Long-Term Responses of Rats to Heat-Treated Dietary Fats: IV. Weight Gains, Food and Energy Efficiencies, Longevity and Histopathology

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ABSTRACT

Representative cottonseed salad oils, corn oils, lards and hydrogenated vegetable shortenings, and portions of the same fats heated at 182 C for 120 hr were fed as 20% of nutritionally adequate diets to weanling albino rats in longevity studies. Differences in the responses of rats fed diets containing the unheated and heated fats were generally small with respect to rates of gain, 12th week and adult weights, efficiencies of utilization of absorbed energy, incidences of grossly detectable diseases and longevities. There were no indications that the feeding of the heated fats had shortened survival times in comparison with the comparable unheated fat. Animals fed hydrogenated vegetable shortening, heated or unheated, survived the longest. However, gains were slightly slower with the heated cottonseed oil diets, and food efficiencies were slightly lower with the heated cottonseed oil and heated lard diets because of decreased digestibilities of these fats. The usual disabilities of old age such as nephritis, respiratory disease and periarteritis were present in all groups. The incidence of mammary tumors was high but did not differ significantly with the kind of fat, heated or unheated. Tumor incidence other than mammary was similar in both sexes and there was no significant difference between fresh and heated fats. Absence of adverse effects attributable to the heated fats during the life span of the rats is further evidence of the safety of these fats of the quality customarily consumed by the human population.

INTRODUCTION

Edible fats undergo chemical and physical changes when exposed to heat/or oxygen or both and, if the treatment is severe enough, some of the products may be harmful to experimental animals when fed under appropriate conditions. The nature and extent of the changes have been investigated extensively during the last few years, yet because of the complexity of the mixtures, there is still much to be learned regarding the composition and the biological effects of heated or oxidized fats. Three general types of chemical changes occur: oxidation, cleavage to smaller compounds and polymerization.

There is considerable evidence that the treatments to which edible fats may be subjected during practical home or institutional use do not cause the formation of deleterious substances in amounts sufficient to impair health (1-15). On the other hand, several teams of scientists have observed adverse biological effects when fats which had been severaly heated (2,11-13,16-25) or extensively oxidized (12,23-42) were fed or injected. Toxic substances can be concentrated in certain fractions of the treated fats by distillation or urea adduction procedures (12,16-21,28,30-37). Fats containing relatively large proportions of unsaturated fatty acids, particularly polyenoic acids, yield more of these toxic substances than the saturated fats (20,21,30). Factors in addition to unsaturation appear to be involved since in our experience different lots of a single type of oil yield reproduceably differing amounts of urea non-adducting fraction (NAF). In general, the biological effects of heated or oxidized fats are in proportion to the NAF content (25,27,31), but the effects differ depending on the nature of the fraction. Concentrates that are predominately monomeric tend to be absorbed more readily and are more toxic than dimers or longer chain polymers (12,20,31).

The small amounts of lipid-derived products developed in fats during cooking ordinarily cannot be detected biologically without extraction and concentration of the NAF. Furthermore, substantial amounts of severely heated fat can be included in the diet without adversely affecting growth or well-being of rats, particularly if associated with some unchanged fat (5,16,35,36).

Nevertheless, the presence in foods of even small amounts of possible deleterious substances, such as NAF, is cause for concern until there is sufficient evidence that these amounts are harmless. As mentioned above, existing data support the belief that fats used in foods or in cooking of foods are safe in this respect, even

though other evidence makes it quite clear that abuse of fats by excessive exposure to heat or oxygen will result in formation of toxic products. For the most part previous biological tests have been of short duration and may not have detected substances that would elicit a response on prolonged administration or in massive doses. It was to study the cumulative and chronic effects of heated fats, if such effects exist, that the present experimental work was designed and conducted. The findings, as reported, confirm the conclusions of the short-term tests, i.e., heat treatments more severe than those used in cooking foods yet in a practical range do not result in formation of sufficient quantities of fat-derived products to cause serious adverse biological effects. They are in substantial agreement with the observations of Nolen et al. (15) who conducted longevity studies with rats fed fats heated in the presence of foods.

