ethyl ester of liquid acids of linseed oil, about 4 mm. deep in basin, open to atmospheric oxygen under 30°. for 50 hours.

2. As shown in Table II, the autoxidized products were fractionated by urea complexes, and the nutritive or retarding effects of each fraction were examined.

3. Fr. 1 separated from the autoxidized products did not give any retarding actions to the rats; while Fr. 2 and Fr. 3 forming urea complexes showed the toxic effects on the animals. From these results it is apparent that aldehydes have no harmful effect upon the rats.

4. On the other hand, polymerized highly unsaturated fatty acids containing a small proportion of peroxide showed the nutritive effects on the rats, but no retarding effect has been discovered.

5. Judging from these results, the toxic effects which were yielded from the autoxidized acids should be attributable mainly to the production of peroxide-structure. In order to prove this assumption, the peroxide of autoxidized products was eliminated by Lea's peroxide determination method. In consequence, peroxide-free products became non-toxic. And the same result was obtained of peroxide-free products of autoxidized highly unsaturated acids.

On the basis of these results, it has come to the conclusion that the most toxic product of autoxidized unsaturated fatty acids is peroxide which has been produced at the biginning of autoxidation.

6. The lethal dose of peroxide oxygen against mice was about 278 mg. of total peroxide oxygen per kg. (LD_{50}).

7. The peroxide was found in liver and muscle fats of rats when the autoxidized unsaturated acids were fed, and if the fat fed to rats contained a large quantity of peroxide, the peroxide value would become higher than that of un-autoxidized acid to rats.

8. The number of mitochondria separated from rats liver was decreased when autoxidized fatty acids were added to the mitochondria solution *in vitro*.

From these findings, it was concluded that the toxicity of peroxide contained in autoxidized unsaturated fatty acids was apparently produced as the result of injuring the tissue of rats.

We hereby highly appreciate the helpful advices given by Dr. Jun'ichi Ozaki, and the constant encouragement by Dr. Hideo Higashi. We also gratefully acknowledge the assistance of Miss Kimie Arai of this laboratory for the feeding of animals.

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rose from 25.6 mg per 100 gm of white cells to 35.2 mg during the first 4 weeks when the intake had reached 57 mg per day, and thereafter remained stationary.

The average serum ascorbic acid level for 13 older women, on the same levels of intake, rose from 0.24 to 1.42 mg during the 10-week period. Their average white cell ascorbic acid rose from 22.2 to 34.9 mg during the first 6 weeks when the intake had reached 72 mg per day, and thereafter remained stationary.

Correlation between serum and white cell ascorbic acid levels was significant only in the young group on intakes of 32 mg and 47 mg of ascorbic acid per day. There was no significant difference in uptake of ascorbic acid by the white cells in the young group as compared with that of the older group. Difference in regression of white cell level on serum level between the two groups was not significant.

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NUTRITIONAL PROPERTIES OF THE MOLECULARLY DISTILLED FRACTIONS OF AUTOXIDIZED FATS[1]

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In previous nutritional work on autoxidized fats, special attention was paid either to the polymer fraction left as a residue after molecular distillation (Kaunitz et al., '55) or to the whole autoxidized fat. It was found that the effects of the polymer fraction or of the whole autoxidized fat could be counteracted by the addition of fresh fat to the diet (Kaunitz et al., '55) and that the caloric requirement of the rat for weight maintenance was increased when such fats were consumed.

The distillate fraction of autoxidized fats, obtained by molecular distillation, had previously been studied only briefly and had not seemed to be particularly remarkable. The further studies to be reported below, however, show that this fraction is also of interest nutritionally.

EXPERIMENTAL

The studies were carried out on albino rats of a homogeneous colony. Weanling males, when they weighed 40 to 50 gm,

[1] Aided by a grant from Schenley Laboratories, Inc., and by a fellowship from Swift and Company.

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were placed on a purified diet containing 30% of lactalbumin and 10% of fresh lard. At the age of 5 weeks, they were distributed into matching groups using procedures reported before (Kaunitz et al., '54).

Commercial lard and refined cottonseed oil were aerated at 95°C. for 200 to 300 hours and then distilled, using alembic distillation for the removal of volatile products, followed by molecular distillation. For the latter, temperatures up to 280°C. were employed. In one instance, a hydrogenated cottonseed oil which had been used for deep fat frying for 80 hours at 190°C. was distilled.

Unless otherwise stated, the experimental diets contained 30% alcohol-extracted casein, 10% fat, 54% dextrose, 4% salts (U.S.P. no. 2), and 2% cellulose, as well as liberal amounts of all known accessory food factors [1] in amounts described before (Kaunitz et al., '54).

RESULTS AND DISCUSSION

In figure 1 are given the average growth curves of groups of 8 male rats which had been maintained on diets containing various fats. The logarithm of the weight in grams is plotted against the reciprocal value of the age; the advantages of this method have been pointed out by Zucker and Zucker ('42).

The animals receiving autoxidized cottonseed oil lost weight rapidly and died after two to 4 weeks. When 10% of fresh fat was added to the diet containing 10% of the oxidized oil, none of the animals died during the period of observation; they were even able to grow. This has previously been described as the protective effect of fresh fat. One group of animals received 10% of the distillate from the molecular distillation of the sample of hydrogenated cottonseed oil which had been used for deep fat frying. These animals grew essentially as well as did those on fresh cottonseed oil. However, when the distillate was combined with oxidized cottonseed oil, growth was significantly below that of the animals receiving both fresh and oxidized cottonseed oils. Also, in contrast to the latter group, some of the animals died toward the end of the period of observation. Therefore, the distillate, while permitting nearly normal growth when included in the diet as the only fat, had lost a high degree of its protective effect.

[1] Doctor Leo A. Pirk of Hoffmann-La Roche, Inc., Nutley, New Jersey, very kindly supplied us with most of the synthetic vitamins used. Vitamin D_3 was supplied by the Sterling-Winthrop Research Institute, Rensselaer, N. Y., and the crystalline beta-carotene, by the Barnett Laboratories, Long Beach, California.

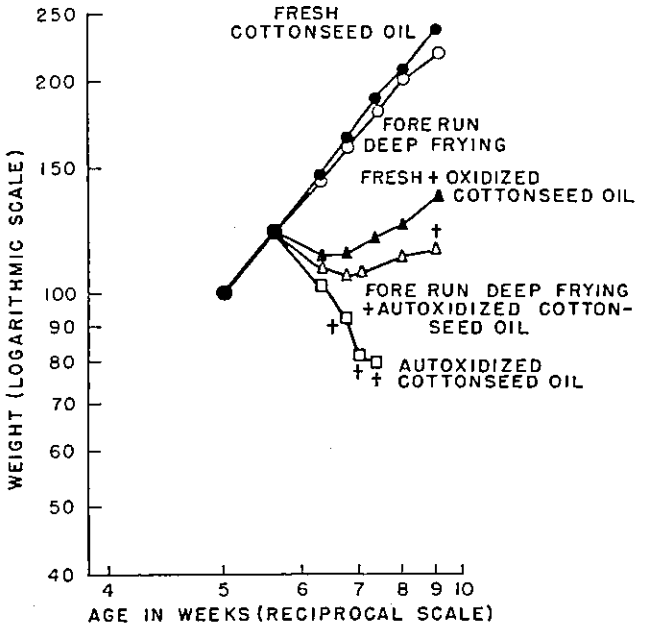

Fig. 1 Influence of the distillate from the molecular distillation of a hydrogenated vegetable oil after its use for deep frying for 80 hours. Each curve is based on the average of 8 well-matched male rats. After the third week of the experiment, the difference in weight of the groups fed oxidized plus fresh fat and oxidized plus distillate is statistically significant.

Six very similar experiments were carried out with the molecular distillation fractions of highly autoxidized cotton-

seed oil or highly autoxidized lard. These distillates usually permitted good, although not quite optimum, growth when used as the sole fat source. Significantly, all of the distillates had lost their protective effect against highly autoxidized cottonseed oil to a degree very similar to that shown in figure 1.

This loss of protective effect could not have been caused by the molecular distillation process. The undistilled autoxidized cottonseed oil containing 40% polymeric "residue" and 60% "distillate" fraction led to rapid deterioration of the animals, whereas a mixture of 40% polymeric residue and 60% fresh oil permitted acceptable growth. Thus, the lack of protective action of the distillate was discernible before the oil had undergone the heating necessary for molecular distillation.

When rats, by daily weighing and restricted feeding, are maintained at a weight constant within 3 gm, it has been observed that the caloric requirements for such weight maintenance decline rapidly within the first few weeks if "good" diets are used (Quimby, '48). It has been shown (Kaunitz et al., '56) that the caloric requirements for weight maintenance do not decrease when the residue fraction of a molecularly distilled autoxidized fat is included in the diet. In figure 2 is shown a similar experiment with fresh fat and the molecular distillate of the hydrogenated vegetable oil which had been used for deep frying. The requirements are expressed as weekly calories per gram of body weight and are the average values for each group of 8 animals. For the calculation of the caloric values of the diets, it was assumed that a factor of 9.2 Cal. per gram could be used for both fats. It seemed reasonable to assume that the caloric value of the distillate did not differ greatly from that of normal fat because, when the distillate was included in a diet as the only fat source and the animals were permitted to eat freely, (1) the resulting growth was only slightly below that of animals fed fresh fat and (2) the food intakes were similar. However, even if

the caloric value of the distillate is slightly below that of the fresh lard, this would not lead to different conclusions.

As can be seen from figure 2, the caloric requirements of both groups declined steeply during the first 4 weeks of observation. The net energy value of the diet containing distillate was lower than that of the diet containing fresh fat, although the difference was not as pronounced as that between the groups fed polymeric residue and fresh fat. However, it

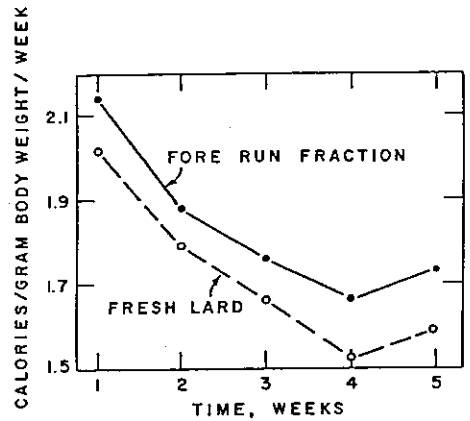

Fig. 2 Influence of fresh lard and the distillate from the molecular distillation of a hydrogenated vegetable oil previously used for deep frying for 80 hours upon the caloric requirements of matching male rats maintained at constant weight. Each curve is based on the average of 8 animals.

may be of some interest that, with a chemically altered but essentially atoxic fat, the animal's caloric requirement for weight maintenance is increased.

