The liver weights (fig. 2 b) showed similar, but somewhat less pronounced, changes. Most of the livers of animals on treated oil were well above those of normal rats; fresh oil prevented the changes. Organ and body weights of rats fed rancid lard have been reported by Greenberg and Frazer ('53). Their average results for kidneys and livers of animals fed rancid or fresh lard fall within the range for normal rats as given in figure 2a and b. It is true, however, that the liver and kidney weights for the rats fed rancid lard tend to be on the upper limit for normal rats while those of the animals fed fresh lard are lower. This is further evidence that heated and aerated lard is much less toxic than similarly treated cottonseed oil.

The scattering of the normal adrenal weights was much greater than that of the liver and kidneys (fig. 2 c). However, in 9 out of 16 cases, the adrenals of those fed treated oil exceeded the maximum weight of the normals while, in the group fed fresh as well as treated oil, this occurred only 4 times.

The majority of the testicular weights of both groups of experimental animals were above those of the normal ones (fig. 2 d). In many instances, therefore, the testes did not lose weight, and some even increased in size despite the loss in body weight resulting from the feeding of the treated oil. This was not changed by the addition of fresh oil to the diet.

The shape of the thymus — body weight relationship in normal animals (fig. 2 e) expresses the involution of the thymus. The curve found in these studies is not appreciably different from that described by Stoerk ('46). Extremely small thymuses were found in the majority of animals fed treated oil. The thymuses of about half of the pair-fed controls receiving treated and fresh oils were also smaller than normal (presumably as an expression of semi-starvation) but were much larger than those fed only the treated oil.

The variation in splenic weights among the animals far exceeded the variations in any of the other organs. Yet, it seems evident that the spleens of about half of the animals fed only treated oil are below the lower limit for normal spleens while those of their pair-fed controls are just within normal limits.

In further studies, an attempt was made to modify the toxic effect of the treated oil by feeding extra amounts of the fat-soluble vitamins and by the feeding of fresh oil by dropper rather than by inclusion in the diet.

When animals receiving 15% of the treated oil were given daily feedings by dropper of about 25 mg of synthetic DL-alpha-tocopherol acetate containing 125 μg of crystalline beta-carotene and 2.5 μg of vitamin D_2, their average weight after two weeks was 75 gm, while that of the rats receiving no supplement was 61, the difference being statistically significant. However, a group receiving 15% of the treated oil and 10% of fresh oil weighed 99 gm on the average at this time. Therefore, the mildy protective effect of tocopherol (and the other fat-soluble factors) does not explain the strongly protective action of fresh oil added to the diet.

Daily feeding by dropper of 0.5 to 0.8 gm of fresh, refined cottonseed oil exerted only a mild protective effect as to growth and survival time in rats fed 15% of the treated oil. After three weeks on the experimental diet, those receiving no supplement weighed, on the average, 60 gm and three out of 12 had died; those fed fresh oil in addition weighed, on the average, 69 gm and none had died. However, when a comparable amount of fresh oil was added to the diet, the average weight was well above 100 gm. We have so far no explanation for this difference.

DISCUSSION

In studies on heated and aerated lard (Kaunitz and Slanetz, '50), it was concluded that the amount of peroxides present is not related to the degree of toxicity. In the current studies, similar results were obtained. In the experiments shown in figure 1a, it was found that a sample of cottonseed oil, heated and aerated to a peroxide number of 191, brought about only very mild growth retardation. When heating and aeration were continued, the peroxide number dropped to 141, but the

sample had become very toxic. It would therefore seem that, if the toxic products are the result of oxidation, they may be breakdown products of peroxides; but it is quite possible that the toxic products are not at all, or only partly, related to oxidative changes. Crampton and his co-workers ('53), in their studies on heated linseed oil advanced this latter viewpoint. They made it seem probable that some products formed during thermal polymerization are toxic.

Concerning the antagonism between treated and fresh oils, we had first believed that an antimetabolite relationship might exist between the toxic products and fresh oil. However, in view of the fact that the protective effect of fresh oil is only mild when it is not added to the diet but is fed separately, the antimetabolite theory may not be a good explanation for the facts observed. The possibility must be considered that addition of fresh oil to heated oil may change the toxic properties of the latter, perhaps by influencing the state of polymerization.[5] But whatever explanation may eventually prove to be correct, it seems noteworthy that fresh oil may, under certain conditions, exert a life-saving effect which is not due to its caloric properties.

SUMMARY

1. The inclusion in a rat diet of 15 to 20% of refined cottonseed oil, aerated and heated to 95°C. for 200 to 300 hours, led to rapid loss of weight and death within three weeks.

