From the Department of Pathology,
College of Physicians & Surgeons, Columbia University, New York, N. Y. (USA)

Differences in effects of dietary fats on survival rate and development of neoplastic and other diseases in rats*) **)

By H. KAUNITZ, R. E. JOHNSON and L. PEGUS

With 5 figures and 4 tables

(Received December 24, 1969)

Considerable interest centers around the problem of whether long-term nutritional effects of dietary fats and oils can be correlated with such chemical properties as their content of saturated and unsaturated fatty acids, their triglyceride structure, melting point, etc. Because the evidence available at present is insufficient for definite conclusions, we have been carrying out long-term studies of fats and oils commonly used in the human diet.

About eight years ago, we began a long-term feeding experiment with rats of the Columbia-Sherman strain (1, 2). Either fresh or mildly oxidized (40 hours of aeration at 60 °C) cottonseed oil, olive oil, corn oil, soybean oil, chicken fat, beef fat, butter oil, and lard were fed at a level of 20 % in a puirfied rat diet containing 30 % alcohol-washed casein, 44 % dextrose, 4 % salt mixture USP XIII, and 2 % cellulose. The salt mixture was low in iodine.

We expected that the aeration would produce no or only disadvantageous effects and were surprised to observe that feeding of mildly oxidized soybean oil, olive oil, and corn oil was associated with life spans similar to those of the rats fed fresh animal fats and longer than those of the groups fed the corresponding fresh vegetable oils. Inasmuch as it seemed unlikely that such an effect could be due to oxidative changes in the triglycerides of these vegetable oils, we concluded that the unsaponifiable fraction contained toxic substances which were inactivated by mild oxidation. This was supported by the occurrence of many enlarged thyroids in the groups fed the vegetable oils, which suggested the presence of goitrogenic materials in these oils.

In view of the fact that these studies had been carried out on iodine-deficient rats in experimental groups of varying size, it seemed desirable to confirm the possible influence of the unsaponifiable fraction on a larger number of animals under study all at the same time. Therefore, a second study was undertaken with 1000 rats of three different strains. The fats and the diet composition were the same as before except that an iodine-sufficient salt mixture was used which contained all other known trace elements (3). In addition, one group was fed randomized, saturated medium chain (C_{6-8}) triglycerides prepared from coconut oil (4), hereafter called MCT; two groups were fed soybean oil and lard which had been given 4.5 megarads of gamma irradiation.

---

*) Lecture given in Nuremberg on September 6, 1969

**) Carried out under Contract No. PH 43-67-731 from the National Cancer Institute, National Institutes of Health, United States Public Health Service

This report will be limited to results obtained with 760 male rats of the Charles River COBS variety. All rats were kept in the same air conditioned (80 °F) room throughout the experiment. The matching groups of 40 rats each were distributed on racks in such a way as to equalize, as much as possible, the effects of temperature and ventilation gradients throughout the room. Rats were inspected twice daily (including weekends), and autopsies were performed as soon as possible after death.

Inasmuch as this experiment is still in progress, only the first twenty rats to die in each group will be discussed in this paper. All data have been analyzed by chi square tests with 8 degrees of freedom (9 fats) which took into account intergroup variation.

Table 1. Mortality of groups of male rats fed various fresh, oxidized, and irradiated fats from weaning. Data include ages at which 50 % (20 rats) of each group had died and the number still alive of the first 20 at 606 days when 20 rats fed fresh cottonseed oil and fresh chicken fat had died.

	Age at which 20th rat died in each group (days)		No. alive at 606 days of first 20 to die in group	
	Fresh	Oxidized	Fresh	Oxidized
Cottonseed oil	606		0	
		648		4
Chicken fat	606		0	
		640		7
Butter	613			5
		620		5
Lard	622		4	
		641		9
(Irradiated)	619		3	
Olive oil	635		10	
		654		6
Corn oil	667		6	
		662		6
Soybean oil	673		7	
		629		7
(Irradiated)	602		0	
MCT	686		9	
Beef fat	689		14	
		670		15

Table 1 gives the ages at which 50 % (20 rats) of each group had died and the number of rats in each group which were alive at 606 days, the earliest age at which 20 had died in any group. The differences between the groups fed fresh and oxidized fats were not sufficiently pronounced to suggest separate treatment of these groups, and the data were combined for statistical analyses. A chi square analysis of the number of rats (of the first twenty to die eventually in each group) alive at 606 days when 20 of the rats fed fresh cottonseed oil and fresh chicken fat had already died showed significant differences among the groups (Chi square = 35.5; $P < .0001$). At this stage of the experiment, the animals fed beef fat had the best survival rate.