When the animals maintained at constant weight were sacrificed at the end of the experiment, their kidneys, livers, and adrenals were weighed. Figure 3 shows log — log plots of organ weights against body weights. The parallel lines give the limits of the spread in organ weight of male rats fed a complete, unrestricted diet. The weights of the livers and

kidneys of the animals on the distillate were within normal limits, although somewhat above those of the animals fed fresh fat. The adrenals of the two groups scarcely differed from one another. In contrast, the livers, kidneys, and adrenals of the animals given the residue fraction substantially

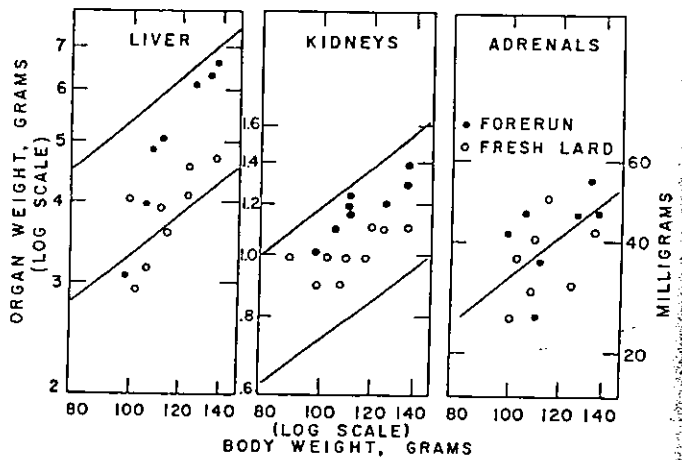

Fig. 3 Influence of fresh lard and distillate from the molecular distillation of a hydrogenated vegetable oil previously used for deep frying for 80 hours upon the organ weight-body weight relationships of male rats kept at constant weight by restricted feeding for 5 weeks. On the log—log plot, the parallel lines indicate the upper and lower limits of the spread in organ weight of rats with unrestricted intakes of a control diet containing fresh lard.

exceeded the upper limit of the normal (Kaunitz et al., '56). These results also show that the distillate itself is hardly toxic.

This low toxicity again became evident in studies with low-protein diets. In earlier work (Kaunitz, '53), it was pointed out that weanling rats placed on diets containing only 5% of casein and fresh fat maintained their weight for several weeks and grew slowly thereafter. Ten per cent of a sample of oxidized lard which was atoxic to rats when included in a diet containing 30% of casein led to rapid weight loss and death when fed in a diet containing only 5% of casein. When 10% of the distillate was included in a diet with 5% of casein, growth of the rats was similar to that of the controls receiving fresh fat.

The chemical changes in the fats responsible for the described effects are not as yet understood. This problem is being actively investigated.

SUMMARY

1. Lard and refined cottonseed oil which had been aerated at 95°C. for 200 to 300 hours and a sample of hydrogenated vegetable oil which had been used commercially for deep fat frying for 80 hours at 190°C. were molecularly distilled at 280°C. The distillates were used in nutritional experiments.

2. When the distillates were included in purified diets containing either 5 or 30% casein, the resulting growth of most of the weanling male rats fed these diets was only slightly below that of matching rats receiving fresh lard.

3. In contrast, distillate added to the nonvolatile polymeric residue from the molecular distillation of autoxidized fats had a protective effect markedly below that of fresh fats.

4. The net energy value of the diet containing distillate was lower than that of the diet containing fresh fat.

5. Liver, kidney and adrenal weights of rats fed distillate were within the normal spread for these organs and were only slightly higher than those of the controls, thereby supplying additional evidence for the low toxicity, if any, of these fractions.

ACKNOWLEDGMENTS

Doctor Waldo C. Ault of the eastern Regional Laboratory of the U. S. Department of Agriculture has greatly helped this work with his advice, suggestions, and criticisms.

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SOME FACTORS AFFECTING CELLULOSE DIGESTION BY RUMEN MICROORGANISMS *IN VITRO*

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A previous report from this laboratory revealed that cellulose digestion by rumen microorganisms *in vitro* was markedly stimulated by such fishery by-products as whale solubles, herring solubles and herring stickwater as well as by a mixture of 18 amino acids (MacLeod and Brumwell, '54). Although much of the effect of the fishery by-products could be ascribed to their amino acid content, some evidence was obtained that other unknown factors might also be present and capable of stimulating cellulose digestion. The present study was undertaken to investigate this possibility further.

In the previous study a marked stimulation of cellulose digestion was obtained with the various supplements tested only when a more dilute inoculum of rumen liquid than it had previously been the custom to use (cf. Burroughs et al., '51) was employed. It was evident that additional information regarding the nutritional requirements of rumen microorganisms could be obtained only if an active inoculum washed as free as posible of rumen liquid was used.

Various compounds or groups of compounds have been reported to stimulate cellulose digestion by rumen microorganisms *in vitro*. These include glucose (Hoflund et al., '48) various water-soluble vitamins, purines and pyrimidines

Table 1

Treatment	No. of subjects	Area of cutaneous histamine wheal (sq. mm.)	Graphic representation of cold vasodilator response (sq. cm.)
Dummy by mouth	4	11·4	37·5
Dummy by mouth	7	10·8	43·8
Iontophoresis of 0·9 per cent saline	3	—	46·9
'Benadryl' (50 mgm.) by mouth	4	8·5	37·3
'Phenergan' (50 mgm.) by mouth	7	3·9	25·6
Iontophoresis of 2·0 per cent 'Pheuergan'	3	—	57·5

time and the results expressed (1) as the mean area enclosed by this curve[2], and (2) as the latent period of the response[3]. A 1/1,000 solution of histamine acid phosphate was placed on the skin of the forearm of the same subjects and a needle-prick was made through the solution. The mean area of the wheal thus produced was measured immediately before each cold immersion.

The results are given in Table 1. 50 mgm. of diphenhydramine hydrochloride, by mouth, reduced the size of the cutaneous histamine wheal ($t = 2{\cdot}21$, $p < 0{\cdot}20 > 0{\cdot}10$) but had no effect on the cold-vasodilator response. 50 mgm. of promethazine hydrochloride given by mouth caused a highly significant reduction both of the histamine wheal ($t = 11{\cdot}473$, $p < 0{\cdot}001$) and of the cold vasodilator response ($t = 3{\cdot}831$, $p < 0{\cdot}01 > 0{\cdot}001$) and it also significantly delayed the onset of the vasodilator response ($t = 3{\cdot}628$, $p < 0{\cdot}01 > 0{\cdot}001$). There was a reflex vasodilator reaction in the index finger of the opposite hand, and this response was also significantly delayed by the oral administration of 50 mgm. of promethazine hydrochloride ($t = 3{\cdot}357$, $p < 0{\cdot}02 > 0{\cdot}01$). When introduced into the finger-tip by iontophoresis, promethazine hydrochloride increased the magnitude of the cold vasodilatation, a result compatible with the local anaesthetic action of promethazine hydrochloride[4,5]. In ten untreated subjects a negative correlation was found between the size of the cutaneous histamine wheal and the magnitude of the cold vasodilatation ($r = 0{\cdot}705$, $p < 0{\cdot}05 > 0{\cdot}02$).

Since systemic administration of promethazine hydrochloride causes central depression[6,7], and since local administration of promethazine hydrochloride did not inhibit the cold vasodilator response, it seems possible that inhibition of the response by the oral administration of 50 mgm. of this drug was due to a central depressant action. These results provide, therefore, no definite evidence for the participation of histamine in the cold vasodilator-response, and this conforms with observations by Duff *et al.*[8].

Experiments are now in progress to investigate the effect of drugs with a central depressant action on the cold vasodilatation in the finger.

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Effect of Vagal Stimulation on the Sinus Venosus of the Frog's Heart

The effect of vagal stimulation or applied acetylcholine on electrically driven heart auricle fibres has been studied with intracellular electrodes by several workers[1]. The purpose of the present experiments was to gain some information about the events during vagal inhibition in spontaneously active pacemaker fibres.

The sinus venosus of a frog's heart was excised together with the two vago-sympathetic nerves, cut open and pinned out. When an electrode was inserted into the region where the beat could be observed to start, a typical pacemaker potential[2] was seen. The membrane potential decreases slowly throughout diastole until the threshold for an action potential is reached. In fibres that showed constant pictures for several beats, the most negative value of the membrane potential during the cycle ranged from 45 to 60 mV.

If the vagi are stimulated briefly during the falling phase of an action potential, the repolarization proceeds to above the most negative point reached in previous cycles, and the next beat is delayed because the slope of the pacemaker potential is reduced (Fig. 1*A*, left). With more prolonged stimulation a slow 'hyperpolarization' suppresses the next pacemaker potential and stops the heart (Fig. 1*A*, right). On removing the vagal stimulus a slow depolarization develops again. In the experiment illustrated, this depolarization was not allowed to reach the threshold; instead, a second train of vagal impulses was sent into the preparation, causing a renewed hyperpolarization. After this procedure was repeated once more, an action potential of shortened duration arose from a now apparently reduced threshold. The maximum increase of the membrane potential, as measured from the foot of the last pacemaker potential, was 8 mV. in this experiment.

In twelve other experiments, the inhibitory hyperpolarization measured 3–13 mV. (mean 8 mV.). On several other sinus fibres, however, vagal stimulation merely depressed the pacemaker potential (Fig. 1*B*). This suggests that the transition from a typical pacemaker to a typical auricle fibre is gradual. It is noteworthy that the first two action potentials after the vagal arrest are not only shortened, but also slightly decreased in amplitude. This reduction of the 'overshoot' amplitude was usually observed, and was particularly striking on occasions when impulses reached the inhibited fibre from an accidentally denervated and still beating part of the sinus.

The events underlying the increase in beat frequency on sympathetic stimulation were studied in a few atropinized preparations. As might be expected, the pacemaker potential increased in steepness after a latent period of several seconds (Fig. 1*C*). The

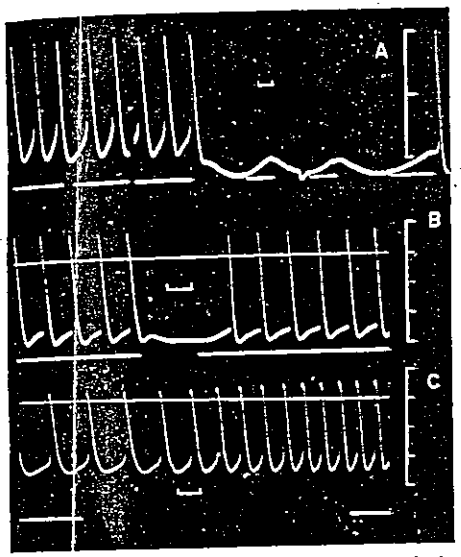

Fig. 1. Intracellular records from the sinus venosus of a frog's heart. Vagal stimulation indicated by breaks in the lower beam. (*A*) Vagi stimulated at 10 per sec. during the repolarization of the third action potential. During the eighth action potential, vagal stimulation starts again (see text). Gap after fifth action potential denotes interval of several seconds. (*B*) Depression of pacemaker potential without hyperpolarization. This preparation was treated with ergotamine, 1 in 10^6. (*C*) Effect of vago-sympathetic stimulation in an atropinized preparation.
Voltage calibration in 20 mV. steps; in (*B*) and (*C*) a line is drawn through zero potential; in (*A*) calibration from −10 mV. downwards. Time: 1 sec.

'overshoot' was increased from 5·5 mV. to 11 mV. on sympathetic stimulation.

These results confirm the results of Gaskell[3], who found that the demarcation potential in turtle auricle was increased during vagal inhibition. They also call to mind the experiments of Howell and Duke[4], and of Lehnartz[5], who showed that during vagal arrest a heart liberated potassium ions; for an outflow of potassium ions could contribute to the inhibitory hyperpolarization. An increase in permeability to potassium, relative to that of sodium, could also account for a decrease in the 'overshoot' and for the faster repolarization of the action potential.

