcinoma.

Cardiovascular system

Focal cardiac myocytic degenerations and interstitial fibrosis were extremely common findings in all groups. These two lesions were frequently coincidental and their incidence increased with age but, with few exceptions, the degree of involvement was only moderate and was generally localized to the left ventricle and its papillary muscles. In practically all cases these lesions were found in rats with moderate to advanced degrees of chronic nephropathy. Focal acute or subacute myocarditis was found in 11 cases. This lesion was associated with acute pneumonia in two instances (groups B and C) with purulent meningitis on one occasion (group B), with otitis–septicemia in another (group E), and the rest in rats with chronic nephropathy as the dominant anatomical finding. Other cardiac lesions were a small focus of acute endocarditis in the wall of the left ventricle of a rat from group B and extensive fibrinous pericarditis in a rat from group E with chronic nephropathy.

No significant changes were observed in the coronary arteries of our rats in any of the groups, and only 4 cases of mild to moderate atherosclerosis of the aorta with some focal calcifications were encountered: one in group A, two in group D and another in group F. However, 8 cases of severe and extensive thrombosis of the mesenteric arteries with conspicuous atherosclerotic changes were found: one in group A, three in group B, three in group D and one in group E. In all these cases of aortic atherosclerosis and mesenteric thrombosis the affected animals had died after the 25th month and all had associated chronic nephropathy grade 3 or 4. No intestinal infarction was detected in cases of mesenteric thrombosis.

TABLE XII

DISTRIBUTION OF ACUTE PNEUMONIA AND FOCAL CHRONIC INTERSTITIAL PNEUMONIA

Group		*Age (months)*					*Total no. lesions*	*Percentage overall incidence*	*Total no. rats*
		<12	*12–18*	*18–24*	*24–30*	*>30*			
A	No. rats	1	3	12	12	2			30
	Acute pneumonia	–	2(66.6)[1]	4(33.3)	–	–	6	20.0	
	Focal interst.	–	–	7(58.3)	2(16.6)	1(50)	10	33.3	
B	No. rats	1	5	10	10	4			30
	Acute pneumonia	1(100)[2]	2(40)[2]	–	1(10)[2]	–	4	13.3	
	Focal interst.	–	2(40)	2(20)	6(60)	2(50)	12	40.0	
C	No. rats	1	5	12	10	2			30
	Acute pneumonia	–	2(40)	4(33.3)[2]	1(10)[2]	–	7	23.3	
	Focal interst.	–	–	3(25)	2(20)	–	5	16.6	
D	No. rats	–	–	10	16	4			30
	Acute pneumonia	–	–	2(20)	4(25)[2]	–	6	20.0	
	Focal interst.	–	–	2(20)	1(6.2)	1(25)	4	13.3	
E	No. rats	–	2	13	14				29
	Acute pneumonia	–	1(50)	4(30.8)	–	–	5	17.2	
	Focal interst.	–	1(50)	3(23.1)	1(7.1)	–	5	17.2	
F	No. rats	–	2	15	9	1			27
	Acute pneumonia	–	–	5(33.3)[2]	1(11.1)	1(100)	7	25.9	
	Focal interst.	–	–	1(6.6)	3(33.3)	–	4	14.8	

[1] Figures in parentheses represent the percentage incidence of rats within the age group with a given lesion.

[2] One of these rats had abscessed pneumonia.

Respiratory system

Besides the congestion and edema of the lungs which, as mentioned above, were almost invariably encountered in rats with advanced chronic nephropathy, the most common lesions found in rats of the different groups were acute pneumonia and focal chronic interstitial pneumonia (Table XII). The former lesion tended to occur in younger rats and usually involved more than one lobe. In a relatively small number of cases with acute pneumonia, the affected lungs had also abscessed formations and on two occasions purulent pleuritis. The focal chronic interstitial pneumonia was generally

mild and consisted of moderate fibrous thickening of the alveolar septa with few mononuclear leucocytes and macrophages. None of the rats in this study had the so-called "chronic murine pneumonia" that is attributed to *Mycoplasma pulmonis* [31]. In several instances, small whitish spots were grossly found in subpleural or internal regions of the lungs. These tiny (~ 1 mm across) and usually multiple lesions did not appear to increase with age and simply consisted of foamy macrophages accumulated in the alveolar spaces.

Digestive system

Moderate to marked dilatation of the esophagus (up to 0.7 cm) was found in a rat from group A, in another from group D and in two rats from group E; all these cases were associated with advanced chronic nephropathy although this association could be simply fortuitous. In one of these cases (group D) there was in addition a bilateral adrenocortical carcinoma, and in another (group E) a marked dilatation of the urinary bladder in a rat that had hindleg paralysis and probably radiculitis. A very marked gastric dilatation was found in a rat from group F that had obstruction of the antrum and pylorus due to the impaction of a large bolus of hair (tricolith).

An ulceration with purulent exudate was found in the hard palate of a rat from group B that also had abscessed pneumonia. Two gastric ulcers with perforation and peritoneal adhesions were encountered: one already mentioned in association with the gastric carcinoma in group C, and the other in a rat from group D having, in addition, advanced chronic nephropathy and a pituitary adenoma. In another rat from group D, also with nephropathy, the entire colon was markedly dilated and had fecal inspissation.