As the experiment has progressed since then, the longer survival rate of the group fed MCT has become pronounced. This is in line with a previous observation on fewer animals (5). It is evident that differences in survival rate do not correlate with linoleate content, melting point, or other obvious properties of triglycerides. A preliminary attempted correlation between body weights and survival rates gave some suggestion that the groups having the highest median weights at a relatively early age had shorter survival rates.

At autopsy, gross anatomical and subsequent histological findings revealed characteristic inflammatory, degenerative, and neoplastic lesions. As was to be expected in laboratory rats, the most frequently observed pathology was lung disease, ranging in severity from small localized pneumonic foci to nearly complete replacement of the lungs by large pus-filled cavities, with lung weights ranging from 3 to 30 grams. Histologically, the lesions were those usually described in old rats: earlier stages were characterized by purulent bronchitis, often associated with bronchiectasis and epithelial proliferation; later stages showed characteristic bronchopneumonia with abscess formation.

When lung weights were correlated with gross and histological signs of pulmonary disease, it appeared that lungs weighing over 8.5 grams had advanced lesions whereas lungs weighing 5.5 grams or less were relatively free of disease. In table 2 is given the distribution of lung weights. Inasmuch as oxidation of the fats did not seem to have any effect, data for rats fed fresh and oxidized fats were combined for statistical purposes. The data suggest that the kind of fat fed did influence the incidence of lung

Table 2. Frequencies of lung weights of 8.6 gm and over and of 5.5 gm and less among the first 20 rats to die in each of groups fed various fresh and oxidized fats from weaning. There are 20 lung weights per group except where noted.

	8.6 gm and over		5.5 gm and less		
	Fresh	Oxidized	Fresh	Oxidized	
Lard	9	12	7	7	
MCT	10		8		
Butter	5	10	6	5	
Chicken fat	8	6	8	6	(18 wts.)
Cottonseed oil	7	7	7	8	
Olive oil	7	4	5	10	(19 wts.)
Soybean oil	4	5	9	11	(19 wts.)
Beef fat	3	5	13	9	(19 wts.)
Corn oil	3	4	9	10	(19 wts.)

infection. (Chi square with 8 d. f. comparing incidence of lungs weighing over 8.5 gm = 20.2; $P < .01$). The high incidence among those fed lard and the low incidence among those fed corn oil contributed nearly 2/3 of the total value of the chi square.

The genitourinary tract was a frequent site of inflammatory lesions. Many of the animals had waxy material obstructing the urethra and seminal ducts associated with severe cellulitis of the prostate and seminal vesicles (figs 1, 2). We observed 25 cases, and oxidation of the fats did not influence the results:

Olive oil	7/40	Butter	2/40
Beef fat	5/40	Corn oil	1/40
Soybean oil	5/60	Chicken fat	1/40
MCT	2/20	Lard	2/60
	Cottonseed oil	0/40	

Fig. 1. Prostatic cellulitis. Waxy material extruded from penis. Greatly enlarged prostate has been bisected.

Chi square (8 d. f.) = 17.09; $P < .05$. The waxy material is probably part of the seminal fluid, and one wonders why certain fats seem to facilitate removal of this material from the genitourinary tract.

What are usually considered to be degenerative diseases occurred mainly in kidneys and hearts. In contrast to the case in man, the aorta was affected only in secondary hyperparathyroidism.