2. The condition was accompanied by diarrhea and by the occurrence of large livers, kidneys, and adrenals and small spleens and thymuses. Histologically, the only change was an occasional intestinal edema.

3. Addition of fresh oil to the diet containing the heated and aerated oil protected the animals against the toxicity. Only growth retardation persisted. This protective effect could also be observed in paired-feeding experments in which the paired rats received the same number of calories and equal amounts of protein and of treated oil. When fresh cottonseed oil was fed by dropper instead of being included in the diet, its protective effect was only slight.

4. Extra feeding of 25 mg of DL-alpha-tocopherol acetate (and other fat-soluble factors) gave mild protection.

5. Peroxides are probably not responsible for the toxic effect of the heated and aerated cottonseed oil; polymerization may be a better explanation. No definite explanation for the antagonism of fresh to treated oil can be given; the effect is probably not due to an antimetabolite relationship but could be caused by a change in the state of polymerization.

[5] This possibility was discussed with a number of workers in the fields of fat and of polymer chemistry. While they agreed that no definite opinion could be given, some of the workers felt that at least changes in the kind of polymerizations could be effected by the addition of fresh to polymerized oil.

LITERATURE CITED

CRAMPTON, E. W., F. A. FARMER AND F. M. BERRYHILL 1951 The effect of heat treatment on the nutritional value of some vegetable oils. J. Nutrition, *43:* 431.

CRAMPTON, E. W., R. H. COMMON, F. A. FARMER, A. F. WELLS AND D. CRAWFORD 1953 Studies to determine the nature of the damage to the nutritive value of some vegetable oils from heat treatment. III. The segregation of toxic and non-toxic material from esters of heat-polymerized linseed oil by distillation and by urea adduct formation. Ibid., *49:* 333.

GREENBERG, S. M., AND A. C. FRAZER 1953 Some factors affecting the growth and development of rats fed rancid fats. Ibid., *50:* 421.

KAUNITZ, H. 1953 Studien über die Ernährung von Ratten mit hochoxydierten Fetten. Arch. exper. Path. u. Pharmakol., *220:* 16.

KAUNITZ, H., AND C. A. SLANETZ 1950 Relation of vitamin A and "lard factor" to disease caused by rancid lard. Proc. Soc. Exp. Biol. Med., *75:* 322.

KAUNITZ, H., H. WIESINGER, F. C. BLODI, R. E. JOHNSON AND C. A. SLANETZ 1954 Relation of protein and fat intake to growth and corneal vascularization in galactoflavin-produced ariboflavinosis. J. Nutrition, *52:* 467.

STOERK, H. C. 1946 Effects of calcium deficiency and pyridoxin deficiency on thymic atrophy (accidental involution). Proc. Soc. Exp. Biol. Med., *62:* 90.

STOERK, H. C., AND T. F. ZUCKER 1946 Relative growth of the kidney in male rats. Proc. Soc. Exp. Biol. Med., *61:* 297.

ZUCKER, T. F., AND L. M. ZUCKER 1942 A simple time-weight relation observed in well nourished rats. J. Gen. Physiol., *25:* 445.

In these two types of salicylate inhibition, they were thus proved to be different in their reaction natures. The relation between the logarithm of the velocity constants of inactivation reaction by higher salicylate concentrations and the reciprocal of absolute temperature are shown in Fig. 6. From the slope of this straight line, the Arrhenius energy was calculated to be 42000 cal. From these results obtained, it may be inferred that the inhibition of cholinesterase by sodium salicylate in its higher concentrations is brought about through the denaturation of enzyme protein or some similar reaction, as it was suggested also in the reaction by urethane.

SUMMARY

1. Sodium salicylate inhibits the cholinesterase in two different ways as the incubated concentration of the former differs.

2. It was indicated that the reversible inhibition, which is observed in the lower concentration range of salicylate, is non competitive. The inhibition seems to be brought about by combination of two molecules of salicylate to one enzyme molecule. The binding of one molecule of substrate to one enzyme molecule may result a hindrance of the binding of salicylate molecule with the latter.

3. In the irreversible inhibition by sodium salicylate which occurs in the higher concentration of the latter, the inactivation reaction of enzyme proceeds according to the first order kinetics, and is independent of substrate concentrations. The Arrhenius energy of this reaction was calculated to be 42,000 cal.

The present investigation was supported by Prof. K. Kaziro, to whom the author's deep gratitude is due.