As mentioned before, the liver of rats with advanced nephropathy almost invariably showed various degrees of hepatomegaly due to subacute and chronic passive congestion. The congested livers had mild to moderate fatty changes midzonally and/or peripherally. Small diaphragmatic herniations of the liver were a relatively common finding, but in two rats from group A a large portion of the superior hepatic lobe was herniated into the thoracic cavity and histologically showed marked diffuse fatty changes. Hepatocellular atrophy was regularly found in animals dying with severe emaciation. Multiple hepatic abscesses were seen in a rat from group B that had abscessed pneumonia. Focal areas of necrosis were observed in another rat from group B that had acute pneumonia and in one rat from group E that had nephropathy, esophageal and urinary bladder dilatations and hindleg paralysis. A mucoid cyst of about 0.5 cm in diameter was encountered in a rat of group E and a small polycystic formation in a rat from group F. Small granulomas in the portal spaces or inside the liver lobules were relatively frequent findings. Another lesion microscopically detected was a bile duct hyperplasia generally associated with periductal fibrosis. This lesion was encountered in two rats from group B, in one from group D and in another from group F. All these animals died after the 23rd month. Finally, in a rat from group B with myxosarcoma of the neck and advanced chronic nephropathy, the vast majority of hepatocytes contained large nuclear and/or cytoplasmic hyalin bodies of undetermined origin.

Pancreatic congestion was a relatively frequent finding in rats with advanced degrees of chronic nephropathy. This was generally associated with moderate pancreatic lobular atrophy and occasionally with mild interstitial fibrosis.

Central nervous system

In addition to the already mentioned massive cerebral hemorrhage in a rat with ruptured pituitary adenoma, and the cerebral displacement and compression in another rat with an internally grown osteoma, one rat from group C and another from group E each had an abscess (3 and 6 mm across, respectively) in the left temporal lobe. Both of these rats had chronic suppurative otitis and there was an obvious connection between these two lesions. Purulent meningitis was found in a rat from group B with acute pneumonia. Two animals had marked cerebral edema: one from group B having an adenocarcinoma of unknown origin but without cerebral metastasis, and the other in a rat from group C with acute pneumonia. Two rats had hindleg and posterior paralysis: one from group E with advanced chronic nephropathy and another from group F with monocytic leukemia. Regretfully, the cauda equina was not examined in these cases for possible presence of radiculitis.

Integument and subcutis

Subcutaneous abscesses of variable dimensions (from 0.5 to 8 cm) and located in the thorax, abdomen, neck and nostrils were encountered; one case in a rat from group A, another in group C, one in each of two rats from group D, one in group E and one in each of two rats from group F. All these animals died after the 22nd month. Some of the abscesses were locally associated with alopecia and dermatitis. Extensive alopecia not associated with abscesses was a relatively uncommon finding since it was only found in a rat from group D and in two from group F. None of the rats had gynecomastia.

Ocular lesions

Unilateral nuclear cataracts were observed in a rat from group B and in another from group E. Another rat from group E had severe suppurative panophthalmitis and this severe infection was the major cause of death.

Miscellaneous findings

A purulent peritonitis found in a rat from group D dying at 17 months appeared the major cause of death. One rat of group B that died at 28 months had bilateral adrenal hemorrhage which was the most probable cause of death. As already mentioned, emaciation was frequently found in rats that had pituitary adenomas but it was also commonly observed in rats with other benign or malignant neoplasms as well as in those with acute pneumonia or other severe infections. Only a minimal proportion of rats dying with chronic nephropathy had emaciation.

DISCUSSION

Changes in body weights

The increase in body weight of all the different dietary groups during the period of rapid increment (weaning to 3 months after initiation of dietary treatment) and during

the rest of the increasing phase (15 months) greatly exceeded the post-weaning weight gains of Wistar male rats fed a stock ration reported by others [32, 33]. While the K values derived from the time–weight relation obtained by Zucker and Zucker [34] in post-weaning male albino rats was 3.44, the values in our rats fed the different regimens ranged from 8.43 to 9.00. Thus the body weights of our rats exceeded those projected from the formulations of Zucker and Zucker by 15–27% at 3 months, by 17–28% at 6 months and by 24–35% at 12 months. These "excess" percentages are much higher than the 10–15% coefficients of variation of body weights observed in rats from weaning to maturity [35, 36]. As judged by this comparative overweight as well as by the size of the epididymal, mesenteric and retroperitoneal fat depots observed in rats killed at these and subsequent periods, the animals became moderately obese. Since it is considered that the rat is in a continual state of growth during its lifetime [32] the decline in weight observed in our animals from 24 months on most probably reflected a reduction in the depot fat due to age-related diseases, although a concomitant cessation of growth cannot be ruled out.

Although the weight gain of rats fed refined diets is generally higher than those fed stock diets [37], the reason for the obesity in our rats is not clear. While obesity has been induced in similar as well as in different strains of rats by feeding *ad libitum* diets high in fat (> 60 g per 100 g of diet) and high in caloric density (> 5 kcal/g of diet) [38–41], this was not the case in our experiment since all the diets contained 15% of fat and had 4.35 kcal/g of diet.