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Nutritive Value of Heated Vegetable Oils

Much of the fat consumed by human beings, particularly in India, has been heated, and the conditions of heating vary widely and also with the nature of the foodstuff into which the oil or fat is incorporated. It is therefore necessary to have scientific data concerning the nutritive value of heated oils and fat.

Heated oils have been shown to be poorly absorbed[1], to produce cancerous tumours[2] and to cause paralysis resembling that due to vitamin E deficiency[3]. Thermal polymerization was found to develop toxic products responsible for growth depression[4]. Kaunitz[5] stated that oxidized fats destroyed vitamins in the diets and thus caused retardation of growth. Chalmers[6] found that injections of heat-polymerized cotton-seed oil did not lead to the growth of tumours.

In most of the above investigations, the oils were heated in a current of carbon dioxide or nitrogen. In India, oils are heated in open pans for long periods and, further, the residual oil from a day's operations is supplemented with fresh oil and re-heated again. This may accelerate the oxidative and other changes. The work reported in this communication was undertaken to elucidate the nutritive value of some of the commonly used oils when they were fed after heating.

Groundnut, sesame and coconut oils were heated in an open iron pan at 270° C. for 8 hr. They were then incorporated into synthetic diets to give 15 per cent fat, and fed to albino rats. There were six groups of six rats each, five weeks old and weighing 40–50 gm. The two groups of rats allotted to any particular oil (unheated and heated) were litter mates and were distributed with due consideration to weight and sex. The diet employed had the following composition: fat, 15 per cent; casein, 12 per cent; sugar, 10 per cent; salt mixture, 4 per cent; and starch, 59 per cent; and the vitamin supplements were: thiamine, 15; riboflavin, 60; pyridoxine, 10; niacin, 10; calcium pantothenate, 50 (all in mgm. per kgm. of the diet); and choline, 1 gm. Vitamins A and D were given in the form of two drops of 'Adexoline' twice a week per rat. The rats were housed in independent cages. The vitamin supplements were added to the diet every day, so as to prevent their destruction by the heated oils. Weekly growth records were maintained. At the end of the sixth week, four rats from each group were opened under chloroform, and liver, spleen, stomach and kidney were removed and weighed. The liver fat was also estimated. The observations are presented in Table 1.

Table 1. Influence of Heating the Oil on its Nutritive Value and Fat Deposition in the Liver

	Average gain per rat per week (gm.)	Feed efficiency	Liver weight as percentage of body weight	Percentage fat in liver
Groundnut oil:				
Unheated	13·0 ± 0·5	1·1	3·8 ± 0·3	3·9 ± 0·3
Heated	5·0 ± 0·9	0·07	5·7 ± 0·6	7·1 ± 0·4
Sesame oil:				
Unheated	10·0 ± 0·8	1·0	4·2 ± 0·7	4·8 ± 0·3
Heated	4·3 ± 0·7	0·7	6·0 ± 0·5	7·8 ± 0·3
Coconut oil:				
Unheated	11·0 ± 1·1	1·1	4·3 ± 0·3	4·5 ± 0·3
Heated	4·5 ± 0·7	0·7	5·8 ± 0·4	7·0 ± 0·3

The results show clearly that in all three cases the heated-oil has adversely affected the gain in weight. The feed efficiency, that is, the increase in weight per gm. of fat consumed, of the heated-oil group of rats is considerably reduced. There were no significant changes in the weights of stomach, kidney and spleen of the two groups of animals. These results are not, therefore, presented here.

It may be seen that the liver weights (fresh) of the heated-oil groups are significantly higher than the controls. The fat content of the livers of the heated-oil group is nearly twice that of the control groups. Heated oil has produced fatty infiltration of the liver. Further, the livers were badly damaged and congested, and were not of the normal colour.

When the heated oil was fed at 30 per cent level, all the rats died within a week, whereas the control rats were quite healthy. In another set of trials, when 15 per cent heated oil (heated at 280° C.) was used in the diet, severe jaundice occurred and four of the six rats died in the sixth week of the experiment. The exact changes brought about by heating the oil and also the other aspects of the problem are being studied.

Our thanks are due to Prof. K. V. Giri for his interest in the investigation.

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Cessation of Tillering in Young Grass Plants

During the early seedling growth of *Lolium perenne* plants in pots, Cooper[1] noted that the number of tillers per plant increased exponentially with time until some date 60–100 days after sowing, when the initial exponential growth was sharply curtailed and tiller production ceased. This phenomenon has been observed in experiments in which the chief concern was with growth as measured by increase in weight. The results obtained in a field experiment where the plants were growing in a sward are summarized in Fig. 1. In this experiment thirty plants of each of twenty-two strains representing seventeen grass species were dug up twice a week, washed free of soil, the tillers counted and then dried and weighed. Each point in the figure is derived from the measurement of 660 plants. In the majority of strains tiller production ceased about the seventieth day after sowing. No sudden change occurred in the rate of increase of the plants (roots plus shoots) or in the rate of increase of the weight of shoot per tiller, that is, the weight of a tiller. Other details of the experiment have been published elsewhere[2].

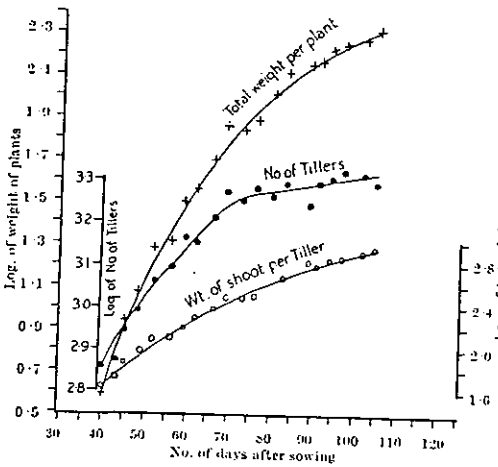

Fig. 1

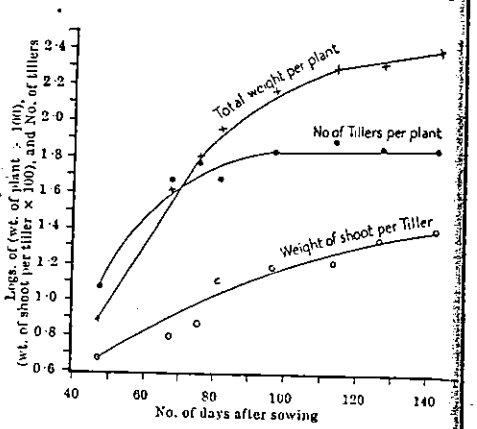

Fig. 2

In another experiment, plants of *Lolium perenne* S.24 were grown in whalehide pots of 6,700 ml. capacity. An equal number of pots containing one, two or three plants were taken at intervals, the plants washed free from soil and then treated as in the other experiment. Usually thirty pots were dealt with on each date. The results are presented in Fig. 2 as the mean of the plants measured on a particular date. Tiller production ceased about the hundredth day after sowing. As in the field experiment, there was no cessation of dry-weight increase comparable to the cessation of tiller production, although a gradual reduction in this rate did occur. The weight of shoot per tiller increased at the same rate throughout the period of the experiment.

This state of inanition of tillering would appear to affect chiefly the meristematic tissues concerned with the production of new tillers; the more mature tissues are unaffected and continued to grow, that is, to gain in weight without any sudden change in the rate of increase for some considerable time after the cessation of tiller production. This is in accordance with Cooper's[3] findings that rate of leaf production on the main tiller was unaffected by cessation of tillering.

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Temporary Destocking of Pastures to aid Control of the Cattle Tick

The tick *Boophilus microplus* Can., is generally considered to be the most serious single cause of loss to the cattle industry in tropical Australia, contributing to the death of many cattle in dry years. Present control measures are costly, and by placing undue reliance on frequent dipping are thought to have increased the tendency of ticks to become resistant to BHC and arsenic[1]. A method involving only two or three dippings per year can be expected to reduce the difficulties of controlling strains of ticks resistant to these and newer acaricides. The results of the following 'pilot-scale' experiment carried out near Rockhampton, Queensland, emphasize the possibility of reducing the frequency of acaricidal treatment by destocking the pastures for periods which some cattle owners do not find inconveniently long.

Two herds of cattle were kept in adjoining comparable paddocks of approximately seventy-five acres. An equal number (10–14) of animals was present in each of the two herds at any one time. The 'control' herd was allowed access to the whole area of paddock *C* continuously, whereas the experimental herd was alternated between the two halves, *A* and *B*, of the other paddock, which was divided by an electric fence. The periods for which the *A* or *B* paddocks were destocked were based, after mid-November 1953, on observations made in the district on the time taken from the date of drop of a replete female tick to the death of its larval progeny (Wilkinson, unpublished data). Fig. 1 shows infestations of adult ticks, exceeding 0·5 cm. in length, on the two herds, the numbers with arrows indicating occasions on which the animals were sprayed with DDT preparation (1 per cent *p,p'*-isomer). It will be seen that, after the beginning of February 1954, infestations of the cattle moved to previously destocked pastures remained low. A few ticks continued to occur on the cattle, and this was considered a desirable feature, because the cattle would be regularly re-infested with *Babesia* carried by the larvæ and thus retain their immunity to 'redwater' fever[2].

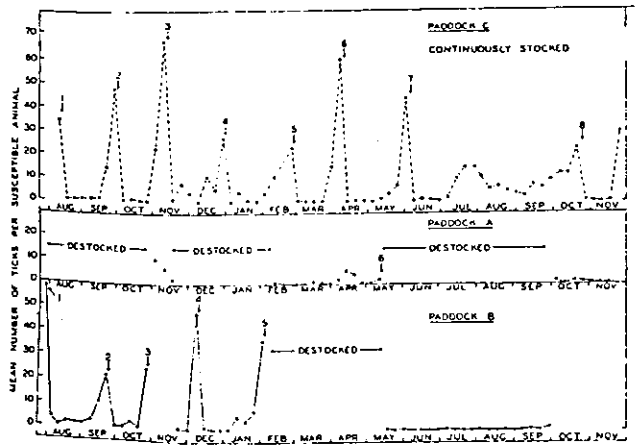

Fig. 1. Comparison of infestations of cattle on a continuously stocked and a pasture spelling paddock. Counts of female ticks, 0·5 cm. and upwards in length, on the right side of the cattle

Destocking of pastures has been practised frequently during tick eradication campaigns, but no experiment on destocking as a control measure has hitherto been described. A fuller account of this work and its application to Queensland conditions will be published elsewhere.

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A New Acaricide, 2,4,5,4'-Tetrachlorodiphenylsulphone

Läuger *et al.*[1], Swingle[2] and Browning *et al.*[3] have stated that the insecticidal value of *p,p'*-dichlorodiphenylsulphone is relatively low; Läuger *et al.*[1] state that diphenylsulphone and monochlorodiphenylsulphone are devoid of any insecticidal activity. On the other hand, Eaton[4] found among various diphenyl compounds some substances with a sulphone link active against spider mites and their eggs; optimal acaricidal activity occurred with mono-*p*-chlorodiphenylsulphone[5].