REFERENCES

(1) Shukuya, R., *J. Biochem.*, **40**, 535 (1953)
(2) Goldstein, A., *Arch. Biochem. Biophys.*, **33**, 22 (1951)
(3) Johnson, F. H., Kanzmann, W. H., and Gensler, R. L., *Arch. Biochem.*, **19**, 229 (1948)
(4) Kikuchi, G., and Tomimura, T., *J. Biochem.*, **41**, 503 (1954)
(5) Tsushima, K., *J. Biochem.*, **41**, 215, 359 (1954)
(6) Shukuya, R., *J. Biochem.*, **38**, 225 (1951)
(7) Alles, G. A., and Hawes, R. C., *J. Biol. Chem.*, **133**, 375 (1950)

The Journal of Biochemistry, Vol. 41, No. 4, 1954

STUDIES ON THE TOXICITY OF FISH OIL

By NOBORU MATSUO

(*From the Biochemical Institute, Tokyo Medical College, Tokyo*)

(Received for publication, March 23, 1954)

It was first depicted by Sahashi (*1*) and Somekawa (*2*) that some sorts of fish oil including whale oil are toxic to rat. But it remains undecided whether they are equally toxic to man or not. Since in Japan, where resource of fat is extremely short, the use of fish oils as nutrient is highly desirable, the wide experiments on man were carried out in many clinical as well as biochemical laboratories. The result of such cooperative works revealed that fish oil is not so toxic to man in dosage of 50–100 gms. per day. But quite recently Kaneda and Ishii (*3*) made an interesting observation that fish oil per se is not so toxic even to rat, but when it is once oxidised simply by being exposed to air, it turns out to be toxic.

In this case it is well assumed that unsaturated fatty acid in fish oil is the substance easily oxidised and at the same time responsible for toxic effect. Using the unsaturated fatty acids obtained from cuttle fish oil, the author carried out the experiment and fully confirmed the results obtained by Kaneda and Ishii (*3*). Further, some chemical nature of the toxic substance was studied, the details of which are partly reported here.

EXPERIMENTAL

Preparations of Unsaturated Fatty Acids

The raw cuttle fish oil produced in Hokkaido was used. Its properties are shown in Table I.

TABLE I

The Properties of Raw Cuttle Fish Oil

Density (15°)	Iodine value (Wijis)	Acid value	Sap. value	Unsap. matter (%)
0.9305	182.5	2.03	189.3	2.19

The unsaturated fatty acids were separated by the soda-acetone process (4). Namely, raw cuttle fish oil was saponified by ethyl alcoholic solution of NaOH, mixed with acetone, left over night at 0° and filtered. From the filtrate, which contained acetone soluble sodium-salts of highly unsaturated acids, acetone was distilled off, and its residue was decomposed by dil. HCl.

The free acids, dissolved in petroleum-ether (b. p. below 65°) were washed several times with water, and dehydrated by anhydrous sodium-sulphate and then petroleum-ether was distilled off, giving the highly unsaturated acids as residue.

Since the sample obtained as above, used to contain somewhat of unsaponificable matter which was existing in raw oil, so it was desirable to remove it as much as we could.

Next, the sample was dissolved in twice the volume of absolute ethyl alcohol containing 2.5 per cent HCl, and heated on the water-bath for about three hours. By this process ethyl esters of the acids were prepared. Then, the ethyl esters were fractionated under a vacuum of 2 mm. Hg at various temperatures. The 205~215° fraction was chiefly used for the author's experiment. This fraction has light yellow colour and no odour. Since these esters are liable to be rapidly oxidised when they are in contact with air, special precaution was paid throughout the preparation.

The container of the esters was always filled with CO_2 gas and was kept in dry-ice during the experiments. An aliquote portion of the sample thus prepared were put into a petri dish and borught into contact with air at the room temperature. As the oxidation proceeded the esters developed light yellow colour. At a certain point of oxidation the reaction was stopped and the ester was kept again in dry-ice avoiding further change.

Ethyl ester of oleic acid was used as control. Some chemical properties of the esters and of their oxidised compounds are indicated in Table II together with those of oleic acid ester for comparison.

TABLE II

Analysis of the Samples Used in the Experiments

	d_4^{15}	n_D^{20}	Iodine value	Sap. value	Unsap. matter (%)
Ethyl ethylesters of highly unsaturated fatty acids	—	1.4823	358.70	163.90	Trace
Oxidised esters of highly unsaturated fatty acids	0.9568	—	264.82	201.49	Trace
Ethyl ester of oleic acid	0.8822	—	82.96	187.69	0

RESULTS

Effect on the Growth of the Rat—The rats of nearly same size and body weight (about 60 g.) were divided into three groups, each comprising 4 rats. To the first group the feed was given containing 5 per cent of the unsaturated fatty acid esters in the basal diet, to the second group that of the oxidised esters and to the third that of oleic acid ester, which considered as control. The composition of the basal diet is given in Table III.

