

sclerosis (Springfield 1953). — 10. DAUBER, D. V., KATZ, L. N., Arch. of Path. **36**, 473 (1943). — 11. JONES, R. J., REISS, O. K., HUFFMAN, S., Proc. Soc. Exp. Biol. **93**, 88 (1956). — 12. STAMLER, J., PICK, R., KATZ, L. N., Circulation Res. **7**, 398 (1959). — 13. DAUBER, D. V., Arch. of Path. **38**, 46 (1944). — 14. WEISS, H. S., Proc. Soc. Exp. Biol. **95**, 487 (1957). — 15. ABELL, L. L., LEVY, B. B., BRODIE, B. B., KENDALL, F. E., J. Biol. Chem. **195**, 357 (1952). — 16. ANDERSON, J. T., KEYS, A., Clin. Chem. **2**, 145 (1956). — 17. MALMROS, H., WIGAND, G., Minnesota Med. **38**, 864 (1955). — 18. MALMROS, H., WIGAND, G., Lancet **1959** II, 749. — 19. REISER, R., J. Nutr. **42**, 319 325 (1950). — 20. BIERI, J. G., BRIGGS, G. M., FOX, M. R., POLLARD, C. J., ORTIZ, L. O., Proc. Soc. Exp. Biol. **93**, 237 (1956). — 21. KINSELL, L. W., PARTRIDGE, J. W., BOLING, L., MARGEN, S., MICHAELS, G. D., J. Clin. Endocrin. **12**, 909 (1952). — 22. AHRENS, E. H., BLANKENHORN, D. H., TSALTAS, T. T., Proc. Soc. Exp. Biol. **86**, 872 (1954). — 23. MALMROS, H., WIGAND, G., Lancet **1957** II, 1. — 24. BEVERIDGE, J. M. R., CONNELL, W. F., MAYER, G., Circulation **12**, 499 (1955). — 25. BRONTE-STEWART, B., ANTONIS, A., EALES, L., BROOK, J. F., Lancet **1956** I, 521. — 26. KINSELL, L. W., MICHAELS, G. D., FUKAYAMA, G., Proc. Soc. Exp. Biol. **98**, 829 (1958). — 27. BRIGGS, G. M., SPIVEY, M. R., KERESZTESY, J. C., SILVERMAN, M., Proc. Soc. Exp. Biol. **81**, 113 (1952). — 28. ALBRITTON, E. C., Standard Values in Nutrition and Metabolism. (Philadelphia and London, 1954).

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Histopathological Changes in Rats and Pigs Fed Rapeseed Oil

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With 4 figures and 1 table

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Numerous investigations have shown that rapeseed oil differs from other commonly used edible oils with respect to certain physiological effects. Experiments with rats have demonstrated that, among other effects, it retards growth (VON BEZNAK et al. 1943, DEUEL et al. 1948, THOMASSON 1955a, THOMASSON & BOLDINGH 1955, WILSKA 1955, ROINE et al. 1958) and increases the cholesterol content of the adrenals (CARROLL 1951). According to THOMASSON (1955b), rats kept on a diet containing 50 cal. per cent of rapeseed oil live 20–25 per cent longer than those fed 50 cal. per cent of butter, but a rapeseed oil content of 73 cal. per cent causes premature death (THOMASSON 1955a). In addition, it has been suggested (CARROLL & NOBLE 1957) that the main component of rapeseed oil, erucic acid, interferes with the reproduction of rats by interfering with the metabolism of the essential fatty acids.

There is so far very little knowledge of the histopathological changes in animal tissues possibly caused by rapeseed oil. THOMASSON (1955b) observed slight liver degeneration in animals fed rapeseed oil, which did not occur in the

corresponding butter group. However, according to MURRAY et al. (1958), a synthetic diet containing 5 per cent of methyl erucate did not produce histological changes, but the growth of the females was below normal.

Since rapeseed oil is gaining increasing use in the human diet, we have considered it necessary to carry out histological studies of animals fed large amounts of rapeseed oil, in an attempt to find out whether this oil produces pathological changes that are not seen in animals kept on a corresponding soybean oil diet.