Diphenylsulphone and mono-*p*-chlorodiphenylsulphone are more or less phytocidal at active concentrations. We have now found that 2,4,5-trichlorodiphenylsulphone ('*V* 17') and 2,4,5,4'-tetrachlorodiphenylsulphone ('Tedion *V* 18') possess strong acaricidal properties without phytocidal side-effects. As can be seen from Tables 1 and 2, the latter substance is more active on the eggs than any of the other compounds listed. As is the case with some newer specific acaricides now in use, such as *CPBS* (*p*-chlorophenyl-benzenesulphonate), *CPCBS* (*p*-chlorophenyl-*p*-chlorobenzenesulphonate) and *p*-chlorophenyl-*p*-chlorobenzylsulphide, 'Tedion *V* 18' also shows activity on all stages except adult spider mites.

The various compounds were tested on *Tetranychus urticae* Koch on bean plants (*Phaseolus*). Potted bean plants with or without eggs were dipped in emulsions containing the active material. After the plants without eggs had dried, these were infected with adult females in order to obtain eggs on the dry residue. After two or three days the adults were removed. In order to evaluate the penetration of 'Tedion' into the leaf, leaves were treated on the upper side and infected with mites on the under side. Solutions and emulsions containing 1,000 and 100 p.p.m. of active material caused in these circumstances a total kill of eggs and larvæ.

The phytotoxic properties of 'Tedion *V* 18' were investigated on seedlings of *Tropaeolum majus*, cucumber, tomato, potato, broad bean (*Vicia faba*), French bean (*Phaseolus vulgaris*) and garden pea (*Pisum sativum*). The plants were thoroughly sprayed with 0·3, 1·0 and 3·0 per cent of active material,

The authors wish to acknowledge that such a heating bath was suggested by the late Alton E. Bailey, and his counsel was most helpful throughout the early part of the development. Comments and suggestions from members of the current AOM Stability Subcommittee of the American Oil Chemists' Society are likewise gratefully acknowledged.

Summary

A constant temperature bath of a type not heretofore reported has been developed. It eliminates the objectionable features of previous apparatus and provides precise control of the very critical temperature factor in AOM stability measurement. The apparatus is easily constructed from readily available materials; it operates reliably, safely and without attention for extended periods of time. Certain matters of technique are discussed, such as submersion of the sample below the top of the bath.

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Biological Effects of the Polymeric Residues Isolated from Autoxidized Fats[1,2]

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FOR A LONG TIME it has been recognized that highly autoxidized (rancid) fats are not the nutritional equivalents of fresh, wholesome fats, but the reason for the difference has not been explicitly stated. This loss has been variously attributed to peroxides or oxygenated products, but in our earlier studies (8) no signs of toxicity resulted from the feeding of adequate diets containing 5% of methyl monohydroxystearate, methyl 9,10-dihydroxystearate, methyl *cis*-epoxystearate, or a methyl oleate peroxide concentrate (85% peroxide). These results suggest that simple oxidized substances are not the principal agents responsible for the growth-depressing effects. It has been assumed that the peroxides destroy the vitamins in the fats, but this can be eliminated as a possible cause by separate administration of an adequate supply of vitamins.

The possibility that the nutritional effect is caused by the destruction of essential fatty acids is not tenable because, as we shall show later, the effect is extremely dramatic when the actual growth-depressing ingredients are fed at a high level to animals which have been receiving a nutritionally adequate diet and continue to receive essential fatty acids.

Information which may eventually be of value in clarifying our present-day ideas concerning autoxidized fats developed from two main sources. In studies of the chemistry of drying oils used for paints and varnishes it was slowly recognized that fats are capable of undergoing autoxidatively produced polymerization. At the same time knowledge was accumulating concerning the biological properties of heated fats (1). Both lines of investigation gradually led to the recognition of the importance of fat polymers and to the need for a distinction between polymerization brought about by heat alone and by autoxidation. It is not yet possible however to distinguish clearly between the bio-responses brought on by the two kinds of polymers because it is difficult to say whether the polymers are the only cause of these effects. Nutritional studies in which concentrates of autoxidatively produced polymers were used have not yet been reported. In any discussion of autoxidized fats however the effects caused by oxygen and by heat often overlap.

As an indication of the lack of agreement concerning the nutritional effects of polymers derived from fats, some workers (1a, 10, 11, 12) have stressed the undesirable nutritional properties of partly polymerized fats while others (3, 4, 13, 15) have deliberately polymerized certain oils to stabilize them for human consumption and reported no ill effects. These differences may result, in part, from the fact that the latter group examined partly polymerized fish oils and the others used fats of different origin.

Earlier (19) we had shown that molecular distillation was useful to isolate autoxidatively produced polymers from autoxidized methyl oleate. It was decided therefore to apply this technique to various autoxidized fats with the hope of removing unoxidized and oxidized monomeric triglycerides (molecular weight 1,000 or less) as the distillate fractions, leaving the autoxidatively produced polymers, if any, as the non-volatile residue. This separation technique was found to be satisfactory. It permitted the isolation of polymeric residues from drastically autoxidized lard, drastically autoxidized cottonseed oil, and a hydrogenated fat which had been used in commercial deep-fat frying.

Present Study

Lard aerated at 95°C. for about 200 hrs. and then molecularly distilled yielded an amber-colored polymeric residue which usually amounted to about 17% of the original fat. Cottonseed oil, similarly treated, yielded about 40% of the polymer. It should be noted

[1] This paper is XXI in the series "Reactions of Fatty Materials with Oxygen." Paper XX is reference 18.
[2] Presented at the Fall meeting of the American Oil Chemists' Society, Philadelphia, Pa., October 10–12, 1955.
[3] A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

that these yields are roughly proportional to the content of polyunsaturated acids in each of the fats. A hydrogenated shortening which had been used for 80 hrs. at 190°C. in the commercial manufacture of French-fried potatoes yielded 7% of polymeric residue.

Our nutritional studies in this field were carried out on albino rats from a colony which had been used for experiments of a similar type for 15 years. We have followed the procedures previously described for setting up well-matched groups and for paired and restricted feeding (6, 7). The basic diet consisted of 30% casein (G.B.I. vitamin-free test casein), 4% salts (U.S.P. No. 2), 2% cellulose (Alphacel), 10–20% fat, and 54–44% dextrose (Cerelose). To this were added liberal amounts of all known vitamins. When highly rancid fats were part of the diet, vitamin suspensions were fed by dropper. Our techniques permit the assumption that eventual differences among the groups of one experimental series are caused by the factors purposely varied. The conclusions arrived at were drawn only if the differences described were statistically significant. To be absolutely certain however, most of the experiments were repeated several times. This work has been carried out during the last three years, and about 3,000 rats have been used.

Results and Discussion

In Figure 1 are shown the results of experiments in which weanling rats were fed diets containing 10% of either fresh lard, the molecular-distillate fractions from autoxidized lard, or the autoxidatively produced polymeric residue. A weight deficit resulting from the feeding of any of the fractions was an expression of its growth-depressing characteristics.

As Figure 1 shows, the volatile fraction obtained by alembic distillation to degas the autoxidized fat was highly toxic when fed to two rats. Since it constituted only 1% of the total autoxidized fat, there was an insufficient quantity for the protracted feeding of additional animals. This fraction, known to consist largely of aldehydes, was not studied further.

Three fractions (M-I, M-II, M-III), obtained by molecular distillation up to a temperature of 280°C., produced no significant weight deficit. A fourth fraction (M-IV), obtained from 280–300°, exerted a mild growth-depressant effect. The non-volatile residue however was significantly active, as shown by the large weight deficit. These results have been confirmed several times.

When 10% of the polymer from autoxidized cottonseed oil was included in the diet, only about half of the rats were alive after four weeks; at the 20% level all of the rats died within one week. Initially, all rats developed diarrhea, a phenomenon also observed in rats fed unfractionated, autoxidized cottonseed oil (containing 40% polymer). When the diarrhea was at its height, all tissues around the anus were highly irritated. The penis was often swollen and deformed, which might have caused some deaths by interfering with urination. Histological examinations of most of the organs of six animals maintained on residues from autoxidized lard and from autoxidized cottonseed oil for four weeks showed no abnormalities except for differences in organ size, as discussed later in this paper. The rats merely showed signs of starvation.

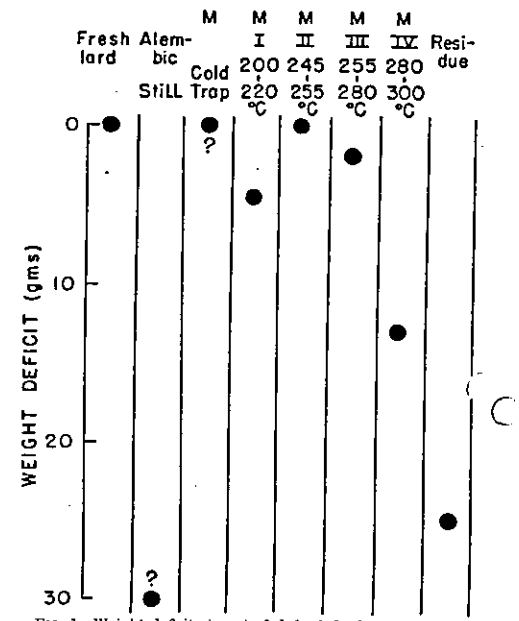

FIG. 1. Weight deficits in rats fed fresh lard, molecular distillates from autoxidized lard (M-I, II, III, IV), and polymeric residue from autoxidized lard.

In our original feeding studies on unfractionated, autoxidized lard and autoxidized cottonseed oil, we assumed that the greater activity of autoxidized cottonseed oil was attributable to the presence of about 2½ times as much polymer as was present in autoxidized lard (8). Further studies however strongly suggest that the polymer from autoxidized cottonseed oil has a greater growth-depressant effect than that from autoxidized lard. This is shown graphically in Figure 2, in which the average growth of rats fed fresh lard or three polymeric residues at a 10% level are compared. In this and subsequent figures giving rat growth, we have followed the procedure of Zucker and Zucker (20) in plotting the logarithm of the rat's weight against the reciprocal value of the age. This results in a straight line for the growth of normal rats, as is shown in the graph for fresh lard. With the residue from autoxidized cottonseed oil the rats lost weight rapidly, and half of them died within four weeks. With the corresponding residue from autoxidized lard the rats were just able to maintain their weight and eventually grow a little, although a significant weight deficit compared to animals fed fresh lard was again noted. All the animals were alive after four weeks on the diet containing polymer from autoxidized lard.

Figure 2 also shows the growth of rats fed a polymer isolated from the hydrogenated shortening used in making French-fried potatoes. Compared to fresh lard, the growth of rats was retarded, but the effect was considerably less than that caused by the other two polymeric residues.

