well as of both fibrinolytic and thrombotic inhibitors. Various changes also occur in pathologic states. It seems reasonable to suspect a possible role of fatty acids in regulation of fibrin formation and digestion.

Summary. We have found that sodium or potassium salts of some long chain fatty acids induce fibrinolytic activity on unheated bovine fibrin films. The influence of chain length and unsaturation on ability to induce fibrinolysis has been studied. Unsaturated fatty acids seem to be more active than the saturated compounds. The presence of one unsaturated bond appears to make little difference in activity of C16 fatty acids. In C18 and C20 acids, it increases activity roughly 10-fold. In C22 and C24 acids, it increases activity more than 100-fold. A possible role of fatty acids in regulation of fibrin formation and digestion is suggested.

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Effect of Mineral Oil Ingestion on Growth and Liver Lipid Composition in the Rat.* (32417)

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Paraffin oils and hydrocarbons have been found to be absorbed and metabolized when supplied with the diet(1,2). The ingestion of such oils has been associated with a decrease in the absorption of carotene, fat soluble vitamins, and other nutrients(3-8). Mineral oil enhanced the development of essential fatty acid deficiency(9) and caused growth retardation, alopecia, priapism and even death in rats when fed at the 10% level(10). However, Oser *et al*(11) found that rats fed petroleum exhibited none of these problems. The gross and microscopic appearance of the tissues and organs remained normal.

In addition to small quantities of mineral oil used for medical purposes, food additives, and food packaging materials in most countries, mineral oils, such as paraffin oil and transformer and spindle oils are used as adulterants in cooking oils, particularly in India. In certain cases, the edible oil may be adulterated with as much as 90% of mineral oil. In the present study, the influence of ingested mineral oils such as paraffin oil, U.S.P.; transformer and spindle oils on the growth rate and lipid class composition of the liver lipid in rats was investigated. Since edible oils adulterated with mineral oil would also be heated during the process of cooking and frying, the effect of mildly thermally oxidized mineral oils admixed with the fresh edible oil was investigated.

Materials and methods. Eight groups each consisting of 4 male weanling rats (Holtzman Strain) were fed *ad libitum* for 4 weeks a purified diet containing 10% by weight

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TABLE I. Effect of Mineral Oils on Growth* and Liver Weight.

Diet supplement	Initial wt (g)	Final wt (g)	Growth per wk (g)	Liver wt (g)	Liver/body wt (%)
2% corn oil	54.6 ± .8	218.3 ± 3.5	40.9 ± .7	10.70 ± .4	4.9 ± .1
8% hydrogenated coconut oil and 2% corn oil	56.5 ± .3	199.0 ±11.2	35.6 ± 2.9	11.01 ± .6	5.6 ± .3
8% paraffin oil and 2% corn oil	55.5 ± .9	184.2 ± 8.8	32.1 ± 2.2	9.08 ± .8	4.9 ± .2
5% oxidized paraffin oil and 4% hydrogenated coconut oil and 1% corn oil	56.5 ± .6	203.0 ± 4.0	36.6 ± 1.3	10.57 ± .4	5.2 ± .1
5% transformer oil and 4% hydrogenated coconut oil and 1% corn oil	56.0 ± .0	116.0 ± 8.8	15.0 ± 2.2	8.87 ± .9	8.4 ± .8
5% oxidized transformer oil and 4% hydrogenated coconut oil and 1% corn oil	55.2 ± .6	136.0 ± 1.4	20.2 ± .4	11.08 ± .5	8.2 ± .3
5% spindle oil and 4% hydrogenated coconut oil and 1% corn oil	55.5 ± .6	120.5 ± 3.9	16.2 ± 1.0	10.65 ± .6	8.8 ± .2
5% oxidized spindle oil and 4% hydrogenated coconut oil and 1% corn oil	56.0 ± .6	132.2 ±13.9	19.0 ± 3.3	11.17 ±1.2	8.5 ± .1

* S.E.M. values—4 animals/group.

of fat‡ (Table I). A level of at least 1% corn oil was used to provide a source of essential fatty acids. A portion of the mineral oil was mildly oxidized by heating it at 200°C for 24 hours and passing dry air through the oil at the rate of 0.22 ml/g/min using a fritted glass dispersion tube. The oil darkened in color, carbonized somewhat and had an acrid odor. It was used after filtering through a Buchner funnel. The animals were weighed at weekly intervals and at the end of 4 weeks were anesthetized, and their livers were immediately removed, dried by pressing between filter paper, weighed, and stored at —20°C until analyzed.