Renal lesions were often associated with secondary hyperparathyroidism. Renal insufficiency is associated with phosphorus retention, causing hypocalcemia; this, in turn acts as a stimulus to the parathyroid gland, which hypertrophies and excretes large amounts of parathormone. This hormone mobilizes calcium from bones, which leads to severe calcification of many organs. The kidney shows tubular calcification, atrophy or enormous dilatation by hyaline casts; areas of interstitial lymphocytic infiltration are quite common. Glomerular changes were mild; thickening and calcification of Bowman's membrane were seen occasionally (fig. 3). In order to correlate incidence of secondary hyperparathyroidism with dietary fat among the first twenty animals dying in each group, those cases were counted which had severe kidney lesions, parathyroid enlargement, and marked heart changes. Oxidation of the dietary fat did not appear to influence the results and data for groups fed fresh,

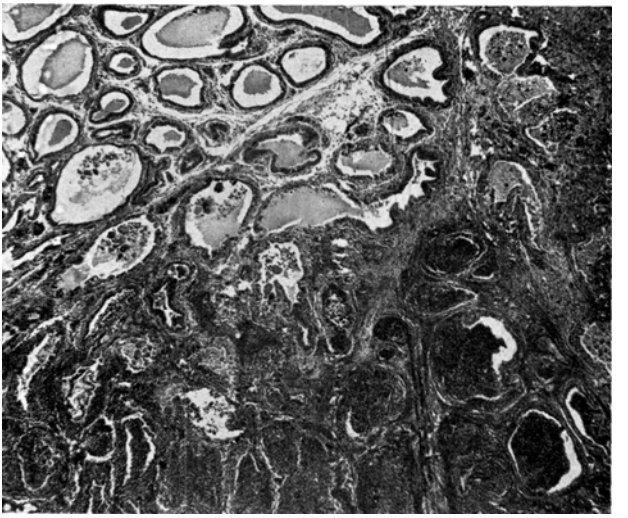

Fig. 2. Prostatic cellulitis. H & E stain; X 30

oxidized, and irradiated fat were combined. The over-all incidence was 14.5 %, and the range was 0 % with MCT to 25 % with olive oil; however, a chi square (8 d. f.) calculation showed no statistical significance. Kidney weights, although ranging from 4 to 10 grams, did not differ significantly among the groups.

In hyperparathyroidism, the heart shows a cell-rich focal myocarditis associated with calcification of muscle and vessels (fig. 4). Probably not all degenerative changes

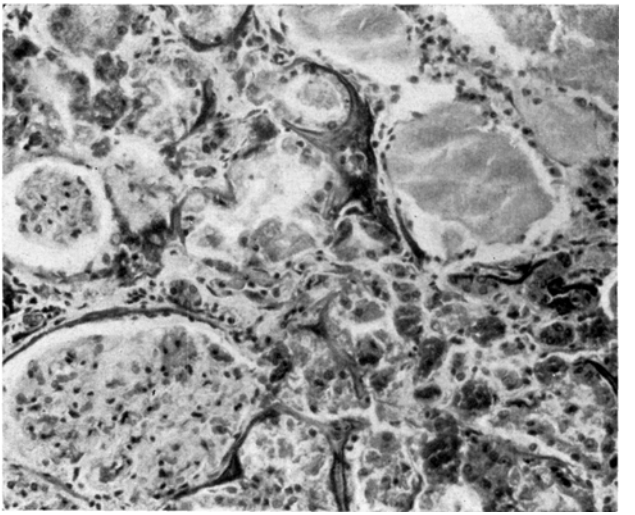

Fig. 3. „Thyroidization" and calcification of kidney in secondary hyperparathyroidism. H & E stain; X 160

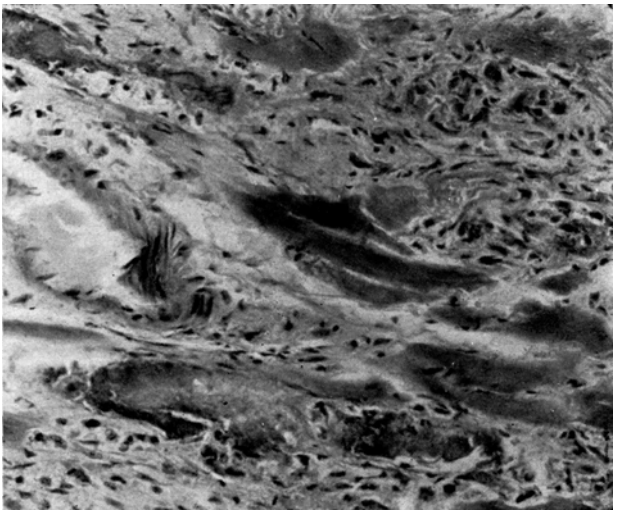

Fig. 4. Myocarditis and calcification in secondary hyperparathroidism. H & E stain; X 240.