As noted earlier, inclusion in the diet of 20% of

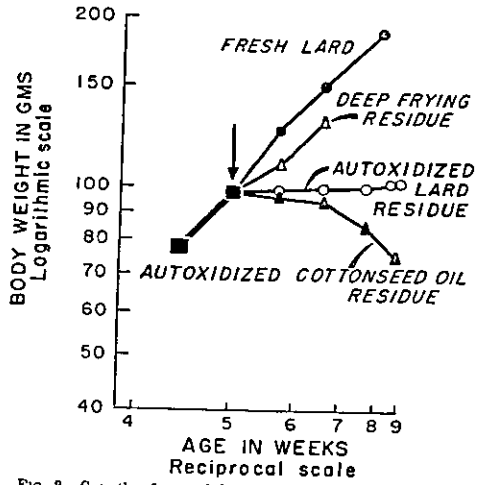

FIG. 2. Growth of rats fed fresh lard and polymeric residues from three autoxidized fats at a 10% level in the diet.

polymer from the autoxidized cottonseed oil led to the rapid death of all the rats. Addition of fresh fat to this diet however exerted a protective effect. A similar protective effect was observed when fresh fat was added to a diet containing autoxidized, unfractionated fat. Thus, with 15% of autoxidized cottonseed oil in the diet, about half of the rats died within three weeks, and all died within six weeks. When the diet also contained 15% of fresh cottonseed oil, all rats were alive after three months. The circumstance that the growth-depressant properties of one fat can be counteracted by the addition of a second fat to the diet has also been noted by Moore and co-workers (14).

Figure 3 compares the growth of rats fed 15% fresh cottonseed oil with those receiving 15% autoxidized cottonseed oil and those receiving 15% of autoxidized cottonsed oil plus 10% fresh cottonseed oil. Rats receiving the autoxidized oil plus fresh oil grew, although not as well as those receiving only fresh fat. Rats fed autoxidized cottonseed oil showed substantially no growth.

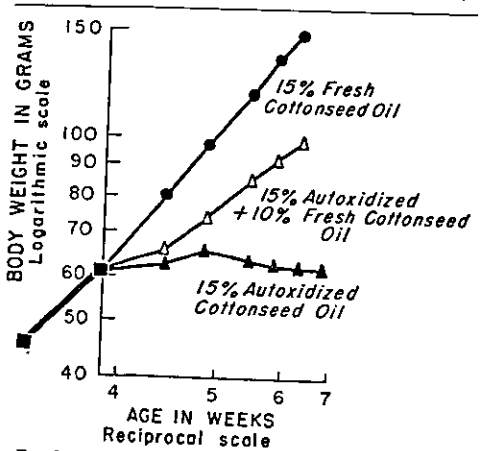

FIG. 3. Growth of rats fed 15% fresh cottonseed oil, 15% autoxidized cottonseed oil, 15% autoxidized cottonseed oil + 10% fresh cottonseed oil.

The results in Figure 3 are all the more remarkable because the food intake of the rats on autoxidized oil was roughly half that of normal rats whereas that of rats eating both fresh and autoxidized oils was nearly normal. Therefore the last-named group was consuming more autoxidized oil than the rats receiving only autoxidized oil. Nevertheless, for some unknown reason, the fresh oil protected the animals. Tocopherol in oral doses of 30 mg. per week exerted hardly any protective effect.

Noticeable changes were also observed in the organ-weight/body-weight relationship, which is shown in Figure 4. Normal organ-weight data were obtained by examining 130 male rats varying in body weight from 18 to 450 g. The limits of the spread in normal organ-weight formed parallel lines on a log-log scale. These data are in line with previous studies (17). For the sake of clarity the lines are given in Figure 4 instead of the individual points. The individual organ-weights of rats fed 15% autoxidized cottonseed oil are indicated by closed circles; those of rats fed 15% autoxidized plus 10% fresh cottonseed oil, by open circles.

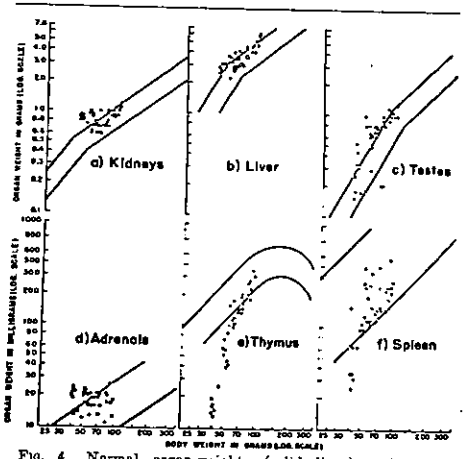

FIG. 4. Normal organ-weights (solid lines) and organ weights of rats fed 15% autoxidized cottonseed oil (closed circles), and 15% autoxidized cottonseed oil + 10% fresh cottonseed oil (open circles).

It is evident that nearly all kidneys, livers, and adrenals from animals on autoxidized oil were above the upper weight limit for normal organs while those from animals receiving autoxidized plus fresh oil were within normal limits. On the other hand, the lymphocytic organs, such as thymus and spleen, were reduced in size after the intake of autoxidized oil. This condition was also improved by fresh oil.

We have also been interested in investigating the caloric requirement for weight maintenance in rats under widely different experimental conditions. Figure 5 shows the caloric requirements of rats fed just enough food to maintain their weight. Under normal circumstances, continued growth requires an increasing caloric intake. The ordinate of Figure 5 indicates the weekly caloric requirement for the maintenance of one gram of body weight; the abscissa gives the time in weeks after the beginning of food restriction.

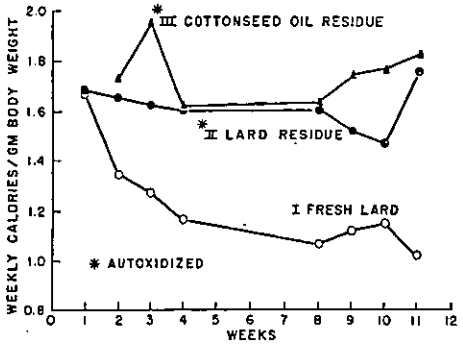

FIG. 5. Average caloric values for weight maintenance of rats fed 10% fresh lard (I), 10% autoxidized lard residue (II), or 10% autoxidized cottonseed oil residue (III).

Curve I, Figure 5, gives the average caloric values for weight maintenance of eight rats on a diet containing 10% fresh lard over a period of nearly three months. The rats were gradually able to get along on less and less food as the experiment progressed, a condition which parallels that in humans. The adaptation to reduced food intake did not occur when the rats were fed 10% of residue from autoxidized lard (Curve II) or residue from autoxidized cottonseed oil (Curve III). Eventually the caloric requirement of the rats fed autoxidized fat residues was more than 50% higher than that of rats given 10% of fresh lard. This tendency toward an increased caloric requirement was also observed when the rats were given diets containing 4 to 7% of the residue fraction.

The mechanism of the effect of polymeric residues on weight maintenance requirements is not clear. One possibility is that the increased caloric requirements are a reflection of feed losses due to diarrhea. The effect however was still found in animals fed 10% of the polymeric residues after the diarrhea had subsided as well as in those receiving 4 or 7% of the residue, which had never had diarrhea.

The fecal output of animals on polymeric residues was increased, perhaps partly because of reduced intestinal absorption, but it is doubtful that this explains the changes sufficiently. Quantitative analysis of the feces, now in progress, may be helpful.

Qualitatively, the changes produced by highly autoxidized (but not molecularly distilled) cottonseed oil are similar to those brought on by the polymeric residues isolated from either autoxidized cottonseed oil or autoxidized lard. There are several facts however which cannot as yet be explained by the assumption that the biological effects of autoxidized fats are produced by the polymeric residues. One of these is that, on a weight-for-weight basis, the growth-depressant effect of residue from the autoxidized cottonseed oil is only slightly greater than that of the undistilled autoxidized oil although the latter contains only 40% polymer. Also, when 15% of the autoxidized cottonseed oil, equivalent to only 6% of the polymeric residue, was included in a diet, all rats were dead at a time when those on a diet containing 10% polymeric residue were still alive. The fact that the activity of the autoxidized, but undistilled, fat is relatively more pronounced than that of the polymeric residue clearly demonstrates that the biological effects are not caused by substances formed in the process of molecular distillation.

Furthermore the volatile fractions obtained by molecular distillation of autoxidized fats, which had little or no growth-depressant properties when fed to rats with an otherwise normal diet, were hardly protective in diets also containing polymeric residues in contrast to fresh fats. A possible explanation for the absence of the protective effect in molecularly distilled fractions from autoxidized fats is their content of *trans* and/or conjugated unsaturated triglycerides. This point is now being investigated.

Some evidence is accumulating that the distribution of the rat's body fat changes under the influence of highly autoxidized fats or their polymeric fractions. Over-all, the emaciated animals lost a great deal of their depot fat, but the liver lipids seemed to increase (9). The composition of the liver lipids in rats after the intake of polymers obtained from autoxidized fat is presently being studied.

When highly autoxidized fats are consumed by rats fed diets deficient in some essential nutrient, such as protein or a vitamin, the deficiency state becomes evident more quickly than when the diet contains fresh fat. Thus it has been reported (11) that the acrodynia in pyridoxine-deficient animals is increased by autoxidized fats. It seems unlikely however that this effect is a specific one because the pyridoxal phosphate content of the liver of the animals fed polymeric residues in this study is normal. Deficiencies in vitamin K (12), vitamin A (16), and riboflavin (6) as well as the effect of cortisone (2) are intensified by autoxidized fat.

In studies with highly autoxidized lard only little effect on the growth of rats was noted when the diet contained 30% casein. With 5% casein animals fed the autoxidized fat lost weight and soon died whereas rats fed fresh fat were able to maintain their weight and eventually grow slowly (5). It may be of great biological importance that some products of autoxidation, which in the presence of an adequate diet produce scarcely any adverse biological effects, may damage the animal severely when fed in a marginal diet.

Finally studies were conducted to determine whether the intake of polymeric residues produced any permanent lesions in rats which had received these fats for several months and were then returned to a normal diet. Figure 6 shows the results of such a study. The broken line represents the growth of controls and the solid line that of the experimental animals. At the age of 33 days (Arrow 1) rats were placed on a diet containing 10% autoxidized cottonseed oil residue and maintained on such a diet for six weeks. As anticipated, no growth was observed during this period. Thereafter (Arrow 2) the rats were replaced on a normal diet. Realimentation occurred rapidly, and the rats finally reached the

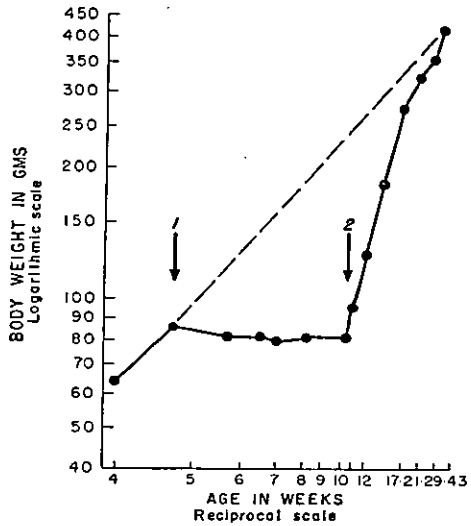

FIG. 6. Growth of rats fed a normal diet to arrow 1, 10% autoxidized cottonseed oil residue from arrow 1 to arrow 2, then returned to normal diet at arrow 2.

same weight as the controls. More than 50 rats which had previously received polymeric residues for several weeks to several months and had then been returned to normal diets have been studied for nearly one year, and in all cases immediate realimentation occurred without signs of subsequent injury from the intake of autoxidatively produced polymer.