Experimental

Experimental Animals. The rats used were 45 young Sprague-Dawley males, mean weight about 50 g at the beginning of the experiment. In the pig experiment the litters of two Yorkshire sows were used, consisting of 5 males and 6 females. At the beginning of the experiment the pigs were 48–60 days old and had a mean weight of 20.6 kg.

Diets. — The following basic diet was used in the rat experiments: 150 g of graham flour, 100 g of dried brewers yeast, 150 g of casein, and 20 g of salt mixture (1000 g sodium chloride, 1000 g calcium lactate, 30 g ferric citrate, 10 g manganese sulfate, 2 g copper sulfate and 0.2 g potassium iodide).

To this basic diet was added rapeseed oil in amounts of 0, 15, 30, 50 or 70 cal. per cent. The control groups were correspondingly given 30 or 70 cal. per cent of soybean oil. When the diet contained less than 70 cal. per cent of oil, sucrose was added to make the total addition of oil and sucrose 70 cal. per cent. To supply the vitamin A and D requirement, each rat was given 6 drops of fish-liver oil once a week.

In the pig experiments the composition of the diet was as follows: 350 g of corn meal, 350 g of wheat bran, 300 g of dried skimmed milk, 150 g of rapeseed oil or soybean oil, and 1500 g of water. The total fat content of the diet was about 34 cal. per cent, the soybean oil and rapeseed oil supplying about 28 cal. per cent. Vitamins A and D were supplied in 0.6 ml of A-Jekol preparation given once a week, corresponding to 24,000 I. U. of vitamin A and 4,800 I. U. of vitamin D₂.

Methods of Histological Examination. — The rats were killed by decapitation and histological specimens were taken from the following organs: Thyroid, heart, liver, spleen, kidneys, adrenals, stomach, small intestine, large intestine, aorta, and striated muscle. Specimens were taken from the same organs in the pigs immediately after slaughter by electric shock. The specimens were fixed in Bouin's solution, sectioned at about 5 μ and stained with hemalum-eosin and van Gieson's staining.

For comparison, a histological examination was made also of 10 rats that had had access *ad libitum* to the laboratory's stock diet without added oil, and of 5 pigs weighing approximately the same as the experimental pigs and fed the normal mixed food of the hog farm.

Experiments and Results

Rat Experiments. — It was originally the intention that the food intake of all rats would have an equivalent energy content. The "70 cal. per cent rapeseed oil" group, however, ate exceptionally little, and when the food given to the other groups of rats was restricted correspondingly, all the rats lost weight. For this reason the 70 per cent rapeseed oil group was thereafter permitted to eat freely, whereas the original feeding program was maintained for the other groups. It was necessary to sacrifice the 70 per cent rapeseed oil group already on the 13th day, since some of the animals had died and the remaining rats appeared to be severely ill. The experimental periods for the various groups are seen in table 1.

Table 1

Group No.	Oil in the diet	Number of animals	Days on the diet	Initial weight, g	Growth in 36 days, g
I	0 cal. %	5	53	46.9	57
II	15 cal. % of rapeseed oil	5	53	47.1	63
III	30 cal. % of rapeseed oil	6	43	46.8	62
IV	50 cal. % of rapeseed oil	7	43	46.9	56
V	70 cal. % of rapeseed oil	7	13	47.4	— 4
VI	30 cal. % of soybean oil	9	53	46.3	63
VII	70 cal. % of soybean oil	6	36	52.5	39