of the heart can be attributed directly to secondary hyperparathyroidism. From the work of others, one can expect that hypertension followed by changes in adrenal hormones are important, but we have no measurements to assess these factors. The earlier lesions, although similar to those associated with secondary hyperparathyroidism, probably differ from the latter in the somewhat different appearance of the modified fibroblasts, in the presence of few fibroblasts, and usually more pronounced focal fibrosis. Eventually, large parts of the heart muscle are replaced by fibrous tissue (fig. 5).

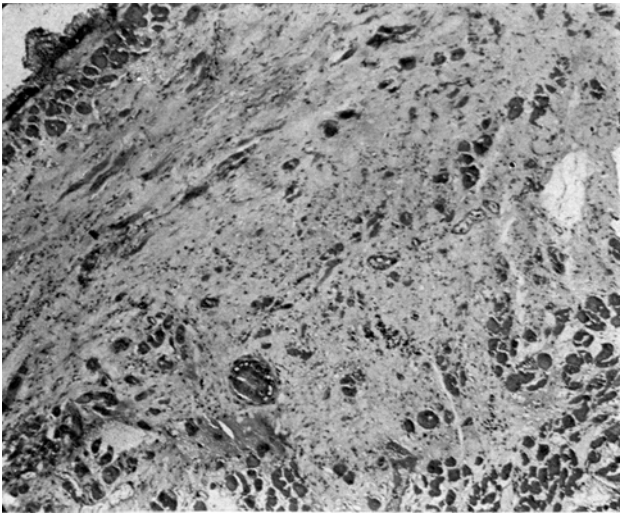

Fig. 5. Replacement of myocardium by fibrous tissue. H & E stain; X 63.

Table 3. Incidence of varying degrees of cardiac fibrosis (evaluated on coded slides) and of unusually heavy hearts among rats fed various fats from the time of weaning. Animals fed fresh and oxidized fats have been combined.

	No. of slides	Frequency of various degrees of cardiac fibrosis			No. of wts.	Frequency of heart wts. of 2.0 gm and over
		4+	3+	0, ±, +		
Corn oil	40	24	9	2	40	18
Cottonseed oil	39	16	9	7	40	9
Soybean oil	39	13	6	9	39	6
Olive oil	40	11	10	8	39	6
Beef fat	35	8	6	10	40	8
Butter	39	8	5	17	39	14
MCT	18	3	5	5	20	6
Chicken fat	40	5	8	11	40	16
Lard	40	7	8	10	40	17

The degree of cardiac fibrosis in the first 20 rats to die in each group was evaluated on coded slides on a scale of 0 to 4+. The results are given in table 3. A chi square calculation (8 d. f.) for pooled data from groups fed fresh and oxidized fats gave 33.1 ($P < .001$). The incidence among rats fed corn oil contributed the major part of the chi square value. Below are given the incidences of hearts weighing 2.0 gm or more. This weight was selected because it was well above the median weight for all hearts. A chi square calculation (8 d. f.) gave a value of 20.7 ($P < .01$). Except in the case of corn oil, there was little correlation between the incidence of heavy hearts and that of advanced fibrosis. One can therefore conclude that the heavy hearts of the animals fed lard, butter, and chicken fat were due to hypertrophy with relatively little scar formation. The fact that corn oil was associated with the heaviest hearts and the most advanced fibrosis may be related to the finding of AVIGAN and STEINBERG (6) and of others that corn oil has a marked serum cholesterol-lowering effect associated with high liver cholesterol values. Whatever it is in corn oil which affected the degree of heart fibrosis was, to a lesser extent, also present in the other vegetable oils. A chi square calculation showed that the incidence of 4 + fibrosis was significantly higher among the animals fed vegetable fats ($P < .0001$). Does the high linoleate content of three of the vegetable oils contribute to this effect?