During the "increasing phase" in body weights (from weaning to 15 months) the rate of increase was generally higher in rats fed CFD (groups E and F) than in those fed SFD (groups A and B) and to a lesser extent than in those fed UFD (groups C and D). These results appear to be in line with those reported by Hopkins *et al.* [1] in weaning rats fed *ad libitum* for 9 weeks diets with various proportions of saturated and unsaturated fatty acids. The relative retarding effect of saturated fat observed in the present studies was not apparently due to substantial differences in food-intake gross energy or in food efficiency, although these latter parameters were only determined in "healthy rats" while the average values of body weights discussed here include healthy as well as sick animals. While there is evidence that the absorption of saturated fats is generally lower than that of unsaturated fats [42, 43], it is unlikely that this possible factor had played a significant role in the observed differences, because the CFD contained more saturated fat that the UFD and still the rats fed the CFD gained more weight than those fed UFD. At any rate, it appears that at long range the ratio of dietary saturated to unsaturated fat is not a very critical determining factor in the rate of increment in body weight and that rats can utilize equally well a fairly wide range of fat mixtures [1, 2, 44].

Organ weights

Although a few statistically significant differences between groups were occasionally found in the relative weights of brain, liver and heart, these differences could not be clearly attributed to any particular influence of the diet and were most probably due to

simple individual variations. On the other hand, neither the relative weights of testes, spleen and kidneys of animals killed at different periods nor the percentages of absolute weight increment with age of kidneys, spleen, liver, brain and heart were in general significantly influenced by diet. The relations between age and the weights or growth patterns of different organs in the albino rat fed stock diets are well-established [35, 45]. For example, the growth or weight increment of kidneys, spleen, brain and heart is nearly uniform after the very early phase of rapid body growth, while the most marked growth of testes appears shortly before puberty. Although in the present experiment the determinations of organ weights began at 3 months after the initiation of dietary treatment, these age-related patterns followed a similar trend and were not significantly influenced by diet. Although it was found that the relative weights of testes in each group significantly decreased from 3 to 18 months and that a later increase occurred at 24 months, these changes simply reflected the changes in body weights already mentioned rather than variations with age.

Food-intake gross energy and food efficiency

The average amounts of calories consumed daily by our rats in all the different groups at the various periods studied were slightly higher than the amounts proposed as normal requirements for young and adult rats, but it is a common observation that the voluntary intake of calories exceeds the proposed requirements [46].

Although the food-intake gross energy of the clinically "healthy" rats at the different periods was in general not drastically or consistently influenced by the type of diet, it was found that at 3, 6 and 9 months the rats of group D fed the UFD at high level of vitamin E ate significantly less amounts of calories than those consumed by the rats of group C offered the UFD at low level of vitamin E. In addition, albeit less consistently, the caloric intake of group D was also lower than that of other groups. The biological significance or consequences of high levels of vitamin E in the particular case of rats fed UFD is difficult to evaluate. Although it has been reported that large doses of vitamin E depressed the growth of chicks [47], neither the food efficiency nor the body weight of rats in group D were in general significantly lower than in group C.

The results on food efficiency showed several significant differences between groups, suggesting *prima facie* an influence of the diet. For example, the food efficiency in group C was significantly higher than the efficiencies in groups B, D, E and F at 3 months but not at other periods. Similarly, the food efficiency of group B was higher than in all other groups only at 6 months, and those in group A higher than in the other groups only at 9 months. Since these food efficiencies were calculated from the determinations in healthy rats only during a week at each of the indicated periods and not on a continuous basis, it appears that the noted effects of diet on food efficiency were only transient variations without significant consequences or direct relations to differences in body weights attained by rats of the various groups at equivalent periods of time.

Life span parameters

These data indicated that the type of diets used in this experiment did not significantly influence the mean and maximum life spans of the rats and therefore these results

appear to be in line with those obtained by Harman [2] for CD male rats fed various diets with different degrees of fat unsaturation. However, our results show in addition that the type of diet did affect the 50% survival time, since this parameter in group D fed the UFD at high level of vitamin E was significantly longer than those in groups fed SFD (A and B), CFD (E and F) and also longer than in group C fed the UFD at low level of vitamin E. Thus, these results suggest that, at least in relation to survival times, the optimal dietary type of fat is the highly unsaturated one (safflower oil), particularly when supplemented with generous amounts of vitamin E. It also appeared that the safflower oil was relatively more important for the prolongation of the 50% survival time than the levels of vitamin E since no significant differences were found between groups A and B or E and F. On the other hand, the beneficial effect of the high level of vitamin E was quite evident in the particular case of UFD (D *vs.* C), but was ineffective, for unclear reasons, in rats fed CFD which contained smaller proportions of safflower oil.

The relationship between dietary unsaturated fatty acids and vitamin E requirements has been, of course, well established in connection with a great variety of morpho-functional events in several animal species as well as in man [48–53], but to our knowledge never before in relation to 50% survival time. The mechanisms whereby the UFD at high levels of vitamin E beneficially affected this life parameter in rats consuming UFD appeared in part related to the onset and incidence of malignant neoplasms. For example, it was found in this study that rats of group D had the lowest incidence of malignant neoplasms and that these malignancies were generally found in animals older than those found in other groups. In relation to the onset and incidence of other common non-neoplastic diseases that may have determined the 50% survival time of the rats, such as severe chronic nephropathy, emaciation, acute pneumonia, *etc.*, no clear beneficial effect of the UFD at high level of vitamin E was observed.

Serum vitamin E

Although the serum levels of vitamin E (total tocopherols) in the present experiment reflected in a way the extreme dietary levels, it should be noted that this was not a proportional correlation, since the levels in the regimens for groups B, D and E were 100 times higher than in those for groups A, C and E, while in the serum of these corresponding groups the differences were only about 2–8-fold. These findings may bear a relation to the demonstration that the intestinal absorption of vitamin E in rats decreases with increasing ingested doses [54].