EXPERIMENTAL PROCEDURES

In order to obtain the information desired, uniform groups of rats were fed diets which contained either a typical commercial fat or portions of this fat which had been subjected to a relatively severe heat treatment. Responses of the animals were compared with respect to: (a) rate of growth, (b) ability to digest the fats, (c) efficiency of food and energy utilization, (d) incidence of various abnormalities during life, (e) longevity, (f) gross changes observed at autopsy, and (g) organ weights. Tissues were taken at autopsy and preserved for subsequent histopathological study in the Human Nutrition Research Division, ARS, USDA.

Four widely used dietary fats were selected: cottonseed salad oil (CS), corn oil (CO), lard (L) and hydrogenated vegetable oil shortening (S). Three prominent brands of each were purchased during the course of the study in the necessary quantities in regular packages (38-50 lb) from commercial suppliers. Equal aliquots of the three brands of a specific fat were blended together for use in the diets and for heating prior to use. Both heated and unheated samples were stored in filled, sealed glass jars at 3 C until used.

Heat Treatment

Fats were heated at 182 C continuously for 120 hr in 46 lb quantities in a commercial, electrically heated deep fat fryer equipped with a chromed steel container and a stainless steel clad heating element. When used in this quantity there were 6.5 sq in. of surface exposed to air for each pound of fat heated, a ratio typical for this type of equipment. During heating the fats were stirred gently to avoid local differences in temperature. The stirring was not vigorous enough to cause entrapment of air bubbles, but it did provide a continuous change of the surface exposed to air and to the heating element. Fats treated in this manner are designated as HCS, HCO, HL and HS to differentiate them from the unheated CS, CO, L and S.

This relatively long and severe heat treatment was considered to be a compromise between short-term, single use of fats which would result in only minimal changes that might not be detectable biologically and more drastic treatments that have no practical counterpart. It was known from previous work (12,25,31) that such a treatment would be severe enough to decrease the digestibility of CS, the fat which had been most extensively studied. On the other hand, rats fed diets containing fats heated under similar conditions were known to grow well and to be apparently healthy for periods up to 12 weeks (11). It was also known that CS treated in this manner would be beyond practical commercial usage, as it would foam violently if attempts were made to introduce foods such as potatoes (12,14,42,43).

Certain analytical characteristics of the fats are shown in Figure 1. The general increases in viscosity and in the content of the urea non-adducting fraction, and the decreases in linoleic acid content and iodine values are greater than is typical for such fats used in practical food frying operations. Peroxide values were low and changed little by the heating procedure.

Preparation and Composition of Diets

Nutritionally complete, synthetic-type diets including 20% of the various test fats were prepared by adding appropriate quantities of the fats and 1% of wheat germ oil to aliquots of a uniform supply of a basal diet mix having the following composition: purified casein, 20.1; purified lactalbumin, 10.0; Jones and Foster salt mixture, 4.0; cellulose (Cellu Flour), 2.0; sucrose, 35.2; primary grown yeast, 5.0; d-α-tocopherol dry mix (Myvamix, 44 I.U./g), 1.0; choline chloride dry mix (25%), 0.4; vitamin A dry mix (Stabmix A, 10,000 I.U./g), 0.2; vitamin D_2 dry mix (Daves Sterol D-2, 1500 I.U./g) 0.2; and vitamin premix, 1.0. The vitamin premix contained, in mg/g, riboflavin, 0.45; niacin, 1.5; d calcium pantothenate, 3.5; pryidoxine hydrochloride, 0.65; biotin, 0.033; 2 methyl naphthoquinone, 1.0; *p*-aminobenzoic acid, 10.0; vitamin B_{12}, 0.001; and sucrose sufficient to bring the total to 1.000 g. The first five com-