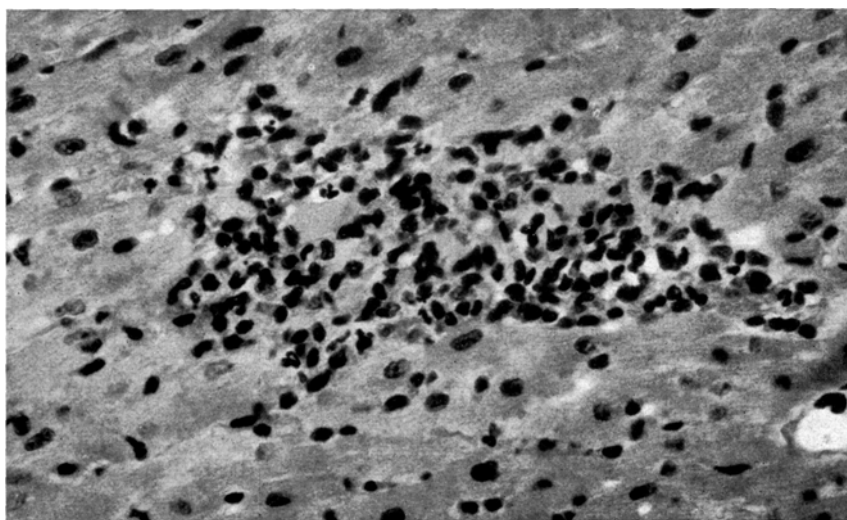


Fig. 1. Circumscribed myocarditic lesion in a rat fed for two weeks with a diet containing 70 cal. % rapeseed oil. 480 x

The increase in the body weights of the rats are also shown in table 1. To render the results comparable, the growth in all groups (group V excluded) is stated for only 36 days. It is seen that growth was best in groups II, III and VI, the diet of which contained 15 or 30 per cent rapeseed oil or soybean oil. A rapeseed oil content of 70 per cent caused loss of weight, sickness and death of several animals in this group. In the group fed 70 per cent of soybean oil the weight increase was below normal although the animals, when judged by their external appearance, did not seem to suffer from the large amount of fat.

The *histological examination* revealed definite inflammatory changes in the myocardium of all the rats given 70 per cent of rapeseed oil (Fig. 1). The interstitial connective tissue was slightly edematous and contained variable numbers of fibroblasts, histiocytes, lymphocytes and plasma cells. Generally there were fairly few granulocytes, which chiefly were neutrophilic; however, some eosinophilic granulocytes were seen. Cloudy swelling and reduced striation were occasionally present in the muscle fibers. Some small necrotic

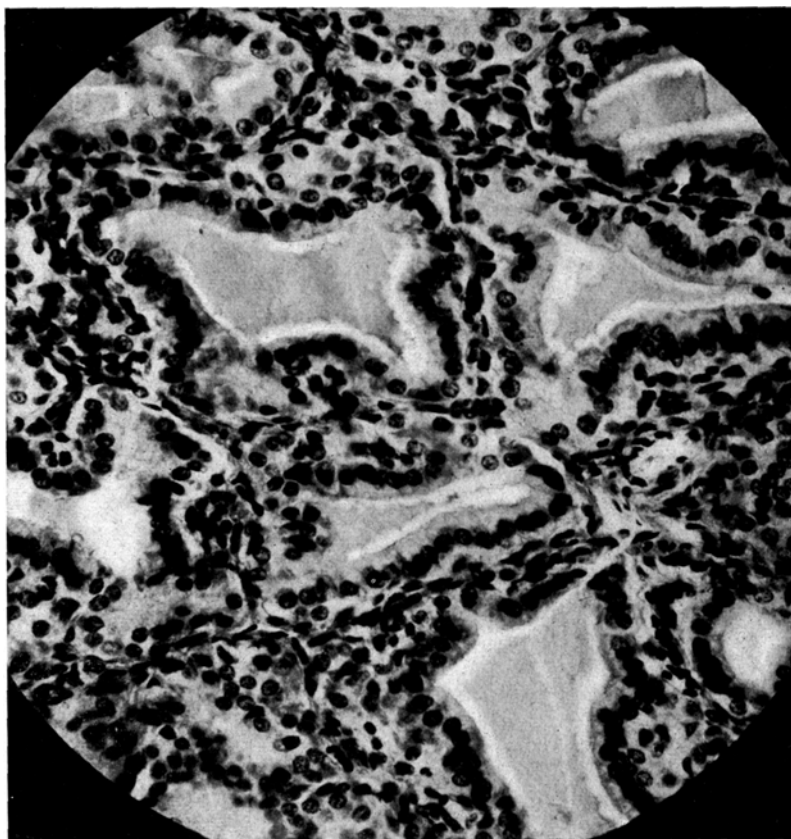


Fig. 2. Hyperfunction of the thyroid gland in a pig fed 28 cal. % rapeseed oil. 460 x

foci were also seen. Frequently the changes were diffuse, but there also were more clearly circumscribed foci. Histologically the condition resembled toxic myocarditis. Similar changes, though milder and more restricted in area, were also present in the myocardium of the rats fed 50 per cent of rapeseed oil.