The livers were homogenized in a blendor and extracted with chloroform:methanol (2:1 v/v). The extract was filtered and the tissue residue again extracted with chloroform:methanol. The combined extracts were dried over sodium sulfate, filtered and the solvent removed under reduced pressure. The lipid classes were separated by preparative thin layer chromatography according to Stahl(12) using petroleum ether-diethyl ether-acetic acid 85:15:1 (v/v/v) as the solvent.

The individual lipid componet absorbed on the silica gel was scraped from the plate and the lipid was eluted with moist diethyl ether. The phospholipid fraction was removed from the silica gel with 2:1 chloroform:methanol. The solvent was evaporated with a rotary vacuum evaporator and the amount of lipid was estimated gravimetrically. The lipid class composition of the livers obtained in this manner is tabulated in Table II.

The phospholipid, triglyceride, and cholesterol ester fractions were saponified with freshly prepared alcoholic potassium hydroxide. The fatty acids were liberated with hydrochloric acid, extracted with distilled hexane, washed, dried over sodium sulfate, and the solvent evaporated to dryness under vacuum. The resultant fatty acids were methylated with a sulfuric acid-methanol 3% (v/v) reagent.

‡ The percentage composition of the diet in addition to the fat was: 15% vitamin free casein; 70.6% cerelose; 4.0% Wesson salt mixture (Science 1932, v75, 339); 0.2% choline chloride and 0.2% of a vitamin mixture with the following composition: Supplied per 100 g diet; inositol 10 mg; niacin 10 mg; thiamin hydrochloride 2 mg; riboflavin 2 mg; p-aminobenzoic acid 10 mg; calcium pantothenate 6 mg; folic acid 0.2 mg; vitamin B_{12} 0.005 mg; pyridoxine hydrochloride 1 mg; biotin 0.05 mg; menadione 1 mg; vitamin A 24,000 IU; vitamin E 10 mg; vitamin D 2,400 IU.

TABLE II. Lipid Class Composition of the Liver.

Dietary Supplement	Corn oil	Coconut oil and corn oil	Paraffin oil	Oxidized paraffin oil	Transformer oil	Oxidized transformer oil	Spindle oil	Oxidized spindle oil
Lipid class								
Phospholipid*	218.8 ± 17.9†	230.2 ± 12.1	243.0 ± 37.0	322.7 ± 20.5	250.5 ± 29.1	354.4 ± 24.8	231.4 ± 27.6	359.4 ± 40.8
Cholesterol and diglyceride	21.9 ± 3.2	36.8 ± 3.2	22.6 ± 5.4	34.2 ± 1.5	27.6 ± 4.7	42.2 ± 2.4	31.0 ± 3.4	45.9 ± 13.8
Free fatty acid	23.3 ± 3.8	34.3 ± 5.6	35.2 ± 6.6	52.1 ± 3.1	30.5 ± 6.4	53.5 ± 5.8	29.2 ± 4.6	59.8 ± 11.8
Triglyceride	52.2 ± 4.4	316.4 ± 30.5	53.1 ± 9.0	73.9 ± 8.8	108.5 ± 14.9	145.7 ± 19.8	85.4 ± 3.6	125.5 ± 9.5
Cholesterol ester	12.4 ± 4.0	31.4 ± 8.3	23.3 ± 6.8	28.9 ± 3.3	31.3 ± 4.9	92.8 ± 1.9	23.5 ± 4.5	28.4 ± 9.5
Total lipid	328.8 ± 25.9	649.9 ± 40.3	377.4 ± 65.4	512.5 ± 28.5	448.5 ± 21.0	638.5 ± 15.9	400.7 ± 40.5	618.0 ± 75.8

* mg total lipid pe r liver.
† S.E.M. Values — 4 animals/group.