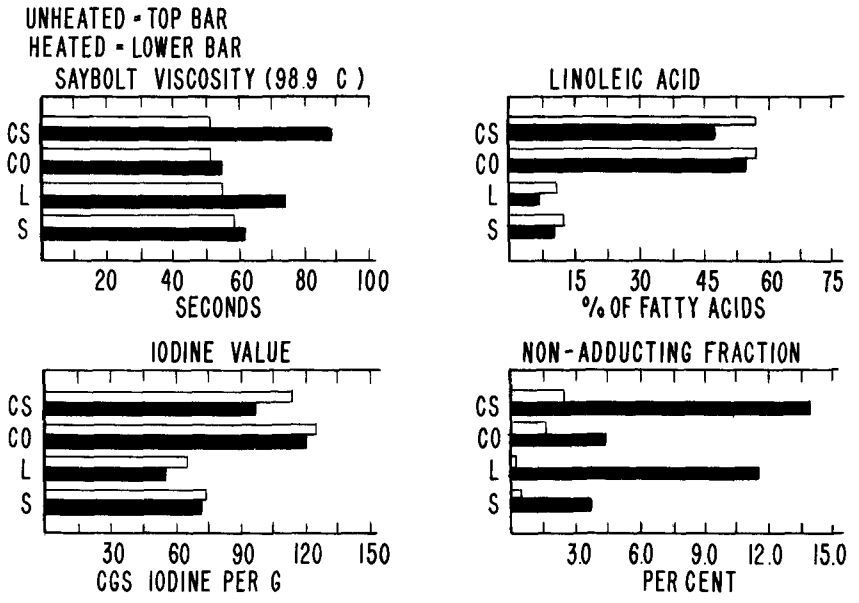

FIG. 1. Analytical values of fats before and after heating.

ponents of the basal diet were premixed in advance (premix 1 stored at room temperature), and the remaining six were likewise premixed (premix 2 stored in brown bottles at 3 C).

The diets were made by first mixing the proper aliquots of premix 1 and premix 2, then adding, during mixing, the appropriate melted fat and, finally, the wheat germ oil. Analyses of the diets gave average values as follows (in per cent): moisture, 3.3; protein, 27.2; fat, 21.9 and ash, 3.6. Fresh batches of each diet were prepared at intervals of about two weeks. Weighed quantities of fresh food in 3 or 6 oz widemouth glass jars were supplied three times weekly to the animals during the growth period and at least weekly thereafter. Food and water were supplied ad libitum. Uneaten food was weighed to determine food usage.

Care of Animals

Fifteen replications, each comprising one male and one female weanling rat (Holtzman albinos) for each unheated fat diet group and two of each sex for each heated fat diet group, were started weekly on the test diets for 15 weeks. Rats were caged individually in drawer-type cages with wire mesh fronts and bottoms. The rats were randomly distributed in cages suspended on racks in an air conditioned room with temperature controlled at 25-26 C and humidity at 45-50%.

Animals were weighed at about the same time each Wednesday. At that time uneaten food was removed for weighing, and each animal was inspected carefully for disease or physical abnormalities. A few rats with rales or suspected middle ear infections were isolated, but no treatment of any kind was administered to the animals except for trimming a few elongated incisors when necessary. Animals which showed severe or consistent losses of weight or other conditions that indicated ill health were observed more frequently. Those which became obviously moribund were decapitated and autopsied immediately to assure tissues suitable for histopathological evaluation. Animals which were found dead were autopsied as soon after death as possible.

During the 2nd, 12th and 52nd week of feeding, complete collections of feces were made for 10 randomly selected rats in each of the dietary groups. Lipid was extracted with ethyl ether from the pooled, acid hydrolyzed sample for each group for each period and weighed following solvent removal. Fat intakes for the same rats were calculated from the food intake and food composition records. The difference between the total dietary fat intake and the total fecal fat output represents gross fat absorption (uncorrected for endogenous fat). The weights of fat absorbed divided by weights of fat consumed gives practical digestibility values.

When the animals were 22 weeks of age, two males and two females which had been pre-

TABLE I

Average Weights of Rats Fed Diets Containing Unheated or Heated Fats for 12, 52 or 78 Weeks

	Average weights of rats in grams					
	Males			Females		
Fat in diet	12 wk	52 wk	78 wk	12 wk	52 wk	78 wk
CS[a]	451	655	681	268	385	458
HCS[b]	427[c]	634	674	253[c]	380	455
(CS/HCS) x 100	106	104	101	106	101	101
CO[a]	432	666	697	260	391	469
HCO[b]	431	653	723	265	394	464
(CO/HCO) x 100	100	102	96	98	99	101
L[a]	436	613	632	257	367	428
HL[b]	430	638	722	256	365	440
(L/HL) x 100	102	96	88	100	101	97
S[a]	440	643	680	262	358	442
HS[b]	428	615	648	254	361	425
(S/HS) x 100	103	105	105	103	99	104
All unheated	440	644	672	262	375	449
All heated	429	635	692	257	375	446
(Unheated/heated) x 100	103	101	97	102	100	101

[a]Fifteen animals started in each group.

[b]Thirty animals started in each group.