Definite pathological changes were not discernible in the other rapeseed oil groups nor in either of the soybean oil groups. Accordingly, myocardial reaction was produced only by the diets containing at least 50 cal. per cent of rapeseed oil.

With the exception of the heart, no other examined organs revealed definite pathological changes in any groups of rats.

Pig Experiments. — During the first 40 days of the experiment the pigs were kept on a restricted diet, each pig being given an equal quantity of food; during the following 20 days they had access to food *ad libitum*.

The pigs fed rapeseed oil showed a mean weight gain of 24.9 kg during the restricted feeding period and of 16.7 kg during the free diet, or a total weight increase of 41.6 kg. The pigs fed soybean oil gained in weight 28.1 kg and 22.3 kg, respectively, or a total of 50.4 kg. Thus the growth of the soybean oil fed pigs was somewhat better than that of the rapeseed oil fed pigs.

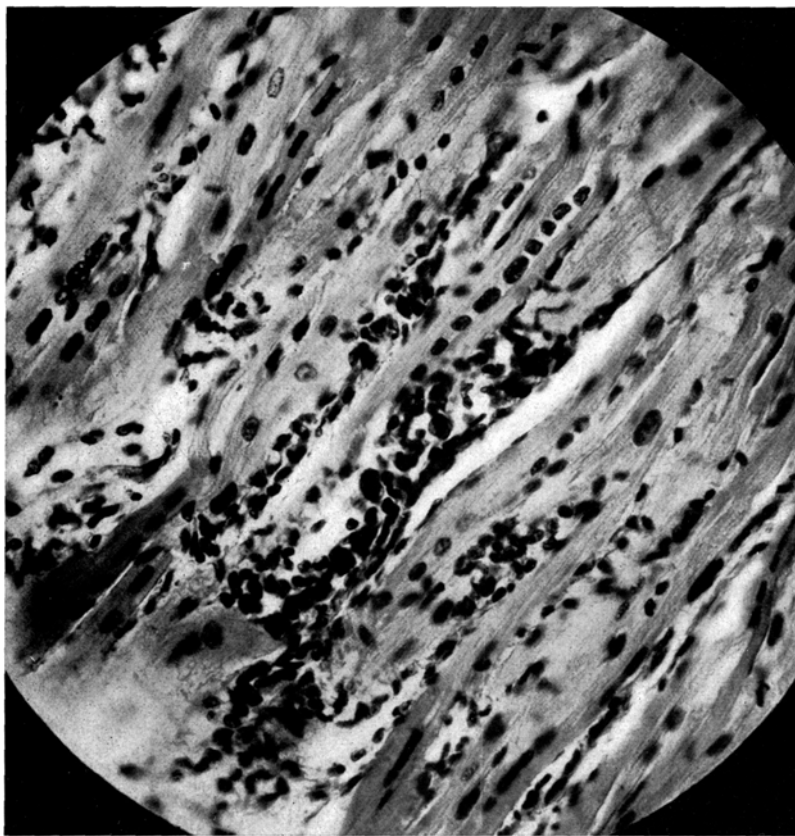


Fig. 3. Diffuse interstitial myocarditis in a pig fed for two months with a diet containing 28 cal. % rapeseed oil. 460 x

The *histological examinations* revealed in the thyroid glands of all the rapeseed oil and soybean oil fed pigs definite changes indicative of hyperfunction (fig. 2). The thyroid follicular epithelium was high and proliferating. Abundant vacuole formation was seen in the colloid, pointing to an active resorptive function. The height of the epithelium of two experimental animals in each group was measured with Abbes projector. The measurements showed no differences between the animals fed rapeseed oil and soybean oil, but in both oil groups the epithelium was higher than in the animals fed normal hog feed, the ratio of height measurements being about 8:5.