Methyl esters were analysed by gas-liquid chromatography on a Barber-Colman Model 10 apparatus with tritium diode ionization detector. The samples were separated on a 6 ft × 4 mm I.D. glass column packed with 15% ethylene glycol succinate polyester on 60-80 mesh chromasorb W at 175°C. Identification was based on comparison with known standards. The fatty acid composition was calculated by the triangulation method.

Results and discussion. The ingestion of 5% transformer and spindle oil admixed with fresh corn and coconut oil as well as diets containing the corresponding oxidized oils resulted in a significant loss in weight as compared to animals receiving only edible oil (Table I). The average growth loss per week was 63%, 60%, 50%, and 53%, respectively, compared to that of rats fed 2% corn oil. When growth was compared to that of rats fed a mixture of 2% corn oil and 8% hydrogenated coconut oil, the average weight loss was 57%, 55%, 43%, and 46%, respectively. Growth data obtained by Ershoff and Hernandez(13) indicated that an average growth rate of about 38 g per week may be achieved on 10% corn oil. We noted an average weight gain of 40 g per week on 2% corn oil.

Rats fed transformer and spindle oils as part of their diets gained approximately one-half as much weight as those fed paraffin oil. However, animals fed the corresponding mildly thermally oxidized mineral oils gained slightly more weight than those fed the non-oxidized oil. This is in contrast to the finding that thermally oxidized edible oils generally depress growth to a considerable degree.

The growth per week of rats fed either paraffin oil or the corresponding oxidized oil was considerably greater than that of animals fed the other mineral oils. Rats fed the diet containing 8% paraffin oil and 2% corn oil developed seborrhea within 48 hours; no seborrhea was noted in animals receiving the other diets.

Ershoff and Hernandez(13) and Greenberg and Ershoff(8) have reported that increasing the fat content of the diet counteracted to some extent the deleterious effect of ingested

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TABLE III. Effect of Paraffin Oil and Oxidized Paraffin Oil on the Fatty Acid Composition of Rat Liver Lipids.*

	Corn Oil				Hydrogenated coconut oil + corn oil			Paraffin oil			Oxidized paraffin oil			
Fatty acid	CE†	TG‡	FFA§	PL‖	CE	TG	FFA	PL	CE	TG	FFA	PL	CE	TG	FFA	PL
12:0	—	—	—	—	1.2	1.2	1.3	.6	1.2	.2	.02	.03	1.1	.3	.3	.1
14:0	11.3	5.7	3.7	2.4	6.7	4.6	8.4	3.9	10.0	3.3	2.9	1.9	6.7	4.1	3.5	1.9
16:0	33.9	37.5	27.9	29.3	52.4	55.6	44.1	46.3	28.3	40.1	25.5	23.2	30.7	38.5	19.8	24.0
16:1	10.3	8.8	8.0	3.6	8.6	3.3	2.8	2.5	13.6	7.6	7.7	3.4	14.4	6.1	8.2	3.1
18:0	9.0	3.5	9.6	22.9	8.1	1.2	5.9	22.7	11.3	4.5	7.8	23.5	9.8	3.6	61.5	23.9
18:1	20.5	38.0	31.8	12.4	13.4	31.5	17.6	9.1	18.7	35.7	34.3	12.0	26.9	42.7	45.0	15.1
18:2	7.7	5.2	8.6	11.3	2.5	2.1	3.5	5.7	6.7	5.1	10.8	11.3	5.4	3.5	7.1	9.2
20:2	—	—	—	—	—	—	—	.1	—	.5	.1	.7	—	—	.8	1.9
20:3	—	—	—	—	1.0	—	4.5	.2	—	.2	1.3	1.0	—	—	.7	2.1
20:4	7.1	1.2	10.2	18.9	5.6	.4	10.3	7.9	9.8	2.8	9.9	22.6	5.0	1.0	8.0	18.6