[c]Significantly less ($P \leq 0.05$) than for rats fed the unheated oil. Data for the 78 week periods were not statistically analyzed because of the small and irregular group sizes.

selected randomly for each group were killed for gross and histopathological examination. This procedure was repeated with other pairs of animals at ages of 36 and 50 weeks.

Except for the six randomly selected animals in each group killed for autopsy and tissue samples, as just described, all animals were maintained on their respective diets until they died or became moribund, or until a preselected termination point based on reduction of the colony to about 10% of the starting number was reached. At this time (928 days of age), all survivors were killed for termination gross and histopathological observations. At the time of autopsy, observations regarding the gross appearance of the animal and its organs and tissues were recorded; designated organs (liver, kidneys, adrenals) were removed, weighed and preserved in 10% neutral formalin for possible future histopathological study. All autopsies for this experiment were coded, i.e., done without knowing the diet group to which the animal belonged.

RESULTS AND DISCUSSION

Two general types of observations were made with respect to the influence of heating on the wholesomeness of fats, those relating to the effectiveness and efficiencies of the several fats tested as measured by growth and food utilization, and those relating to the health and longevity of the animals. Neither of these criteria gave any indication that fats heated for 120 hr at 182 C adversely affected the general health or well-being of rats to which they had been fed for their full life span. There were some minor differences between groups when heated-unheated comparisons were made, but these tended to balance out, some being in favor of the heated, others in favor of the unheated.

Growth

Animals fed heated fats grew well and at about the same rates as their counterparts fed unheated fats and maintained their weights over the long-term, as shown by the data in Table I. The only effect noted was a slightly slower rate of gain for each sex in the HCS diet group ($p \leq 0.05$ at 12 weeks, not significant thereafter). The slightly slower rate of growth for HCS fed rats is consistent with previous observations (21,25,31). There was generally more variation

TABLE II

Food and Calorie Efficiencies of Rats Fed Unheated or Heated Fats for 12 Weeks and Digestibility of Fats Fed

1	2	3	4	5	6	7	8	9
Sex and diet type	Average 12 wk gain, g	Average total 12 week food disappearance, g[a]	Grams food consumed per gram gain	Calories absorbed per gram gain[b]	Digestibilities[c] of fats in the diet, %			Digestible energy per gram diet, calories[d]
					2nd wk	12th wk	52nd wk	
Males								
CS	398	1219	3.06	14.4	91.6	92.3	93.7	4.70
HCS	376	1231	3.28	14.3	80.0	76.3	65.9	4.37
CO	381	1175	3.08	14.6	93.6	92.8	93.3	4.71
HCO	380	1192	3.14	14.6	91.1	90.2	89.8	4.66
L	385	1186	3.08	14.6	91.0	92.0	93.7	4.73
HL	378	1265	3.34	15.0	76.1	79.9	77.4	4.48
S	385	1244	3.23	14.6	86.0	83.7	89.3	4.52
HS	376	1216	3.23	14.6	86.7	84.1	76.8	4.53
All unheated	387	1206	3.11	14.5	---	---	---	4.66
All heated	378	1226	3.25	14.6	---	---	---	4.51
Females								
CS	217	874	4.02	19.1	93.7	94.2	95.0	4.74
HCS	203	922	4.55	20.0	79.7	78.1	72.6	4.41
CO	210	869	4.14	19.6	95.4	94.8	94.2	4.75
HCO	214	900	4.21	19.7	92.2	91.9	91.7	4.69
L	206	864	4.19	20.0	92.6	94.4	94.6	4.78
HL	206	915	4.44	20.1	75.9	82.3	79.1	4.53
S	211	906	4.30	20.0	88.6	91.0	79.0	4.67
HS	204	883	4.33	20.0	86.4	88.6	82.2	4.62
All unheated	211	878	4.16	19.7	---	---	---	4.73
All heated	207	905	4.38	19.9	---	---	---	4.56

[a]Food disappearance corresponds closely with food consumption, as only a few crumbs were scattered and not weighed.

[b]Calculations based on digestible energies shown in the last column in this table.

[c]See text.

[d]Calories were calculated using average diet composition and factors of 3.95 for sucrose, 4.27 for protein, 9.30 for vegetable oil and 9.50 for lard, according to USDA Handbook 8, (44). The 9.30 and 9.50 combustible energy values were used for fat, because digestibility was based on data for 12 week fat digestibilities listed in Column 7.

between the types of unheated fats fed than there was between the heated vs. unheated variants of each type. The long-term weight differences between the four types of unheated fats fed were never more than 9% and were not significant statistically at any age.