The myocardium of all the rapeseed oil and soybean oil fed pigs showed interstitial inflammation, which varied in intensity but was, on the whole, fairly mild; in some cases there was a slight cloudy swelling in the muscle. In similarity to the myocardium of the rats, the changes were both diffuse (fig. 3) and focal (fig. 4). No difference could be observed in this respect between the groups fed different oils. Changes were not found in the heart muscle of the pigs given normal mixed food.

The stomachs of all the examined rapeseed oil fed and soybean oil fed pigs showed inflammatory changes pointing to mild gastritis.

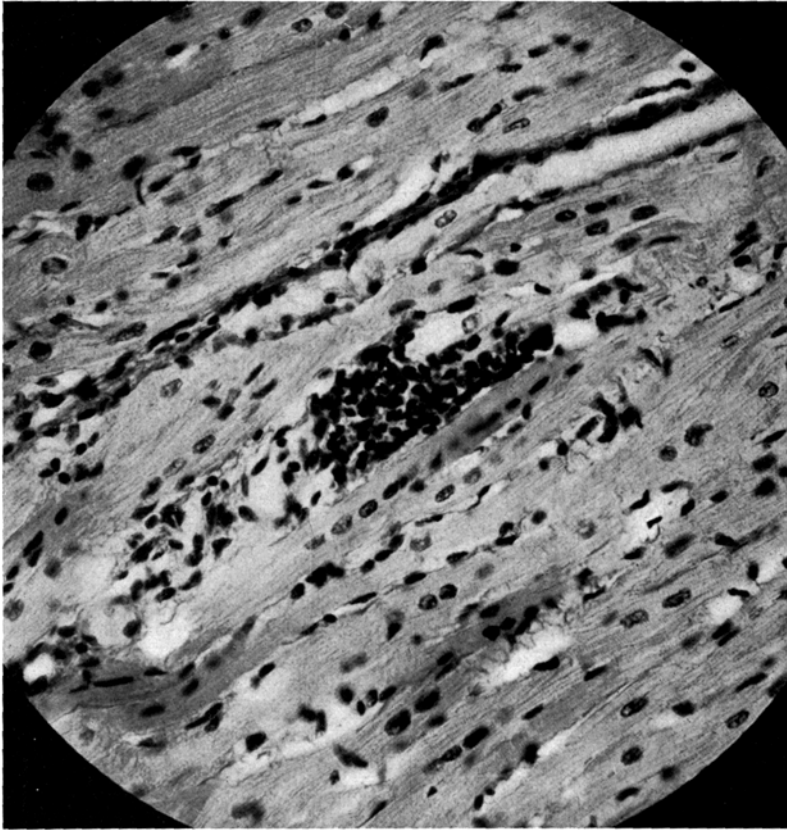


Fig. 4. Circumscribed interstitial myocarditis and cloudy swelling of muscle fibers in a pig fed for two months with a diet containing 28 cal. % rapeseed oil. 460 x

In the other examined organs, on the other hand, pathological features could not be definitely demonstrated, any changes seen being within physiological limits.

Summary

Rats were fed diets in which rapeseed oil provided 0, 15, 30, 50 or 70 per cent of the calories. The controls were rats whose diet contained 30 or 70 cal. per cent of soybean oil. The diet containing 50 and especially that containing 70 per cent of rapeseed oil clearly retarded the growth of the animals. These groups also showed interstitial inflammatory changes in the myocardium. Similar changes were not found when 30 cal. per cent or less rapeseed oil was given. The histological changes were evidently produced by the rapeseed oil, since soybean oil even at the level of 70 cal. per cent produced no changes.

In the pig experiments no histological difference was seen between the animals given 28 cal. per cent of rapeseed oil and the same amount of soybean oil. However, the pigs appeared to be in general more sensitive than rats to the high fat content of the diet, for all the examined pigs showed histological evidence of thyroid hyperfunction, interstitial myocarditis, and inflammatory reaction in the gastric mucosa.