* All values are numerical averages of 4 animals/group; † cholesterol ester; ‡ triglyceride; § free fatty acid; in terms of weight percent of total fatty acids in fractions.
‖ phospholipid.

mineral oil. Our results show that in spite of the presence of 4% hydrogenated coconut oil and 1% corn oil (constituting 50% adulteration of the edible oil) there was a significant depression in growth rate, indicating that normal growth was not supported by edible oils adulterated with various mineral oils such as transformer, spindle, oxidized transformer and oxidized spindle oils. As previously noted in rats which had been fed oxidized edible oils(14), the oxidized mineral oils also increased the liver to body weight ratio. This ratio was approximately 8 to 5 in those fed the oxidized and unoxidized mineral oils respectively.

The level of edible fat as well as the presence of oxidized mineral oil was important to the level of liver lipid (Table II). The animals fed a total of 10% corn and hydrogenated coconut oil contained more triglycerides than those fed only 2% corn oil. Such divergent influences of dietary fat has been demonstrated by Blank *et al*(15) who found that the relative amounts of phospholipid decreased markedly and the triglyceride increased in the liver lipids of rats fed corn oil or lard when compared to the lipid in rats on a fat free diet.

The increase in weight of the lipid components in the liver from animals fed oxidized mineral oils was probably a reflection of the increase in liver size. The differences in weight in the phospholipid component seemed to indicate that this lipid component was more responsive to the presence of dietary oxidized mineral oil than the cholesterol, free fatty acids, triglycerides or cholesterol ester components.

The distribution of fatty acids between phospholipids, fatty acid, triglyceride and cholesterol ester fractions (Table III), indicated a well defined pattern which was influenced only slightly by the dietary fatty acids.‖ This is in accordance with the general observations of other investigators(16,17). Data are given only for the liver lipids of animals fed hy-

‖ The fatty acid composition of hydrogenated coconut oil and corn oil expressed as wt. per cent was respectively: decanoic, 1.3, --; lauric, 48.9, --; myristic, 20.0, 0.2; palmitic, 10.7, 13.4; stearic, 12.9, 2.3; oleic, 3.1, 26.4; linoleic, 2.9, 51.2.

drogenated coconut oil, paraffin and oxidized paraffin oil since the data obtained upon feeding the other hydrocarbon oils were similar to those fed paraffin oil. Although lauric acid and myristic acid accounted for nearly 70% of the total dietary fatty acids in hydrogenated coconut oil, neither of these acids were present in appreciable amounts in the lipid classes of liver. These results confirm previous studies(18,19). However, in rats fed corn oil or paraffin oil, the myristic acid content in the cholesterol ester fractions was found to be higher than that of the animals fed hydrogenated coconut oil. Even though corn oil contained only a trace of myristic acid, the increase in myristic acid in the liver lipid was considerable.

Palmitic acid was found in higher concentration in the triglyceride and cholesterol esters than in the phospholipids and free fatty acid from the liver lipids of rats fed the different dietary fats. However, palmitic acid was observed to be the predominant acid in all of the lipid classes from animals fed hydrogenated coconut oil, while this was not the case in the animals in other groups. This is in agreement with the results of Glende and Cornatzer who reported that animals fed hydrogenated vegetable oil appeared to have higher percentages of palmitic acid in all of the liver lipid classes(17).