The serum levels of vitamin E found in our rats fed the diets low in vitamin E (groups A, C and E) are comparable to those found in rats fed diets with almost equivalent amounts of this vitamin [16]. Although a direct relationship between serum tocopherol and serum lipid levels is well documented [55], no significant correlations were found at any of the periods studied in the present experiment between serum levels of vitamin E and those of cholesterol, triglycerides or phospholipids.

Since it is well known that the vitamin E status and requirements in various animal species and man are primarily determined by the amounts of dietary polyunsaturated fatty acids [56], it was intriguing to find in our study that, at least among rats fed diets

low in vitamin E, the SFD rather than the UFD and CFD consistently affected the serum levels of this vitamin. For example, the level of group A was significantly lower than those of groups C and E in practically all periods studied. This effect could not be attributed to differences in food intake between these groups and it seems unlikely that the coconut oil might have adversely affected the absorption of vitamin E. While we do not have a satisfactory explanation for this unexpected finding, it is recognized that the serum levels of vitamin E may not accurately reflect the vitamin status or tissue storage [57]. It should be noted also in this regard that, while the levels of vitamin E in certain tissues of rats increased with age in this study as well as in previous reports of others [57, 58], the serum levels of our rats did not show this secular trend, except in group C but only between 18 and 24 months.

Serum lipids

Serum cholesterol

The levels of serum cholesterol in the present experiment appeared almost immutable to diet and practically unchanged with age. There were, of course, some significant variations with time in some groups, but these variations from one period to another did not follow any consistent trend. Other studies on Wistar, Fischer 344, CFE and Sprague–Dawley rats showed increased serum cholesterol with advancing age [59–62], while in rats of the Lewis BN and BA strains no changes were observed [59]. It seems, therefore, that these conflicting results could be attributed to differences in dietary regimens and to the strain of rats used.

While most of the studies in man indicate that serum cholesterol is substantially affected by the type of dietary fat [63], the results obtained in diverse strains of rats comparing the effects of various types of saturated and unsaturated fats have been also conflicting. It should be noted, however, that previous studies in rats were generally of short duration. The lack of hypercholesterolemic effect of UFD in our long-term study is, therefore, in line with the results obtained by some investigators [64–66], but differ from those of others [67–72].

It has been also reported that the dietary excess of vitamin E lowers the serum total lipids and/or the serum cholesterol [63, 73–75]. Although in our rats killed at 12 months the level of serum cholesterol in group F (CFD, high vitamin E) was indeed lower than in group E (CFD, low vitamin E), this effect was only transient and was not seen in other groups fed different types of dietary fat in any of the periods studied.

Serum triglycerides

Although a hypotriglyceridemic effect of dietary safflower oil has been observed in short-term experiments (3–10 weeks) in young male and female rats [64, 65], the results of the present study suggest that this effect may be transient and probably depends also on other dietary factors. The influence of age on the serum triglyceride levels is still unclear. In Fischer rats, for example, the serum triglyceride levels remained unchanged with age in one experiment and increased in another conducted in the same laboratory [62]. To complicate this situation, our results showed a decrease in these levels from 3 to

18 months in practically all groups, followed by subsequent rebound in older rats (24 months).

Serum phospholipids

The results for serum phospholipids indicate a transient hypophospholipidemic effect of safflower oil (groups C and D) as well as a transient lowering effect (at 6 and 12 months) of excess of dietary vitamin E, but only in rats fed CFD (groups E *vs* F). Although several significant variations with time occurred in some groups, the most conspicuous were the elevations of serum phospholipids of groups D, E and F at 24 months over the levels detected at 18 months. The significance of these variations in relation to aging is not clear.

Serum albumin/globulin ratios

Previous studies in rats fed a commercial diet showed that the serum levels of albumin decreased with age [76]. This age-related decrease has been attributed to urinary loss associated with nephropathy rather than to a decrease in serum half-life or rate of production [77]. It has been shown, in fact, that the albumin synthesis in rats increases with age [78, 79]. The reasons for the unchanged levels of serum albumin with age found in our rats are unknown since the incidence and severity of nephropathy was comparable to that seen in other experiments [76] and no studies on albumin synthesis and catabolism were performed in the present experiment. It was clear, however, that despite the high prevalance of chronic nephropathy, our animals did not develop severe nephrotic syndrome which is characterized, among other manifestations, by reversal of the albumin/globulin ratio and hypercholesterolemia [80]. It is also evident that the serum albumin levels in the present study were not influenced by the type of dietary fat or by the levels of vitamin E.

While the levels of serum total globulin were also unaffected by age and diet, the levels of the different globulin fractions were not measured in this experiment. Other authors have observed that the alpha-1-globulin fraction increases with age [76], but the gamma-globulin fraction was found unchanged with age in one study [76] and increased in others [77, 80].

Pathological changes

It is now amply recognized that for the interpretation of results on aging research in experimental animals it is imperative to have comprehensive information on the spontaneous age-associated lesions in specific stocks and strains [76, 81–83]. Our study provides, therefore, this baseline information for evaluating the data presented in this and subsequent communications of this series.