TABLE III

Ingredients of Basal Diet

Starch (rice powder)	80%	Yeast	3%
Casein (ether extracted)	9%	Liver oil	1 drop/day
McCollum salt mixture	3%		

Total amount of diet per day is 10 g. (from 1st day to 5th day), 12.5 g. (from 6th day to 10th day), 15 g. (from 11th day to the 30th day).

The experimental results are recorded in Table IV and also illustrated in the accompanying Fig. 1 and photographs (Figs. 2, 3, and 4). It is apparent that the rats fed on the unsaturated fatty acid ester containing diet showed the same satisfactory growth as those on the oleic acid ester diet. But it is quite remarkable that all rats that fed on the oxidised ester diet not only showed retarded growth but died within few days after the beginning of the experiment. It was also noticed in this group that white hair turned into brown.

TABLE IV

Feeding Records

Kind of ester fed	Sex	Increase of body weight (g.) 10th day	average	20th day	average	30th day	average
Ethyl ester of oleic acid.	♀	+11.0	+13.1	+34.0	+43.3	+61.0	+82.1
	♂	+14.0		+51.2		+113.5	
	♀	+15.0		+40.5		+70.8	
	♀	+12.5		+47.5		+83.0	
Ethyl ester of unsaturated fatty acid.	♂	+ 7.7	+ 6.3	+41.0	+28.1	+85.0	+75.8
	♀	+ 7.0		+38.5		+77.0	
	♂	+ 7.0		+44.5		+75.0	
	♀	+ 3.2		+28.5		+66.0	
Oxidised form of the above ester.	♀	−3.8 (died on 6th day)					
	♀	−5.0 (died on 7th day)					
	♀	−5.0 (died on 8th day)					
	♂	−8.5 (died on 3rd day)					

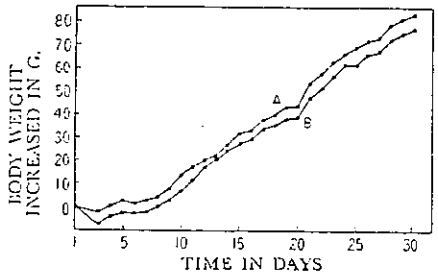

FIG. 1. The curve of average body weight increased. A (Control): average body weight increment of the rats fed on the diet containing 5 per cent of the ethyl ester of oleic acid. B: that of rats fed on the diet containing 5 per cent of the ethyl ester of highly unsaturated fatty acids.

FIG. 2. The rat fed on the diet containing 5 per cent of the ethyl ester of oleic acid. (Control) On the 27th day after the commencement of the experiment.

FIG. 3. The rat fed on the diet containing 5 per cent of the ethyl ester of highly unsaturated acid. On the 27th day after the commencement of the experiment.

Effect of More Mild and Chronic Intoxication by the Oxidised Ester—For this purpose the grown-up rats were fed on the diet containing 5 per cent of the oxidised ester not daily but at some intervals.

One of the typical examples is illustrated in Fig. V with regard to the change of body weight.

As shown in Fig. 5. the retarted growth of the animal continued during the feeding of the toxic diet. but somewhat recovering was observed when that feeding was stopped. It ended, however, in fatal at the end of 23th day after the commencement

FIG. 4. The rat fed on the diet containing 5 per cent of the oxidised ethyl ester of highly unsaturated acid. On the 4th day after the commencement of the experiment.

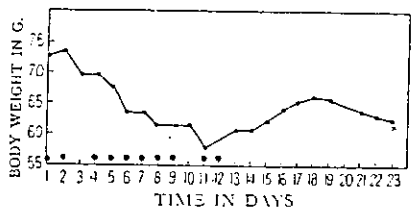

FIG. 5. The curve of body weight changed.
The mark • shows the day on which the oxidised ester diet was given.

of the experiment. The appearance of the animal was quite particular. The hair of the face was diplated off, the mouth and rear legs were swollen, the remaining hair changed into brown, and hemorrhages were seen here and there in the skin. (see Figs. 6 and 7.)