Food Efficiency and Digestibility

At the end of 12 weeks on diet the rats fed HCS or HL had consumed more food per gram gain than animals on diets containing unheated fats of the same types, but those fed HCO or HS had not consumed more than their counterparts (Table II, Column 4). The increased food consumptions were statistically significant ($P \leq 0.01$) for the males and females fed HL and for the females fed HCS. However, when the comparisons were made on a per calorie absorbed basis rather than on a per gram of food consumed basis, the differences between the heated and unheated fats disappear (Table II, Column 5). It seems probable that the decreased food efficiencies of animals fed diets containing HCS or HL are due to nondigestible substances in the fats, presumably polymers originating during the heating of the fats (19,20,31). The presence of nondigestible lipids is demonstrated by the digestibility values for each of the three time periods (Table II, Columns 6, 7 and 8). Substantial decreases in digestibility for HCS vs. CS and HL vs. L were observed for all periods. The digestibility values are similar for the 2nd, 12th and 52nd week for each unheated fat, however, there is some indication that the heated fats were progressively less well digested as the animals aged. The statistical significance of the differences observed could not be determined because it was necessary to pool feces for rats in each group to provide sufficient sample for analysis.

The equal utilizations of energy other than that of the nondigestible portions of the fats is evidence that digestibilities of the other components of the diets were not affected. These observations differ from those of Friedman et al. (31) who, using cottonseed oil stirred for 190 hr at 225 C, found that, "This difference in nutritive value (i.e., energy) cannot be accounted for by the decreased digestibility of the heated cottonseed oil." However, their fat, having been much more severely treated, was not comparable to those used in our tests.

Incidence of Diseases or Abnormalities

In general the animals remained healthy and lived long, relatively disease-free lives but, as expected with a group of aging animals, a variety of physical abnormalities was noted. These observations were recorded in detail and tabulated according to type and treatment of the fats and sex of the animal. The abnormalities were typical of those occurring in the rat and there were no substantial differences in frequencies between types of fat or treatments.

TABLE III

Survival of Rats Fed Diets Containing Unheated or Heated Fats

Dietary fats	Average survival in rat days		
	M	F	M & F
CS	635	738	687
HCS	650	706	678
Both	646	715	680
CO	656	683	669
HCO	657	706	681
Both	656	700	678
L	551	808	679
HL	662	696	679
Both	632	727	679
S	667	767	717
HS	691	765	728
Both	684	766	725
All unheated	627	749	688
All heated	665	718	692
Entire experimental colony	646	734	691

Longevity

The overall survival of the rats in this experiment was good, an average of 646 days for the males and 734 days for the females, and there were no indications that animals fed heated fats had shortened lifespans. In fact, in five of the eight heated-unheated comparisons, the average survival was greatest in the groups fed heated fats although the differences were not statistically significant (Table III). When data for both sexes and all fats are combined the average survival periods for the heated and unheated fat-fed animals are almost identical, i.e., 688 vs. 692 days.

There is a substantial indication that the subgroups fed diets containing S or HS survived longer than the subgroups fed the other fats, although the differences are not statistically significant at the $P \leq 0.05$ level. The lard-fed rats were the least consistent in their responses to the treatments, the males fed heated lard surviving longer than those fed unheated lard, whereas the females reversed this order. Furthermore, the lard-fed males had the shortest average survival period of all the male

TABLE IV

Incidence of Tumors in Rats Fed Throughout Life Diets Containing Different Fats

Type fat	Number of rats fed[a]	Percent of animals showing tumors				
		Females			Males	
			Nonmammary		Nonmammary	
		Mammary	Benign	Malign.	Benign	Malign.
CS	9	44	22	11	33	0
HCS	24	50	4	4	12	8
CO	9	22	0	33	0	22
HCO	24	50	4	13	4	21
L	9	56	11	11	0	11
HL	24	46	0	13	8	13
S	9	45	11	0	0	0
HS	24	46	0	17	4	4
Totals						
Unheated	36	42	11	14	8	8
Heated	96	48	2	12	7	12

[a]For each sex.

subgroups. At least part of the shorter survival of the unheated lard-fed males may be attributed to unexplained early deaths of three of the nine-member life-survivor subgroup. The expected longer survivals of all female subgroups in comparison with the corresponding male subgroups is highly significant ($P \leq 0.01$).