Several studies on the age-associated lesions in similar and in different strains of rats fed commercial diets have been previously reported [76, 83–86]. Although some of the most recent studies have been conducted in barrier-reared inbred stocks of rats, the comparison of our results with those of other investigators still offers points of obvious interest. It appears, for example, that the incidence of total benign and malignant neo-

plasms in our Wistar outbred male rats housed in a conventional environment (pooled data from all groups and with values corrected for numbers of animals at risk) is comparable to that reported in Wistar-derived inbred rats kept under conventional or barrier-reared conditions [84] and almost similar to the incidence observed in barrier-reared Fischer 344 male inbred rats and in Sprague–Dawley-derived outbred male rats [76, 83]. Our results indicate, in addition, that the type of dietary fat and levels of vitamin E may substantially modify the incidence of malignant neoplasms. On the other hand, the prevalence of specific types of neoplasms in our rats, such as pituitary adenomas (30%, pooled data), was higher than that found in some studies [76, 83], but lower than in other reports [84, 87, 88]. The structural and ultrastructural features of the pituitary adenomas encountered in our rats are similar to those previously described by others [87–90]. The cells of these adenomas gave variable immunocytochemical reactions for prolactin [87, 90] but they probably also produced growth hormone. Therefore, these tumors are considered to be mammosomatotropic [89]. Although in the present study about 25% of the pituitary adenomas were associated with endocrine tumors (*i.e.* adrenocortical adenomas and islet cell adenoma) as well as with other benign and malignant neoplasms, gynecomastia was never detected. The incidence of adrenocortical adenoma was also higher in our study (9%, pooled data) than in other reports [76, 83].

In relation to other types of benign and malignant neoplasms, the incidences in our rats differed somewhat from those found in other strains of rats [76, 83, 84]. Furthermore, certain malignant tumors found in our rats were not observed in other studies and vice versa [76, 83, 84].

It is well known that among the non-neoplastic lesions of rats, chronic nephropathy is the most prevalent and serious disease, and that practically 100% of the animals develop diverse degrees of involvement, a fact confirmed in the present study [76, 80–84]. Several recent studies have dealt with the pathogenesis and ultrastructural features of this disease [91–93]. Our studies suggest that the different types of diets do not substantially affect the incidence of chronic nephropathy.

The incidence of acute pneumonia was relatively high in our rats (20%, pooled data) when compared with the almost total absence of this disease in barrier-reared animals [76, 83]. Conversely, chronic murine pneumonia, attributed to *Mycoplasma pneumonis* [31] and frequently seen in conventionally housed rats and also occasionally found even in barrier-reared Wistar rats [84, 94], was never observed in our animals. The incidence of focal chronic interstitial pneumonia was somewhat higher than that reported in barrier-reared rats [76], but this difference may be due perhaps to the criteria used to diagnose this lesion.

Although focal myocytic degeneration associated with focal cardiac fibrosis was frequently found in our rats, these lesions were generally very small and most probably had little functional implications. Arteriosclerotic lesions were very rarely encountered, and when present they particularly involved the mesenteric arteries.

Since in aging research it is often difficult if not impossible to delineate the innate changes of aging from those produced by arteriosclerosis (*i.e.* loss of cells, fibrosis, *etc.*),

the paucity of the former in rats [95] makes this animal the ideal model for this type of study, particularly in investigations on the effect of diet on aging, since rats generally eat practically everything that is offered, and more is known about nutrition in rats than in any other animal species. The information on differences in biochemical and pathological changes between various strains and stocks of rats (inbred or outbred), as well as between conventionally and barrier-reared rats, will undoubtedly facilitate the selection of the rat pedigree and the environmental conditions most adequate for a particular study in aging research.

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REFERENCES

1 C. Y. Hopkins, T. K. Murray and J. A. Campbell, Optimum ratio of saturated to monounsaturated fatty acids in rat diet. *Can. J. Biochem. Physiol., 33* (1955) 1047–1054.
2 D. Harman, Free radical theory of aging: effect of the amount and degree of unsaturation of dietary fat on mortality rate. *J. Gerontol., 26* (1971) 451–457.
3 D. Harman, Free radical theory of aging: dietary implication. *Am. J. Clin. Nutr., 25* (1972) 839–843.
4 R. J. Morin, Longevity, hepatic lipid peroxidation and hepatic fatty acid composition of mice fed saturated or unsaturated fat-supplemented diets. *Experientia, 23* (1967) 1002–1004.
5 D. Harman, Free radical theory of aging: effect of free radical reaction inhibitors on the mortality rate of male LAF_1 mice. *Gerontologist, 6* (1968) 13.
6 A. Comfort, J. Youhotsky-Gore and K. Pathmanathan, The effect of ethoxyquin on the longevity of C3H strain mice. *Nature, 229* (1971) 254–255.
7 R. R. Khon, Effects of antioxidants on life span of C57b1 mice. *J. Gerontol., 26* (1971) 378 380.
8 Z. Deyl, M. Jurĭcová and E. Stuclĭková, The effect of nutritional regimens upon collagen concentration and survival of rats. In V. J. Cristofalo and E. Holečková (eds.), *Cell Impairment in Aging and Development,* Plenum Press, New York, 1974, pp. 359–369.
9 K. P. Buu-Hoi and A. R. Ratsimamanga, Retarding action of nordihydroquaiaralic acid on aging in the rat. *C.R. Soc. Biol., 153* (1965) 1180–1182.
10 S. Oeriu and E. Vochitu, The effect of administration of compounds which contain sulfhydryl groups on the survival rates of mice, rats and guinea pigs. *J. Gerontol., 20* (1965) 417–419.
11 H. A. Shroeder and M. Mitchener, Selenium and tellurium in rats. Effects on growth, survival and tumors. *J. Nutr., 101* (1971) 1531–1546.
12 D. Harman, Aging: a theory based on free radical and radiation chemistry. *J. Gerontol., 11* (1956) 298–300.
13 A. Comfort, *The Biology of Senescence,* Reinhart, New York, 1956, p. 1.
14 B. L. Strehler, *Time, Cells and Aging,* Academic Press, New York, 1962, p. 11.
15 H. J. Deuel, Jr., S. M. Greenber, C. E. Calbert, E. E. Savage and T. Rukui, The effect of fat levels of the diets on general nutrition V. The relationship of the linoleic acid requirements to optimum fat level. *J. Nutr., 40* (1950) 351–360.