Acknowledgments

We are indebted to Waldo C. Ault for his unceasing interest in this work. We also wish to thank Herbert Stoerk of the Merck Institute for Therapeutic Research, Rahway, N. J., for the histological examinations and George Boxer of the same Institute for pyridoxal phosphate determinations. Leo A. Pirk of Hoffman-La Roche Inc., Nutley, N. J., provided us with most of the synthetic vitamins used in the experiments. We are indebted to the Sterling-Winthrop Research Institute, Rensselaer, N. Y., for crystalline vitamin D_2, to the Barnett Laboratories, Long Beach, Calif., for crystalline beta-carotene, and to Merck and Company, Rahway, N. J., for vitamin B_{12}.

Summary

There is increasing evidence that the abnormal nutritional properties of highly autoxidized fats are related to the polymers which develop during autoxidation. Lard and cottonseed oil were aerated at 95°C. for 200 hrs. and molecularly distilled; and the residue fractions, non-volatile at 275 to 300°C., were studied.

Diets containing 20% of autoxidatively produced polymeric residue, fed to albino rats, led to diarrhea and rapid death, but when this residue was reduced to 10%, most of the animals were gradually able to tolerate it. At the 4 or 7% level it was well tolerated, but growth was reduced. There were no distinctive histological lesions, and withdrawal of the polymer permitted immediate realimentation without evidence of subsequent injuries.

The polymeric residue from autoxidized cottonseed oil exerted a greater growth-depressant effect than that from lard, and the latter, more than that from a hydrogenated vegetable oil used for deep-fat frying for 80 hrs. at 190°C. Addition of fresh fat to the polymeric residues decreased their growth-depressant effect.

When rats were fed a measured amount of diet sufficient to maintain their weight, the caloric requirement necessary for weight maintenance gradually decreased. When the dietary fat source consisted of polymeric residue to the extent of 4 to 10%, the caloric requirement for weight maintenance decreased relatively little, if at all. The polymeric residue from autoxidized lard was, in this respect, as effective as that from autoxidized cottonseed oil.

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The Chemistry of Polymerized Oils. VII. Cyclization of Methyl Beta-Eleostearate

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RECENTLY PASCHKE AND WHEELER (5) studied the cyclic monomer obtained on polymerization of a 10% solution of mixed methyl eleostearates in methyl laurate at 250°C. for 48 hrs.

Following our earlier work on the thermal dimer of methyl β-eleostearate (1), we examined the cyclic monomer. From our previous investigations we had concluded that it was important in thermal polymerization studies to keep the reaction conditions as mild as possible, in order to minimize secondary changes. For this reason the cyclic monomer was separated from material which had only been heated at 180° for 4½ hrs.

Experimental

Preparation of Methyl β-Eleostearate Cyclic Monomer. Methyl β-eleostearate (m.p. 12.4 to 13.6°), $E^{1\%}_{1cm.} = 1{,}850$ in ethanol at 268 mμ, was prepared by esterification from the acid, m.p. 71°, with *p*-toluenesulfonic acid as catalyst.[1] Some 115 g. of this ester were heated in a sealed evacuated ampoule for 4½ hrs. at 180°. Monomeric material (69 g.) was separated by repeated passages through a falling-film molecular still at 105°. A solution of 66.2 g. of this distillate in 50 ml. of methanol was added at 40° to a solution of 330 g. of urea in 1,500 ml. of methanol. After 24 hrs. at room temperature the precipitate (which on decomposition with water gave 37.0 g. of oil) was filtered off, and the filtrate was concentrated to 800 ml. After two days the precipitate (which on decomposition with water gave 10.5 g. of oil) was removed, and the filtrate was concentrated to 500 ml. This solution was left at 5° over-night, and the precipitate was filtered off. On treatment with water the latter afforded 0.55 g. of oil. The filtrate, after removal of urea and methanol, gave 11.2 g. of ester. On distillation in a small falling-film molecular still at 105°, this material yielded 10.0 g. of cyclic monomer, or 9% based on the original eleostearate. Its ultraviolet spectrum, which is shown in Figure 1(a), is very different from that recorded by Paschke and Wheeler (5). It had the following properties: Number of double bonds (Adams' catalyst in ethanol) = 2.00 (calc. 2.00); C = 77.42% (calc. 78.03%); H = 11.32% (calc. 11.04%).

Conversion of the Cyclic Monomer to Orthophthalic Acid. Four grams (0.0134 mole) of this cyclic monomer were refluxed with 2.45 g. of N-bromosuccinimide (0.0134 mole) in 30 ml. of carbon tetrachloride for 3 hrs. The solution was cooled, and the solid succinimide (1.27 g.) was filtered off. The filtrate was evaporated under vacuum, and the residue was heated with 5 ml. of redistilled diethylaniline at 130° under nitrogen for 1 hr. to dehydrobrominate the diene to the benzene derivative. When cooled, the mixture set semi-solid. Water and hexane were added, and the mixture was shaken. The hexane layer was separated and washed three times with dilute HCl, then four times with water. The hexane solution was dried over Na_2SO_4, and the solvent was removed to give 4.26 g. of dark oil, which was molecularly distilled at 105° to afford 2.58 g. of a straw-yellow distillate. The ultraviolet absorption curve of this material is shown in Figure 1(b).

[1]Ester prepared from the acid by treatment with diazomethane in ether had $E^{1\%}_{1cm.} = 1{,}980$, m.p. 14.0–15.1°. Pure ester should have $E^{1\%}_{1cm.} = 2{,}060$ based on $E^{1\%}_{1cm.} = 2{,}161$ for the acid (4).

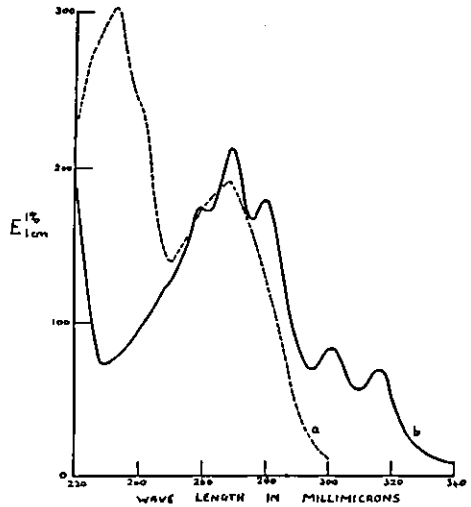

FIG. 1. Ultraviolet absorption spectra. a) Cyclic monomer. b) Material obtained by substitutive bromination and dehydrobromination of cyclic monomer.

A mixture of 0.67 g. (0.0023 mole) of the straw-yellow distillate, 0.82 g. (0.0046 mole) of N-bromosuccinimide and 12 ml. of carbon tetrachloride, was refluxed for 12 hrs. Solvent was removed under vacuum, and the residue was extracted several times with boiling hexane. After evaporation of the hexane 1.07 g. of brominated ester was obtained. This was refluxed with 2.5 g. of Na_2CO_3 in 50 ml. of water for 6 hrs. (*cf.* [5]). The solution was acidified with diluted H_2SO_4 and extracted continuously with ether for ½ hr. The ethereal extract was washed several times with water and evaporated to dryness under vacuum to give 0.72 g. of resinous material. This was dissolved in 100 ml. of acetone (previously treated with $KMnO_4$ and dried over K_2CO_3) and 2.0 g. of $KMnO_4$ added in portions, with shaking, over several hours. Fnally the solution was refluxed until the permanganate color had disappeared. The acetone was removed in vacuum, and the residue was extracted three times with 150 ml. of boiling water

STUDIES TO DETERMINE THE NATURE OF THE DAMAGE TO THE NUTRITIVE VALUE OF MENHADEN OIL FROM HEAT TREATMENT[1]

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INTRODUCTION

Crampton et al. ('56) have compared the nutritive values of various fractions of the ethyl esters prepared from heat-polymerized linseed, soybean and sunflower seed oils. Particular attention was paid to the fraction of the distillable ethyl esters that failed to form urea adducts (the NAFD fraction). The NAFD from linseed oil was highly injurious to the well-being of young growing rats, despite the fact that the material was a bland, neutral oil. The corresponding fraction from soybean oil was injurious, though less so than linseed NAFD. The NAFD from sunflower seed oil was much less injurious than that from the other two oils. The adduct-forming fractions of the distillable esters (AFD) from all three oils were nutritionally innocuous.

Crampton et al. ('53) and Wells and Common ('53) have considered the possibility that failure of the NAFD fraction to form urea adducts might be due to formation of cyclic monomeric acids during the thermal polymerization of the triglyceride. Since that time considerable evidence has been forthcoming to demonstrate that cyclized monomeric acids are formed during heating of methyl eleostearate (Paschke and Wheeler,

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec, Canada, Journal Series no. 398.

'55) and of linseed oil (Macdonald, '56). We ourselves have applied the procedure described by Paschke and Wheeler ('55) to the NAFD fraction from linseed oil and have secured confirmatory evidence, from infrared spectrophotometry, of the presence of aromatic material in the product. Our use of the term "cyclic monomers" in our previous papers (Crampton et al., '53, '55) is justified, therefore, to the extent that this fraction has been shown to contain some cyclized material, though it is probably not composed entirely of "cyclic monomers." *Cis-trans* isomerizations may account, in part at least, for failure of NAFD fractions to form urea adducts; and comparable evidence for the presence or absence of cyclic monomeric acids in the NAFD fractions of soybean and sunflower seed oils is not yet available.

The results of comparison of NAFD fractions from linseed, soybean and sunflower seed oils, however, suggested that it could be of interest to conduct similar studies on an oil containing acids of higher unsaturation. Menhaden oil was chosen for this purpose. The results are presented in the present paper.

EXPERIMENTAL

The sample of commercial alkali-refined menhaden oil used in this work had the following characteristics: sp.gr. (25°C.), 0.9251; refractive index (25°C.), 1.4784; iodine no. (Benham and Klee, '50), 180; saponification no. 188; mean molecular weight (cryoscopic), 839; unsaponifiable, 1.04%. This oil was heated in batches of 500 gm for 15 hours at a temperature of 275°C. while passing a strong current of CO_2. The time of heating was selected on the basis of the results of preliminary kinetic studies. The heated oil was converted to the ethyl esters by transesterification in the presence of hydrochloric acid gas as catalyst (see appendix). The use of acid catalysts was preferred because of the sensitivity of the polyene acids of menhaden oil to alkali isomerization. Fractionation of the esters by urea adduct formation and distillation were performed as described by Crampton et al. ('53). In addition,

TABLE I

Yields and characteristics of fractions of ethyl esters of menhaden oil used in feeding trial

FRACTION[1]	YIELD AS % OF TOTAL ESTERS OF HEATED OIL		REFRACTIVE INDEX 25°C.	IODINE NO.[2]	FREE FATTY ACIDS	SAP. NO.[3]	PEROXIDE VALUE[4]	
	Linseed[5] (12 hr. at 275°C.)	Menhaden (15 hr. at 275°C.)			%		(a)	(b)
							mg/peroxide O_2/kg oil	
AF	46 (293)[6]	45 (299)	1.4483	86	0.73	183	276	6712
NAF	54 (472)	45 (497)	1.4964	151	1.23	151	0	1047
DE	60 (294)	60 (296)	1.4517	84	1.15	186	0	861
AFD	49 (293)	34 (290)	1.4420	45	0.41	189	360	1792
NAFD	11 (300)	20 (310)	1.4659	143	1.92	169	0	1215
NDE	40 (550)	..	.....	...	...	...	...	....
WE	..	100 (314)	1.4598	156	1.32	182	0	1100

[1] AF = Adduct-forming fraction of total esters.
NAF = Non-adduct-forming fraction of total esters.
DE = "Distillable" esters.
AFD = Adduct-forming fraction of distillable esters.
NAFD = Non-adduct-forming fraction of distillable esters.
NDE = Non-distillable esters.
WE = Whole esters of unheated oil.
[2] Data for linseed oil quoted from Crampton et al. ('53).
[3] Iodine numbers determined by the method of Benham and Klee ('50).
[4] Saponification numbers determined by the method of the A.O.A.C. ('55).
[5] Peroxide values determined by the method of Skellon and Wills ('48).
(a) Value of ester fraction before its inclusion in the diet.
(b) Value determined on oil fraction extracted from the diets by cold chloroform at the conclusion of the 28-day feeding trial.
[6] Figures within parentheses are cryoscopic mean molecular weights determined in cyclohexane.

ethyl esters of the whole unheated oil were prepared by acid-catalyzed transesterification. The yields and characteristics of the various fractions are presented in table 1. It will be noted that the yield of the NAFD ester fraction was 20%, based on weight of whole esters.