Gross Observations at Autopsy: Organ Weights

The detailed gross observations made at autopsy were distributed rather uniformly among the groups with no unusual observations or conditions attributable to heating of the fats.

The consumption of diets including heated fats did not result in significantly different kidney or adrenal weights when compared with organs from animals fed unheated fats. For those rats routinely killed for tissue samples during the first year of the experiment, the liver-body weight ratios were higher for animals fed heated fat diets than for those fed the corresponding unheated fat diets for all comparisons except that for the S-HS male subgroups. The increases were significant ($P \leq 0.05$) for the male and female subgroups fed the HCS diet, and for the female subgroup fed the HL diet. No significant differences attributable to the heating of the fat components of the diets were found for livers removed from rats found dead or dying, but the liver weights and the liver-body weight ratios of male rats fed the HCS diet were significantly heavier ($P \leq 0.05$) than those for male rats fed diets containing HCO, HL or HS.

Histopathology

Pathological changes were those commonly found in old animals and included respiratory disease, chronic nephritis and periarteritis, the latter occurring mostly in males without relation to diet. In the animals with tumors, the incidence of nephritis was low in those fed hydrogenated vegetable oil (less than 8% compared to 21% on the other diets and present whether fats were heated or unheated).

Table IV gives the incidence of tumors in both sexes and characterizes them as benign or malignant. Of 132 male rats, 23 had tumors, of 132 female rats, 77 were tumor bearers, 61 had mammary tumors and 5 of these had additional tumors located elsewhere.

Mammary tumors were found in 46% of the female rats with a similar incidence among the groups fed different fats. Because of their superficial location they were observed early and were mostly of long duration. The average age at death for mammary tumor bearers varied from 728 days for those fed corn oil to 797 days for those fed hydrogenated vegetable shortening. Tumor weights varied greatly; 15 were under 50 g and 25 were over 250 g. The largest tumor, weighing 835 g, was found in a rat dying at 539 days and had been noted first at 296 days. The body weight after the tumor was removed was 242 g. This was the youngest rat of those dying with mammary tumors. Mammary tumors were often multiple and some were bilateral. The usual picture was that of a benign fibroadenoma. Some showed chronic inflammatory reaction and occasionally abscess formation. Adrenal enlargement was associated with these tumors, of 33 adrenals examined, 24 showed extensive hemorrhage often with cortical lipoidosis. Nonmammary tumors were as frequent in males as in females.

Relatively few tumors developed with diets containing hydrogenated vegetable shortening. Benign tumors predominated in the rats fed cottonseed oil. The highest incidence of malignancy was observed in the animals fed corn oil but too few tumors were involved to establish the significance of these findings. Neoplasms in these rats were mainly of connective tissue origin (sarcomas) rather than of epithelial tissues (carcinomas). Skin tumors, five in male rats and two in females were listed as epitheliomas since they showed excessive growth of cells, formation of keratinized epithelial pearls and local infiltration, but were of low malignancy and probably secondary to chronic ulceration. No distant spread was present except in one 928 day old female with a large tumor between the legs which had some nodules in the mesentery. Subcutaneous tumors were found in nine males and five females. In the male, seven were benign and two sarcomatous. In the female all but one were malignant, of these one was carcinomatous. An adenocarcinoma of the salivary gland might also be added to this group. In none of these were distant metastases found. Abdominal tumors, including those of the gastrointestinal, endocrine and reproductive systems, were found in 10 males and 14 females. In male rats, three were benign and seven malignant, mostly diffuse and involving the gastrointestinal tract; five of these were sarcomatous and two carcinomatous. In female rats there were five benign and nine malignant tumors. The benign tumors included two adenomas of the adrenal gland, two of the pancreas and one uterine fibromyoma. In contrast to males, tumors of the reproductive system were common, six occurring in the uterus and adnexas of which five were malignant. A pancreatic duct carcinoma was the only tumor with distant metastases to the lung. The only primary tumor in the liver was a bile duct carcinoma. Two diffuse lymphosarcomas were found involving most of the abdominal viscera.

Age Incidence of Nonmammary Tumors

The average age at death for males with benign or malignant tumors was over 675 days. Female rats with benign tumors averaged 691 days, those with malignant tumors 546 days. The earlier deaths occurred mostly in those with tumors of the reproductive system.

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