Twenty-six per cent of the 380 animals discussed in this report had neoplasms. Myeloid leukemia (chloroma) was seen 42 times; other blood dyskrasias were reticulocellsarcomas and various lymphomas, the latter often involving the mediastinum. Large fibrosarcomas occurred frequently.

The most frequent ectodermal lesions were epidermoid tumors, which were often ulcerated and were of low grade malignancy. Few endodermal tumors and such mesodermal tumors as those of prostate and testis were seen.

Tumors of the pituitary (weighing 100 mg or more) were observed 25 times in the animals fed vegetable oils and 11 times among those fed animal fats. Other endocrine tumors were benign enlargements of the mammary glands which often led to the formation of "milk cysts", adrenal medullary tumors, and thyroid adenomas and adenocarcinomas. However, we have excluded endocrine tumors from the statistical evaluation of neoplasms given in this report. The endocrine tumors will be treated in a separate report in collaboration with JACOB FURTH.

Table 4. Incidence of chloromatous leukemia and other non-endocrine neoplasms among rats fed one of various fats. Data from groups fed fresh, oxidized, and irradiated forms of the same fat have been combined.

	Chloroma	Other non-endocrine neoplasms	Total incidence
Chicken fat	11	6	17/40 43 %
Beef fat	4	9	13/40 33 %
Soybean oil	8	12	20/60 33 %
Lard	7	12	19/60 32 %
MCT	0	5	5/20 25 %
Olive oil	4	4	8/40 20 %
Butter	3	4	7/40 18 %
Corn oil	4	3	7/40 18 %
Cottonseed oil	1	2	3/40 8 %

Table 4 shows the incidence of non-endocrine neoplasms. Chloroma occurred most frequently among rats fed chicken fat; there were no cases among the rats fed MCT and only one among those fed cottonseed oil. A chi square calculation (8 d. f.) on pooled data (fresh, oxidized, and irradiated) gave a value of 17.8 or $P < .05$. Other malignancies occurred more particulary after the rats were 550 days old. A chi square analysis (8. d. f.) on pooled data (fresh, oxidized, and irradiated) showed that there were significant differences in the incidence of all non-endocrine neoplasms among the groups fed various fats (Chi square = 20.2; $P < .01$).

The present data show that the survival rate and diseases of the Charles River strain of rat have been strongly influenced by the kind of dietary fat, confirming although not in detail, the results of our previous studies with iodine-deficient rats of the Columbia-Sherman strain.

The question immediately arises as to whether the results of this study have any relevance for man. Although direct extrapolation is not possible because many of the diseases of the rat are different from those of man, some general conclusions are probably directly applicable to human beings – especially the overriding impression that all fats have their individual properties compounded of their fatty acid composition, triglyceride structure, melting point, unsaponifiable fraction, trans acid content, and perhaps other unknown attributes. This view has been given too small consideration in recent years with the exponential development of lipid research based on the techniques of gas-liquid and silicic acid chromatography with the attendant emphasis on fatty acid composition and triglyceride composition.

Our data thus far tend to show that over-all life span and the kinds of diseases rats die from can not be linked to any of the currently fashionable chemical parameters. Lower mortality rates have been associated with two fats having a low linoleate content and with a high melting point (beef fat) and a low melting point (MCT), whereas another fat with a low linoleate content and an intermediate melting point (butter) has been associated with a higher death rate. Animals fed the highly unsaturated corn oil have had a lower mortality rate but, at the same time, have had severe cardiac changes.

The significant differences in tumor incidence raise the question as to the nature of the agents responsible. The work done by BORNEFF (7) and by FRANZKE (8) showed the presence of such carcinogens as benzpyrenes in soybean and other vegetable oils,

but the levels may not be biologically active. PETER MARQUARDT has suggested nitrosamines as a possibility, and we have obtained some evidence for the presence of amines in the unsaponifiable fraction of soybean oil. However, whether they are nitrosamines and whether they are present in sufficient amounts remains to be seen. In the case of chicken fat, it is perhaps not impossible that the high incidence of leukemias in the animals fed this fat was related to the fact that commercially raised chickens carry leukosis viruses. It may not be a coincidence that rats fed MCT, a mixture of pure triglycerides, did not have this disease.