The basal diet for the nutritional experiment was similar to that used in our previous experiments, except that wheat flour was replaced by ground whole wheat. The percentage compositions of the diets were as follows: — 10% level diet: whole wheat 54; dried skimmilk 19; casein 12; ester fraction 10; dried yeast 3; dicalcic phosphate 1; iodized salt 0.5; ferrous sulphate 0.1; vitamin A and D supplement 0.15; chromic oxide 0.25. Twenty per cent level diet: as above, but with 44% ground wheat and 20% ester fraction.

The experimental animals comprised 12 groups each of 10 rats. Six of these groups received each a different ester fraction at a level of 10% of the diet and the other 6 groups received the same ester fractions at a level of 20%. Ester fraction 6 (see table 1) was not included in the feeding experiment. Its place was taken by the esters of whole unheated menhaden oil which were also fed both at the 10% and the 20% level. The various diets were offered ad libitum.

The results for survival, food intake, liveweight gain, digestibility of the ether extract and gain per 1000 digested calories are presented in table 2. Tables 1 and 2 include comparable data for similar fractions from heated linseed oil. The latter data have been cited from Crampton et al. ('53).

The results presented in table 2 may be summarized in the general statement that the various fractions from heated menhaden oil had about the same effects on the well-being of the rats as had similar fractions from linseed oil in previous comparable experiments (Crampton et al., '53). A slight apparent superiority of the NAFD from menhaden oil was not sufficiently marked to warrant any assertion as to its superiority over the NAFD from linseed oil. The menhaden NAFD,

TABLE 2

Effects of esters of heated menhaden oil on survival and live weight gains of rats and the digestibility of ether extracts of the diets

FRACTION	RATS SURVIVING 28-DAY TEST		AV. LIVE WEIGHT GAINS FOR 28-DAY PERIOD		AV. FOOD INTAKE PER RAT PER 28-DAY PERIOD		APPARENT DIGESTIBILITY OF ETHER EXTRACT		AV. LIGHT WEIGHT GAIN PER 1000 CAL. DIGESTED	
	20%[1]	10%	20%	10%	20%	10%	20%	10%	20%	10%
	%	%	gm	gm	gm	gm	%	%	gm	gm
AP	100	100	118	113	238	286	94	87	105	95
	(100)[2]	(100)	(101)	(107)	(266)	(300)	(96)	(95)	(61)	(61)
NAP	0	90	...	3	...	132	...	73	...	37
	(20)	(100)	(-32)	(30)	(141)	(205)	(75)	(77)	(9)	(50)
DE	80	100	2	46	95	152	95	88	43	67
	(90)	(100)	(4)	(66)	(118)	(228)	(97)	(92)	(43)	(63)
AFD	100	100	111	122	233	261	94	91	92	103
	(100)	(100)	(57)	(108)	(103)	(209)	(96)	(96)	(51)	(62)
NAFD	10	60	-50	-13	81	96	85	87	...	...
	(0)	(0)	...	...	...	...	...	(93)	...	...
WE	100	100	61	98	165	233	93	94	73	87
	...	...	...	...	...	...	...	...	...	...
LSD			18	18	30	30			18	18
(P = 0.05)			(23)	(23)	(36)	(36)			(16)	(16)

[1] The ester fractions were fed at 20 and 10% of the diet.

[2] Figures within parentheses related to comparable data for linseed oil cited from Crampton et al. ('53).

however, was definitely and markedly inferior nutritionally to the NAFD from soybean or sunflower seed oil.

The chief interest of the present work lies in the additional evidence that it provides for the association of toxicity of the NAFD fraction of heat-polymerized oils with the presence of polyene acids in the original oil.

SUMMARY

The non-adduct-forming fraction (NAFD) of the distillable esters from heated menhaden oil was toxic to rats to a degree comparable with the toxicity of the similar fraction from heated linseed oil.

The adduct-forming fraction (AFD) of the distillable esters from the heated oil was nutritionally innocuous.

The results provide some additional evidence for an association between the toxicity of the NAFD fraction and the presence of polyene acids in the original oil.

ACKNOWLEDGMENTS

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APPENDIX

A. Preparation of ethyl esters. One kilogram absolute ethanol was weighed into a tared 2-liter round bottomed flask with ground glass joint. Dry hydrochloric acid gas was passed in until the weight had increased by 20 gm.

The oil (500 gm) was then added and the mixture was refluxed for 24 hours. The mixture was then allowed to cool, finally in a large separatory funnel. The upper layer was separated and distilled from a steam bath until the volume was reduced by one half. The concentrate was poured into 4 volumes of water, and to the mixture the lower layer from the separation was added.

The esters were extracted from the aqueous mixture with peroxide-free ethyl ether. The ether was removed under reduced pressure using a stream of N_2 instead of air. The esters thus prepared were stored under CO_2 in tightly stoppered flasks in the freezer until required.

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greater part of his professional life, he advised on national medical problems, wrote for medical journals, and spoke to medical audiences. As he took part in the development of nutrition he became convinced, and so urged, that it be looked upon as an exceedingly important factor in preventive medicine. He was frequently consulted by the practitioner regarding clinical problems; the discussion usually revolved about normal physiology and chemistry upon the basis of which guidance to the solution of the problem was usually given.

During his lifetime many honors came to Dr. Mendel. He was proud to have been a charter member of the Yale Chapter of Sigma Xi. Later, honorary degrees were conferred by the University of Michigan, Rutgers University, and Western Reserve University. He was long a member of the National Academy of Sciences and of the American Philosophical Society. In 1929 he was elected to membership in the Societe de Biologie in Paris and became a member of the American Academy of Arts and Sciences a year later. His academic service was recognized by a gold medal given by the American Institute of Chemists and a year before his death, the Chemists' Club of New York conferred upon him the Conne Medal for outstanding chemical service to medicine. When he was 60, his friends, students, and professional associates presented him with his portrait and for the same occasion, there was published an Anniversary Number of the *Yale Journal of Biology and Medicine* containing articles by some of his former pupils. While he valued these various honors, he prized most of all the successes of his many former students.

In Dr. Mendel's death on December 9, 1935, at the age of 63, there passed not only one of the pioneers in the science of nutrition, but also a gentle friend to many whose lives were enriched by contact with him.

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STUDIES TO DETERMINE THE NATURE OF THE DAMAGE TO THE NUTRITIVE VALUE OF SOME VEGETABLE OILS FROM HEAT TREATMENT[1]

IV. ETHYL ESTERS OF HEAT-POLYMERIZED LINSEED, SOYBEAN AND SUNFLOWER SEED OILS

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(Received for publication March 31, 1956)

INTRODUCTION

The literature on the effects of polymerization temperature on the nutritive value of edible oils has been reviewed up until 1952 by Crampton et al. ('53). Since then Frahm et al. ('53) have reported deleterious effects of heat-polymerized whale oil when it is fed to mice. Raju and Ragagopalan ('55) have reported the results of feeding rats with diets containing 15% of peanut or sesame or cocoanut oil which had been heated at 270°C. in open pans in contact with air. The effects included depression of live weight gain, decrease in food efficiency and increases in liver weight as percentage of body weight and in percentage of liver fat. However, although the temperature used by Raju and Ragagopalan suggests that there was polymerization, the experiments of these workers are not comparable with those carried out in our laboratories where the oils were heated in a current of CO_2. Kaunitz et al. ('55) have found that cottonseed oil heated and aerated at 90 to

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Province of Quebec, Canada. Journal Series no. 394.

95°C. for periods of up to 300 hours became injurious to rats. Incorporation of fresh oil gave a degree of protection against some of the deleterious effects; peroxides were considered not likely to be responsible for the ill effects. The conditions of heating differed greatly from those used in our laboratories.

In a previous paper (Crampton et al., '53) we have reported a study of the nutritional properties of certain fractions prepared from the ethyl esters of heat-polymerized linseed oil by distillation and urea adduct formation. The preparation and the designations of the different fractions are described in this previous paper, as are also the diets and plan of the feeding trials. For convenience of reference the flow sheet for fractionation is reproduced in figure 1.

It should be pointed out here that polymerization of triglyceride oils is now known definitely to include formation of significant amounts of trimeric, and even of some higher polymeric acyl radicals, as well as of dimeric acyl radicals (Paschke and Wheeler, '54). In the present paper, therefore, fraction G is designated "polymer" rather than "dimer."

From this earlier work it seemed reasonable to suppose that heated linseed oil was nutritionally injurious, firstly, because of the presence of polymerized material that is poorly absorbed, if at all, and secondly, because of the presence of monomeric acyl radicals incapable of forming urea adducts by reason of some structural feature, possibly a cyclization. In this connection it is noteworthy that Paschke and Wheeler ('55) have now demonstrated the formation of a cyclic monomer during heat polymerization of methyl eleostearate and have shown that this cyclic monomer is mainly an ortho-disubstituted cyclohexadiene.