16 J. G. Bieri, Kinetics of tissue α-tocopherol depletion and repletion. *Ann. N. Y. Acad. Sci., 203* (1972) 181–191.
17 R. B. Alfin-Slater, L. Aftergood and S. Kishinoff, Investigation on hypervitaminosis E in rats. *IX International Congress of Nutrition,* Karger, Basel, 1972, p. 191.
18 R. Hill, J. M. Linazasoro, F. Chevalier and I. L. Chaikoff, Regulation of hepatic lipogenesis: The influence of dietary fats. *J. Biol. Chem., 233* (1958) 305–310.
19 C. E. French, R. H. Ingram, J. A. Uram, G. P. Barron and R. W. Swift, The influence of dietary fat and carbohydrate on growth and longevity in rats. *J. Nutr., 51* (1953) 329–339.
20 R. O. Recknagel and A. K. Ghoshal, Quantitative estimation of peroxidative degeneration of rat liver microsomal and mitochondrial lipids after carbon tetrachloride. *Exp. Mol. Pathol., 5* (1966) 413–426.
21 K. Yagi, A simple fluorometric assay for lipoperoxides in blood plasma. *Biochem. Med., 15* (1976) 212–216.
22 L. F. Rodkey, Separation and determination of the total globulins of human serum. *Clin. Chem., 11* (1965) 488–494.
23 E. Van Handel and D. B. Zilversmit, Micromethod for the direct determination of serum triglycerides. *J. Lab. Clin. Med., 50* (1957) 152–157.
24 R. O. Hurst, The determination of nucleotide phosphorus with stannous chloride–hydrazine sulphate reagent. *Can. J. Biochem., 42* (1964) 287–292.
25 B. Zack, R. C. Dickerman, E. G. White, H. Burnett and P. J. Cherney, Rapid determination of free and total cholesterol. *Am. J. Clin. Pathol., 24* (1954) 1307–1315.
26 M. L. Quaife, N. S. Scrimshaw and O. H. Lowry, A micromethod for assay of total tocopherols in blood and serum. *J. Biol. Chem., 180* (1949) 1229–1235.
27 P. Armitage, *Statistical Methods in Medical Research,* Wiley, New York, 1971, pp. 408–415.
28 W. J. Dixon and F. J. Massey, Jr., *Introduction to Statistical Analysis,* McGraw-Hill, New York, 1957, pp. 145–152.
29 D. J. Finney, *Probit Analysis. A Statistical Treatment of the Sigmoid Response Curve,* University Press, Cambridge, 1952, pp. 20–35.
30 A. Goldstein, *Biostatistics. An Introductory Text,* Macmillan, New York, 1964, p. 156.
31 G. C. Jersey, C. K. Whitehair and G. R. Carter, *Mycoplasma pulmonis* as the primary cause of chronic respiratory disease in rats. *J. Am. Vet. Med. Assoc., 163* (1973) 599–604.
32 M. S. Dunn, E. A. Murphy and L. B. Rockland, Optimal growth of the rat. *Physiol. Rev., 27* (1947) 72–94.
33 W. S. Spector, *Handbook of Biological Data,* W. D. Saunders, Philadelphia, 1956, p. 160.
34 L. M. Zucker and T. F. Zucker, A simple time weight relation observed in well-nourished rats. *J. Gen. Physiol., 25* (1942) 445–463.
35 C. B. Freudenberger, Variability in body weight, body length and organ weights of the rat. *Anat. Rec., 56* (1933) 47–56.
36 T. F. Zucker, L. Hall, M. Young and L. Zucker, The growth curve of the albino rat in relation to diet. *J. Nutr., 22* (1941) 123–138.
37 T. F. Zucker and L. M. Zucker, Fat accretion and growth in the rat. *J. Nutr., 80* (1963) 6–19.
38 O. Michelsen, S. Takanashi and C. Craig, Experimental obesity. I. Production of obesity in rats by feeding high-fat diets. *J. Nutr., 57* (1955) 541–554.
39 J. J. Barboriak, W. A. Krehl, G. R. Cowgill and A. D. Whedon, Influence of high-fat diets on growth and development of obesity in the albino rat. *J. Nutr., 64* (1958) 241–249.
40 S. C. Peckman, C. Entenman and H. N. Carroll, The influence of a hypercaloric diet on gross body and adipose tissue composition in the rat. *J. Nutr., 77* (1962) 187–197.
41 D. Lemonnier, Obésité par des régimes hyperlipidiques chez le rat et la souris. *Nutr. Dieta, 9* (1967) 27–42.
42 A. D. Barbour, The effect of the saturated fatty acid content of the diet on the composition of the body fat. *J. Biol. Chem., 106* (1934) 281–288.
43 V. Augur, H. S. Rollman and H. J. Deuel, Jr., The effect of crude lecithin on the coefficient of digestability and the rate of absorption of fat. *J. Nutr., 33* (1947) 177–186.
44 H. J. Thomasson, The biological value of oils and fats. I. Growth and food intake on feeding with natural oils and fats. *J. Nutr., 56* (1955) 455–468.