References

- VON BEZNAK, A., VON BEZNAK, M. and HAJDU, M., Ernährung 8, 236, (1943); Ref.: Chem. Abstr. 39, 2544 (1945). — CARROLL, K. K., Endocrinology 48, 101 (1951). — CARROLL, K. K. and NOBLE, R. L., Can. J. Biochem. Physiol. 35, 1093 (1957). — DEUEL, H. J. Jr., GREENBERG, S. M., STRAUB, E. E., JUE, D., GOODING, C. M. and BROWN, C. F., J. Nutrition 35, 301 (1948). — MURRAY, T. K., CAMPBELL, J. A., HOPKINS, C. Y. and CHISHOLM, M. J., J. Am. Oil Chemists Soc. 35, 156 (1958). — ROINE, P. and UKSILA, E. Acta Agr. Fenniac 94, 11 (1959). — THOMASSON, H. J., J. Nutrition 56, 455 (1955 a.). — THOMASSON, H. J., J. Nutrition 57, 17 (1955 b.). — THOMASSON, H. J. and BOLDING, J., J. Nutrition 56, 496 (1955). — WILSKA, A., Suomen Kemistilehti 28 A, 283 (1955).

BUCHBESPRECHUNGEN

Radioisotope Studies of Fatty Acid Metabolism. (Untersuchungen des Lipidstoffwechsels mittels Radioisotopen.) Von J. F. MEAD und D. R. HOWTON - Los Angeles. VIII, 141 Seiten mit 2 Abbildungen und 2 Tabellen. (London 1960, Pergamon Press, Ltd.) Preis: geb. 42. s

Die Möglichkeit des Arbeitens mit radioaktiven Isotopen hat der Stoffwechsel-forschung einen mächtigen Impuls gegeben. Dies trifft in besonders hohem Maße für den Lipidstoffwechsel zu, der — nicht zuletzt wegen seiner großen Bedeutung für die praktische Medizin — in der neueren Zeit eine intensive Bearbeitung erfahren hat. Die vorliegende, relativ wenig umfangreiche Monographie vermittelt einen ganz ausgezeichneten Überblick über den Lipidstoffwechsel. Die Verfasser haben es in vorbildlicher Weise verstanden, die wesentlichen Gesichtspunkte klar herauszuarbeiten ohne das Buch mit überflüssigem Ballast zu belasten. Behandelt werden Resorption, Transport, Speicherung und intermediärer Stoffwechsel unter Berücksichtigung der Verhältnisse bei Tier, Pflanze und Mikroorganismen, wobei der Schwerpunkt jedoch auf den Stoffwechsel im tierischen Organismus gelegen ist. Bei der Darstellung sind auch die Phosphatide und Sterinester mit berücksichtigt. Das Buch enthält weiterhin noch Kapitel über die Synthese markierter Fettsäuren und deren Abbau zwecks Konstitutionsermittlung. Dem Buch ist eine weite Verbreitung zu wünschen.

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Zur Besprechung eingegangene Bücher

(Besprechung vorbehalten)

Aktuelle Probleme der Ernährung. (Bibliotheca Nutritio et Dieta, fasc. 1 — Schriftenreihe des Instituts für Ernährungsforschung, Band 1.) VIII, 224 Seiten mit 50 Abb. und zahlr. Tab. (Basel 1960, S. Karger Verlag.) Preis: kart. sfr 34,—.

Kochsalzarme Kost. Wirkung und Anwendung, Richtlinien, Bestimmungen und Tabellen mit Milliäquivalentwerten für den Mineralgehalt der Nahrungsmittel. Von H.-J. HOLZMEIER-Bonn. VIII, 416 Seiten mit 25 Abb. und 63 Tagesmenükarten. (Stuttgart 1960, Georg Thieme Verlag.) Preis: geb. DM 39,—.

Polypeptides which Affect Smooth Muscles and Blood Vessels. Herausgegeben von M. SCHACHTER-London (Proceedings of a symposium held in London on 23rd and 24th March 1959). XV, 336 Seiten mit zahlr. Abb. und Tab. (London 1960, Pergamon Press, Ltd.) Preis: geb. 50 s.

USA-Studienreise eines Milchwirtschaftlers. Von M. E. SCHULZ-Kiel. 216 Seiten mit 116 z. T. farbigen Abb. (Nürnberg 1959, Verlag Hans Carl.) Preis: geb. DM 18,50.

Vitamin B 12. Von L. E. SMITH. XII, 196 Seiten mit zahlr. Abb. und Tab. (London 1960, Methuen Co., Ltd.) Preis: geb. 15 s.