Palmitoleic acid was concentrated in the cholesterol esters of the liver lipid. The cholesterol ester fractions from rats fed paraffin, transformer, and oxidized paraffin and transformer oils contained 32 to 72% more palmitoleic acid than those fed corn oil. The amount of palmitoleic acid in the free fatty acid and triglyceride fractions was double that in the phospholipids in all groups except in those fed hydrogenated coconut oil. Since none of the test fats contained more than traces of palmitoleic acid, its origin is probably endogenous. Stearic acid was present in large amounts in the phospholipids of all of the liver lipid fractions. An increased level of oleic acid from 25% to 31% in the free fatty acids and 12% to 22% in the triglyceride was observed in animals fed transformer, spindle, and oxidized paraffin, transformer and spindle oils, when compared with the fractions from the rats fed corn oil.

The arachidonic acid content in the phospholipids from animals fed corn oil, various mineral and oxidized mineral oils was significantly greater than in those fed hydrogenated coconut oil. This is in agreement with Glende and Cornatzer(17) who observed that hydrogenated vegetable oil produced a lowering of linoleic and arachidonic acid levels. However, the amount of arachidonic acid was almost evenly distributed in the free fatty acid and cholesterol ester fractions of all the liver lipids, the concentration being slightly higher in the free fatty acid than in the cholesterol ester fraction.

Summary. The effect of paraffin, transformer, spindle and the respective oxidized oils on growth rate and percentage composition of liver lipid in rats was studied for a period of 4 weeks. The results indicated that transformer, spindle, oxidized transformer and oxidized spindle oils significantly depressed growth rate in rats. A decreased percentage in phospholipids and an increased percentage in triglycerides was noted in the liver lipids from these animals. Paraffin oil and oxidized paraffin oils decreased the growth rate per week slightly; there was no change in the percentage of the various lipid classes in the liver in these animals. Increased levels of palmitoleic acid were found in the cholesterol esters fraction of rats fed paraffin, transformer, oxidized paraffin and transformer oils. More oleic acid was detected in free fatty acids and triglycerides of rats fed transformer, spindle, oxidized paraffin, transformer and spindle oils.

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Genetically Determined Differences in the Amylase Activity of Mouse Submandibular Glands.* (32418)

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Lacassagne in 1940 demonstrated the sexual dimorphism of the submandibular glands in mice and other rodents. Since that time numerous investigators have examined the effect of various endocrine manipulations such as castration, thyroidectomy, castration and thyroidectomy and hypophysectomy upon rodent salivary glands. Much of this work has been reviewed by Shafer and Muhler(1). Several reported studies have been concerned specifically with the influence of the sex hormones on the amylase content of rat or mouse submandibular glands(2,3,4). Schneyer (5) has shown that the concentration of amylase in the submandibular-sublingual gland complex varied significantly in males from 4 different strains of mice. She concluded that the enzyme level was genetically determined. Our research supported her findings. Further, we suspected that the influence of sex hormones on salivary gland amylase was not the same for all strains of mice.

The purpose of this study was 2-fold: first, to examine the normal amylase levels in submandibular glands from both sexes of several inbred strains and 2 hybrids; second, to observe the effect of castration and ovariectomy on submandibular gland amylase levels in these strains.

Methods. Adult male and female mice from 10 inbred strains (C3H/HeJ, AKR/J, CBA/J, C57BL/6J, A/WySn, CE/J, DE/J, DBA/2J, BALB/cJ, LP/J) and 2 hybrids ($DED2F_1$ and $D2DEF_1$, $D2CEF_1$ and $CED2F_1$) were obtained from The Jackson Laboratory, Bar Harbor, Maine. All the mice used were between 7 and 9 months of age. At that time 10 to 12 individuals from each sex, strain and hybrid were gonadectomized. Thirty-five days later the animals were killed by cervical dislocation, the submandibular glands removed and dissected free from the closely adhering major sublingual glands, frozen at dry ice temperature, and freeze-dried. The lyophilized glands were used for both dry weight and amylase determinations. It has been shown(4) that freeze-drying the tissues does not change the amylase levels from those of fresh material.

To serve as controls, 10 to 12 intact males and females from each of the strains and hybrids were sacrificed and their submandibular glands removed, weighed and lyophilized. Amylase activities were determined spectrophotometrically using Van Loon's(6)

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