45 H. H. Donaldson, *The Rat,* 2nd edn., The Wistar Institute of Anatomy and Biology, Philadelphia, 1924, pp. 206–223.
46 R. G. Warner and L. H. Breuer, Jr., Nutrient requirement of the laboratory rat. In *Nutrient Requirements of Laboratory Animals,* 2nd edn., National Academy of Sciences, Washington, D.C., 1972, pp. 56–93.
47 K. E. Masson and M. K. Horwitt, Tocopherols. X. Effects of deficiency in animals. In W. H. Sebrell and R. S. Harris (eds.), *The Vitamins,* Academic Press, New York, 1972, p. 272.
48 H. Dam, Effect of cod liver oil and rancidity on certain vitamin E deficient symptoms. *Proc. Soc. Exp. Biol. Med., 52* (1943) 285–287.
49 L. J. Filer, R. E. Rumery and K. E. Masson, Specific unsaturated fatty acids in the production of acid fast pigment in the vitamin E deficient rat and the protective action of tocopherols. In C. G. Mackenzie (ed.), *Biological Antioxidants, Transactions of the First Conference,* J. Macy, Jr. Foundation, New York, 1946, pp. 67–77.
50 E. L. Hove and P. L. Harris, Relative activity of the tocopherols in curing muscular dystrophy in rabbits. *J. Nutr., 33* (1947) 95–106.
51 K. L. Blaxter, F. Brown and A. M. MacDonald, The nutrition of the young Ayrshire calf. 13. The toxicity of the unsaturated acids of cod liver oil. *Br. J. Nutr., 7* (1953) 287–298.
52 M. K. Horwitt, C. C. Harvey, G. D. Duncan and W. C. Wilson, Effects of limited tocopherol intake in man with relationships to erythrocyte hemolysis and lipid oxidations. *Am. J. Clin. Nutr., 4* (1956) 408–419.
53 P. L. Harris and N. D. Embree, Quantitative consideration of the effect of polyunsaturated fatty acid content of the diet upon the requirements for vitamin E. *Am. J. Clin. Nutr., 13* (1963) 385–392.
54 M. S. Losowsky, J. Kelleher, B. E. Walker, T. Davis and C. L. Smith, Intake and absorption of tocopherol. *Ann. N. Y. Acad. Sci., 203* (1972) 212–222.
55 M. K. Horwitt, C. C. Harvey, C. H. Dahm and M. T. Searcy, Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Ann. N. Y. Acad. Sci., 203* (1972) 223–236.
56 M. K. Horwitt, Vitamin E. In R. S. Goodhart and M. E. Shils (eds.), *Modern Nutrition in Health and Disease,* 5th edn., Lea and Febiger, Philadelphia, 1973, pp. 175–185.
57 J. G. Bieri, In *Present Knowledge in Nutrition,* 4th edn., The Nutrition Foundation, Inc., New York, 1976, pp. 98–110.
58 W. B. Weglicki, Z. Luna and P. P. Nair, Sex and tissue specific differences in concentrations of α-tocopherol in mature and senescent rats. *Nature, 221* (1969) 185–186.
59 D. Kritchevsky and S. A. Tepper, Serum cholesterol levels in inbred rats. *Am. J. Physiol., 207* (1964) 631–633.
60 L. A. Carlson, S. O. Froberg and E. R. Nye, Effect of age on blood and tissue levels in the male rat. *Gerontologia, 14* (1968) 65–79.
61 J. Dupont, M. M. Mathias and N. B. Cabacungan, Dietary lipid, fatty acid synthesis and cholesterol metabolism in aging rats. *Lipids, 7* (1972) 576–589.
62 J. A. Storey, S. A. Tepper and D. Kritchevsky, Age related changes in the lipid metabolism of Fischer 344 rats. *Lipids, 11* (1976) 623–627.
63 A. Keys, F. Grande and J. T. Anderson, Bias and misrepresentation revisited: "Perspective" on saturated fat. *Am. J. Clin. Nutr., 27* (1974) 188–212.
64 B. C. O'Brien, C. L. Skutches, G. R. Henderson and R. Reiser, Interrelated effects of food lipids on steroid metabolism in rats. *J. Nutr., 107* (1977) 1444–1454.
65 R. A. Watterman, D. R. Romsos, A. C. Tsai, E. R. Miller and G. A. Leveille, Influence of dietary safflower oil and tallow on growth, plasma lipids and lipogenesis in rats, pigs and chicks. *Proc. Soc. Exp. Biol. Med., 150* (1975) 347–351.
66 L. H. Chen, S. Liar and L. V. Packett, Interaction of dietary vitamin E and protein level or lipid source with serum cholesterol level in rats. *J. Nutr., 102* (1972) 729–732.
67 R. Okey and M. M. Lyman, Dietary fat and cholesterol metabolism. I. Comparative effects of coconut and cottonseed oils at three levels of intake. *J. Nutr., 61* (1957) 523–533.
68 J. Avignan and D. Steinberg, Effects of saturated and unsaturated fat on cholesterol metabolism in the rat. *Proc. Soc. Exp. Biol. Med., 97* (1958) 814–816.