The question that next presented itself was the degree to which formation of non-adduct-forming monomeric material could be related to the fatty acid composition of the original oil itself. Accordingly, in 1953-'54 further rat feeding trials were carried out involving 24 lots of 10 animals each in order to study the nutritional value of similar fractions prepared from soybean oil and sunflower seed oil. Soybean oil was

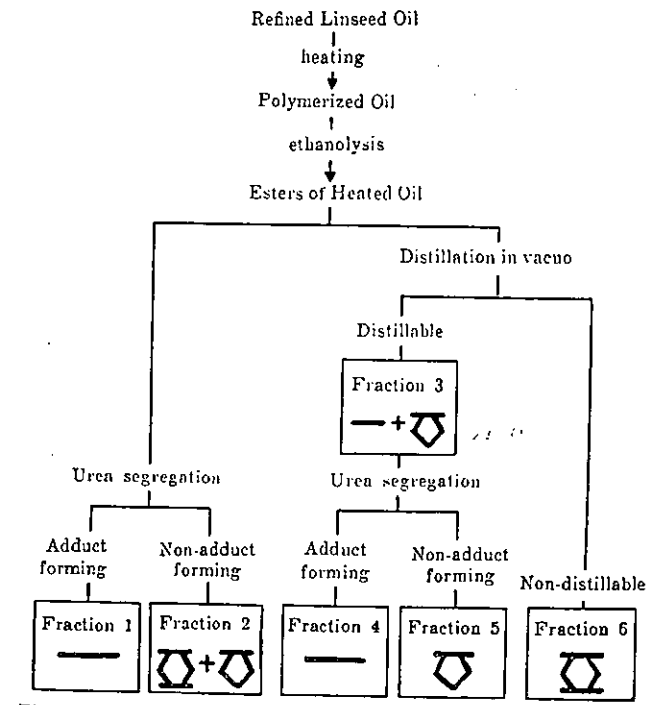


Fig. 1 Flow sheet illustrating preparation of fractions of esters of heated linseed oil used in feeding trials.

chosen because it is a food oil that contains some linolenic acid. Sunflower seed oil was chosen because of its high linoleic acid content and negligible linolenic acid content. Typical data for the fatty acid composition of the three oils are summarized in table 1.

Preliminary experiments with soybean and sunflower seed oils showed that non-adduct-forming distillable ester (NAFD) fractions could be obtained from both oils using the same polymerization temperature (275°C.) and heating in a current of CO_2. It was necessary to heat these oils for longer times

TABLE 1

Fatty acid composition by weight of linseed, soybean and sunflower seed oils

	SATURATED			UNSATURATED		
	C_{16}	C_{18}	C_{20}	Oleic	Linoleic	Linolenic
	%	%	%	%	%	%
Linseed N. American (I No. 179.8)[1]	6.3	2.5	0.5	19.0	24.1	47.4
Soybean (I No. 132.5)[2]	10.6	2.4	2.4	23.5	51.2	8.5
Sunflower seed[3]	6.4	1.3	4.0	21.3	66.2	..

[1] Rose and Jamieson ('41), cited by Bailey ('52).

[2] Hilditch, Meara and Holmberg ('47), cited by Bailey ('52).

[3] Barker, Crossley and Hilditch ('50), cited by Bailey ('52).

TABLE 2

Yields of fractions of ethyl esters used in feeding trials

FRACTION	YIELD AS PER CENT OF TOTAL ETHYL ESTERS OF HEATED OIL		
	Linseed[1] (12 hr. at 275°C.)	Soybean (24 hr. at 275°C.)	Sunflower seed (26 hr. at 275°C.)
1. Adduct-forming fraction of total esters	46 (293)[2]	63 (296)	65 (..)
2. Non-adduct-forming fraction of total esters	54 (472)	37 (415)	35 (..)
3. "Distillable" esters	60 (294)	74 (296)	75 (303)
4. Adduct-forming fraction of distillable esters	49 (293)	64 (294)	65 (295)
5. Non-adduct-forming fraction of distillable esters	11 (300)	10 (294)	10 (296)
6. Non-distillable esters	40 (550)	26 (415)	25 (613)

[1] Data for linseed oil quoted from Crampton et al. ('53).

[2] Figures in parentheses are cryoscopic mean molecular weights.

than linseed oil in order to attain reasonable yields of this fraction. The times used for bulk preparation of fractions are given in table 2, together with approximate yields of the various fractions. The data for linseed oil are quoted from Crampton et al. ('53).

RESULTS

Tables 3 to 7 summarize the results of the feeding experiments with soybean oil and sunflower seed oil, the data for linseed oil being cited from Crampton et al. ('53). In the discussion that follows, the data for linseed oil are considered along with those for the two other oils; the latter sets of data are here reported for the first time.

Survival of rats. The percentages of the rats surviving the 28-day feeding are shown in table 3.

TABLE 3

Comparison of the nutritional effects of ethyl esters of linseed, soybean and sunflower seed oils

(Ten rats per lot)

Percentage of rats surviving 28-day test

DIET	ESTER FRACTION	LINSEED		SOYBEAN		SUNFLOWER SEED	
		20%	10%	20%	10%	20%	10%
1	—	100	100	100	100	100	100
2	◇ + ◇	20	100	0[1]	100	50[2]	50[2]
3	— + ◇	90	100	100	100	100	100
4	—	100	100	100	100	100	100
5	◇	0	0	100	80[3]	100	100
6	◇	70	100	0[1]	10[4]	100	100

[1] All animals removed after 10 days because of diarrhea and extreme viscosity of feces.

[2] Five animals removed because of diarrhea.

[3] Remaining rats in poor condition.

[4] Some diarrhea.

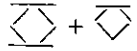
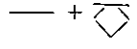
The only deaths recorded were in lots where the diets contained either non-adduct-forming monomers or dimeric or higher polymers, with the former displaying the greater toxicity. Also, in every lot receiving esters of polymeric acids there was diarrhea and the feces were varnish-like. This material was so sticky that at morning inspection the feet and tails were often found to be inseparable without washing. In some cases the animals were stuck to the wire floor of their cages. The diets, however, did not appear to be toxic in the usual sense of the term.

The ester fraction which consisted entirely of urea adduct-forming monomers had no harmful effect on survival. The one death in lot 3 may have been due to the "cyclic" monomers of linseed oil also present, since all rats died in lot 5 even where the diet contained but 10% of such material.

Gain of rats. The gain figures shown in table 4 are not directly comparable as between oils. For each ester source, however, figures are strictly comparable as between diets and between levels.

TABLE 4

Live weight gains — 28 days

DIET	ESTER FRACTION	LINSEED		SOYBEAN		SUNFLOWER SEED	
		20%	10%	20%	10%	20%	10%
		gm	*gm*	*gm*	*gm*	*gm*	*gm*
1	——	101	107	93	99	143	150
2		32	39	..	63	107	150
3		4	66	83	101	162	162
4	——	57	108	91	99	151	159
5		..	..	16	36	97	142
6		13	77	..	69	90	143
Least significant difference (P = 0.05)		23	23	10	10	20	20

The results leave little doubt that esters of both "cyclic" monomeric and polymeric acids from the heat polymerization of these three oils are undesirable components of rat diets. However, there would seem to be a difference in the degree of toxicity of the non-adduct-forming distillable fractions, insofar as that from sunflower seed oil was less damaging than those derived from flaxseed or soybean oil. These oils differ chiefly in their contents of the trienoic linolenic acid and there is a temptation to ascribe to this fatty acid the origin of the toxic "cyclic" monomers. Such an ascription requires an assumption that some non-adduct-forming material arises on heat polymerization from some fatty acid other than linolenic and perhaps also that this is a less toxic material than that formed from trienoic fatty acids.

Food intake. Fractions 1 and 4 were equally readily eaten, except at the 20% level of the linseed oil fractions. The polymer fraction 6 was practically equally acceptable, except at the 20% level of the linseed oil fraction; this is remarkable in view of the unattractive varnish-like nature of the materials in question. The "cyclic" monomer fraction (fraction 5) was less readily eaten than the other fractions; and here it is to be noted that these fractions were either colorless or very pale, bland oils. Fractions 2 and 3, containing some "cyclic" monomer, were as readily accepted as fractions 1 and 6, in the case of the soybean and sunflower seed oil fractions, but less readily in the case of the linseed fraction. On the whole, these results suggest that the only fraction that was definitely poorly acceptable was fraction 5.

Digestibility of the oils. There was reasonably clear evidence that the non-adduct-forming polymers were poorly digestible. This was predictable from the abnormal feces. It is probable that low digestibility was a major causal factor in the slower gain of the animals fed this oil fraction. The digestibility of other fractions was above 90%.

Efficiency of utilization of dietary calories (gain per 1000 digested calories). The data suggest that, in general, the rats used that portion of the calories which they absorbed about

equally well excepting for the "cyclic polymers." In the case of the linseed and soybean oils, the digested polymeric esters were in some cases as efficient as the straight-chain materials, but with sunflower seed oil this fraction was as unsatisfactory as the cyclic monomers (table 5).

TABLE 5

Gains per 1000 digested calories

DIET	ESTER FRACTION	LINSEED		SOYBEAN		SUNFLOWER SEED	
		20%	10%	20%	10%	20%	10%
		gm	*gm*	*gm*	*gm*	*gm*	*gm*
1	——	61	61	99	99	82	89
2	⬡ + ◇	9	50	..	88	69	101
3	—— + ◇	43	63	92	104	95	90
4	——	54	62	99	106	92	87
5	◇	..	..	85	85	77	93
6	⬡	61	56	..	88	65	84
Least significant difference (P = 0.05)		16	16	10	10	14	14

DISCUSSION

Certain characteristics of the non-adduct-forming distillable ester fractions from the three oils are presented in table 6. The order of decreasing injuriousness was also the order of decreasing iodine value and refractive index, while the cryoscopic mean molecular weights were, for practical purposes, the same and corresponded to a preponderance of C_{18} acids. The most marked difference between the three NAFD fractions was in respect to their behaviour on alkali isomerization (fig. 2). Linseed NAFD displayed a relatively low absorption at 233 mμ, while both soybean NAFD and sunflower seed

TABLE 6

Iodine values and refractive indices (n^{25}_d) of fractions of ethyl esters of linseed, soybean and sunflower seed oils

FRACTION		LINSEED	SOYBEAN	SUNFLOWER SEED
1. Adduct-forming fraction of total esters	Iodine no.	118.2	99	..
	n^{25}_d	1.45345	1.45302	..
2. Non-adduct-forming fraction of total esters	Iodine no.	162.7	125	..
	n^{25}_d	1.47561	1.46998	..
3. "Distillable" esters	Iodine no.	130.1	106	110
	n^{25}_d	1.45684	..	..
4. Adduct-forming fraction of "distillable" esters	Iodine no.	124.8	92	107
	n^{25}_d	1.45494	1.44935	1.45254
5. Non-adduct-forming fraction of "distillable" esters	Iodine no.	170.7	143	130
	n^{25}_d	1.46986	1.47001	1.45671
6. "Non-distillable" esters	Iodine no.	159.9	114	106
	n^{25}_d	1.48017	.	1.47655

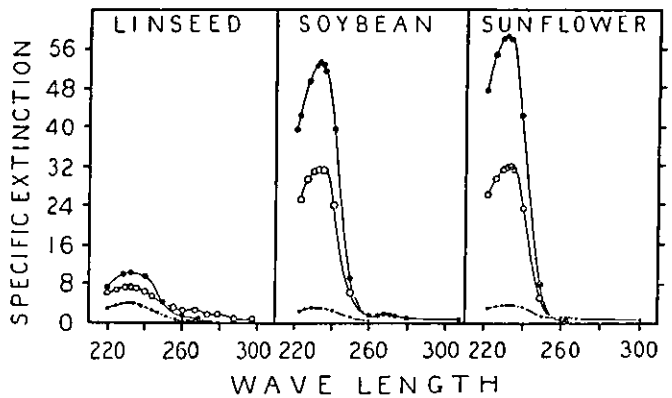


Fig. 2 Ultraviolet absorption spectra of non-adduct-forming distillable fractions of the esters of heat polymerized linseed, soybean and sunflower seed oils.

●—●—● unisomerized.
○—○—○ alkali-isomerized for 25 minutes.
●—●—● alkali-isomerized for 6 hours.