If it is true that the non-triglyceride fraction of these fats is as important as the studies suggest, considerable effort will have to be invested in trying to find so far unknown pharmacologically active substances in this fraction. It may also become necessary to introduce changes in the processing of certain of the fats.

We are most grateful to Dr. JACOB FURTH of the Cancer Research Institute of Columbia University for his guidance in our histological work.

Summary

Groups of 40 male rats of the Charles River CD strain were fed a purified complete diet containing 20% of cottonseed oil, olive oil, corn oil, soybean oil, chicken fat, beef fat, butter, lard, or medium chain, saturated triglycerides (MCT). Except for the latter, the fats were fed either fresh or after 40 hours of aeration at 60 °C. The rats were observed for weight gain, life span, and terminal pathological changes. There were significant differences in survival rate, occurrence of malignant non- endocrine tumors, and degenerative and inflammatory diseases, such as the high incidence of chloromatous leukemias among the rats fed chicken fat, or the severe myocardial lesions among the groups fed vegetable oils. Inasmuch as the results could not be accounted for by the usual properties of triglycerides, it was concluded that minor constituents in fats play an important part in their individual nutritional effects. It was speculated that the processing of some fats may have to be changed.

Zusammenfassung

Gruppen von vierzig Rattenmännchen der „Charles River CD" Zucht wurde eine hochgereinigte Nahrung verabreicht, die 20% Baumwollsamenöl, Olivenöl, Maisöl, Soyaöl, Hühnerfett, Rindsfett, Schweineschmalz oder gesättigte Triglyceride mittlerer Kettenlänge (MCT) enthielt. MCT wurde ohne Vorbehandlung , die anderen Fette entweder frisch oder nach vierzig stündigem Luftdurchblasen bei 60 °C verwendet. Gewicht, Überlebenszeit und histopathologische Veränderungen wurden beobachtet. Signifikante Unterschiede zwischen den Gruppen wurden beobachtet. Es erschien z. B. beachtenswert, daß die mit Hühnerfett ernährten Tiere eine erhöhte Zahl von myeloischen Leukaemien aufwiesen und daß die mit Pflanzenfetten ernährten Tiere besonders ausgeprägte myokardiale Veränderungen hatten. Da die Resultate nicht durch die Eigenschaften der Triglyceride erklärt werden konnten, muß man annehmen, daß Substanzen in der Nicht-Triglyceridefraktion von größter Bedeutung für die biologischen Unterschiede dieser Fette sind. Verbesserte Verfahren zur Herstellung von Nahrungsfetten sind zu empfehlen.

References:

1. KAUNITZ, H., R. E. JOHNSON and L. PEGUS, Proc. Soc. Exp. Biol. Med. **123**, 204 (1966). — 2. KAUNITZ, H. and R. E. JOHNSON, J. Nutrition **91**, 55 (1967). — 3. BERNHART, F. W. and R. M. Tomarelli, J. Nutrition **89**, 495 (1966). — 4. BABAYAN, V. K., J. Amer. Oil Chem.

Soc. **45**, 23 (1968). — 5. KAUNITZ, H., C. A. SLANETZ, R. E. JOHNSON, V. K. BABAYAN and G. BARSKY, J. Amer. Oil Chem. Soc. **35**, 10 (1958). — 6. AVIGAN, J and D. STEINBERG, Proc. Soc. Exp. Biol. Med. **97**, 814 (1958). — 7. BORNEFF, J. and B. Fabian, Arch. Hygiene Bakteri. **150**, 485 (1966). — 8. FRANZKE, C. and W. FRITZ, Fette Seifen **71**, 23 (1969).

Authors' address:
Department of Pathology
College of Physicians and Surgeons
Columbia University, New York N. Y. 10032 (USA)