69 S. S. Pawar and H. C. Tidwell, Cholesterol and lipid levels in blood and livers of rats fed unsaturated fat. *Am. J. Physiol., 213* (1967) 1350–1352.
70 R. Okey, M. M. Lyman, A. G. Harris, B. Einset and W. Hain, Dietary fat and cholesterol metabolism; effects of unsaturation of dietary fats on liver and serum lipids. *Metabolism, 8* (1959) 241–255.
71 D. K. Bloomfield, Cholesterol metabolism. III. Enhancement of cholesterol absorption and accumulation in safflower oil-fed rats. *J. Lab. Clin. Med., 64* (1963) 613–623.
72 H. C. Tidwell, J. L. Pope and P. Gifford, Metabolism of polyunsaturated fatty acids and serum cholesterol levels in rats. *J. Nutr., 88* (1966) 111–114.
73 I. Herril, G. Minarek and E. D. Gifford, Effects of vitamins A and E on lipids of selected rat tissues. *J. Nutr., 87* (1965) 424–428.
74 K. N. Prodouz and R. M. Navari, Effects of vitamin A and E on rat tissue lipids. *Nutr. Rep. Int., 11* (1975) 17–28.
75 N. Y. Yang and J. D. Desai, Effect of high levels of dietary vitamin E on liver and plasma lipids and fat soluble vitamins in rats. *J. Nutr., 107* (1977) 1418–1426.
76 G. L. Coleman, S. W. Barthold, G. W. Osbaldiston, S. J. Foster and A. M. Jonas, Pathological changes during aging in barrier-reared Fischer 344 male rats. *J. Gerontol., 32* (1977) 258–278.
77 K. Salatka, D. Kresge, L. Harris, D. Eldestein and E. Ove, Rat serum protein changes with age. *Exp. Gerontol., 6* (1971) 25–35.
78 R. E. Beauchene, L. M. Roeder and C. H. Barrows, The interrelationship of age, tissue protein synthesis and proteinuria. *J. Gerontol., 25* (1970) 359–363.
79 C. F. van Bezooijen, T. Grell and D. L. Knook, Albumin synthesis by liver parenchymal cells isolated from young, adult and old rats. *Biochem. Biophys. Res. Commun., 71* (1976) 513–519.
80 B. N. Berg, Spontaneous nephrosis, with proteinuria, hyperglobulinemia and hypercholesterolemia in the rat. *Proc. Soc. Exp. Biol. Med., 119* (1965) 417–420.
81 C. E. Finch, Guest editorial. *J. Gerontol., 32* (1977) 257.
82 S. Levine, O. Bayat and R. Rehman, Letter to the Editor. *J. Gerontol., 32* (1977) 358.
83 B. J. Cohen, M. R. Anver, D. H. Ringler and R. C. Adelman, Age-associated pathological changes in male rats. *Fed. Proc., 38* (1978) 2848–2850.
84 G. E. Paget and P. G. Lemon, The interpretation of pathological data. In W. E. Ribelin and J. R. McCoy (eds.), *The Pathology of Laboratory Animals,* C. C. Thomas, Springfield, IL, 1965, pp. 382–405.
85 B. N. Berg, Longevity studies in rats. II. Pathology of aging rats. In E. Cotchin and F. J. C. Roe (eds.), *Pathology of Laboratory Rats and Mice,* Blackwell Scientific Publications, Oxford, 1967, pp. 749–786.
86 K. C. Snell, Spontaneous lesions of the rat. In W. E. Ribelin and J. R. McCoy (eds.), *The Pathology of Laboratory Animals,* C. C. Thomas, Springfield, IL, 1965, pp. 241–302.
87 A. Ito, P. Moy, H. Kautnitz, K. Kortwright, S. Clarke, J. Furth and J. Meites, Incidence and character of the spontaneous pituitary tumors in the strain CR and W/F male rats. *J. Natl. Cancer Inst., 49* (1972) 701–711.
88 J. A. Saxton and J. B. Graham, Chromophobe adenoma-like lesions of the rat hypophysis. *Cancer Res., 4* (1944) 168–175.
89 U. Kim, K. H. Clifton and J. Furth, A highly inbred line of Wistar rats yielding spontaneous mammo-somatotropic pituitary and other tumors. *J. Natl. Cancer Inst., 24* (1960) 1031–1053.
90 K. Kovacs, E. Horvath, R. G. Ilse, C. Ezrin and D. Ilse, Spontaneous pituitary adenomas in aging rats. A light microscopic, immunocytological and fine structural study. *Beitr. Pathol., 161* (1977) 1–16.
91 J. E. Gray, R. N. Wearer and A. Purmalis, Ultrastructural observations of chronic progressive nephrosis in the Sprague–Dawley rat. *Vet. Pathol., 11* (1974) 153–164.
92 W. G. Couser and M. M. Stilmant, Mesangial lesions and focal glomerular sclerosis in the aging rat. *Lab. Invest., 33* (1975) 491–501.
93 J. D. Elema and A. Arends, Focal and segmantal glomerular hyalinosis and sclerosis in the rat. *Lab. Invest., 33* (1975) 554–561.
94 J. R. M. Innes, F. M. Garner and J. L. Stookey, Respiratory disease in rats. In E. Cotchin and F. J. C. Roe (eds.), *Pathology of Laboratory Rats and Mice,* Blackwell Scientific Publications, Oxford, 1967, pp. 229–257.
95 B. C. Bullock, K. L. Banks and P. J. Manning, Common lesions in the aged rat. In *The Laboratory Animal in Gerontological Research,* National Academy of Sciences, Washington, D.C., pp. 62–82.