DISCUSSION

The above results substantially proved the finding first made by Kaneda and Ishii that the highly unsaturated acid from fish oil is not toxic but rather its oxidised form is toxic. As it is usually assumed that unsaturated fatty acid absorbs oxygen forming peroxide at double bonds in the molecule, so it might be plausible that the toxicity of the oxidised acid is caused by peroxide. In this regard the next paper will give full details. At any rate it is promising that fish oil can be utilised

Fig. 6

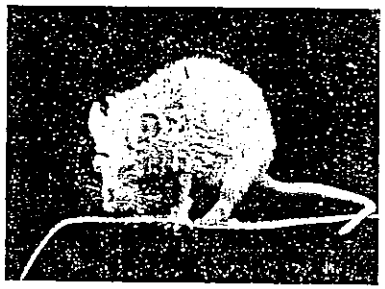

Fig. 7

as human nutrient when it is prepared and preserved avoiding spontaneous oxidation. Or it may be more safe to hydrogenate fish oil to certain extent to get rid of double bonds.

Besides such important problems of fish oil as fat resources in nutrition, the essential feature of toxic effect of the oxidised unsaturated fatty acid from fish oil is interesting to investigate. In this line the work of Kaunitz and Johnson (*5*) is noteworthy. They observed that rat fell into flavin-shortage when rancid lard was given. But it seems to the author that the toxicity is too acute to ascribe it solely to riboflavin shortage.

SUMMARY

1. When the diet containing 5 per cent ethyl esters of highly unsaturated fatty acids obtained from cuttle fish oil was given to rat, it showed quite the same good nutritive effect as the diet containing oleic acid ester.

2. To the contrary the oxidised form of the above unsaturated fatty acid esters showed an extremely toxic effect to rat.

REFERENCES

(1933)

(*2*) Somekawa, E., *Sci. Pap. Inst. Phys. Chem. Research (Tokyo)*, **20**, 149 (1933)

(*3*) Kaneda, T., and Ishii, S., *Bull. Jap. Soc. Sci. Fisheries*, **19**, 171 (1953)

(*4*) Toyama, S., and Tsuchiya, T., *J. Soc. Chem. Ind. Japan*, **28**, 963 (1925)

(*5*) Kaunitz, H., Johnson, R. E., and Slanetz, C. A., *J. Nutr.*, **46**, 151 (1952)

reginous substance.

When various amounts of dimethyl 5-hydroxykynurenamine hydrochloride were injected intravenously to rabbits, the result as indicated in the following table was obtained.

TABLE I

The compound tested	Blood pressure lowering activity	Duration of the activity
	$\mu g./kg.$	*mm. Hg*	
Dimethyl 5-hydroxy-kynurenamine		
	50	14	18 *sec.*
	100	20-22	
	200	32	1 *min.* 40 *sec.*
	500	40	3 *min.* 50 *sec.*
Dimethyl kynurenamine		
	100	15	
	200	28	
	500	30	

Judging from this result, it seems that the action of dimethyl 5-hydroxykynurenamine is stronger than that of dimethyl kynurenamine.

By interrupting the incubation of 5-hydroxykynurenine with liver homogenate at the early stage and fractionating the cleavage products by paper chromatography we obtained a substance, which was judged to be 5-hydroxykynurenamine from its properties. The diluted solution of this substance showed also the blood pressure lowering action.

This work was aided by a grant from the scientific research fund of the Ministry of Education of Japan. We wish to express our thanks to the Takeda Research Laboratory for performing elementary analysis.

REFERENCES

(*1*) Makino, K., and Takahashi, H., *Science*, **120**, 544 (1954)
(*2*) Makino, K., and Takahashi, H., *J. Am. Chem. Soc.*, 76, 6193 (1954)

The Journal of Biochemistry, Vol. 42, No. 5, 1955

NUTRITIVE VALUE OR TOXICITY OF HIGHLY UNSATURATED FATTY ACIDS. II

By TAKASHI KANEDA, HISAE SAKAI and SEINOSUKE ISHII

(*From the Tokai Regional Fisheries Laboratory, Tokyo*)

(Received for publication, June 5, 1955)

In the previous paper (*1*), the authors have established that highly unsaturated fatty acids are not harmful at all when the fatty acids are thoroughly refined so as not to autoxidize themselves. Moreover, it has been found that the widely-believed toxic effects of highly unsaturated acids are primarily caused, not by the unsaturation of fatty acids, but by the autoxidized products which are yielded from highly unsaturated acids.

Since the authors presumed that this conclusion might apply not only to the highly unsaturated acids but to all of the members of natural unsaturated fatty acids, the same course of feeding experiments was carried out, using ethyl esters of liquid acids of linseed oil.

The results of experiment were entirely identical as expected. Based upon these results, it is considered that all the unsaturated acids, which are not easily autoxidized, become harmful once they autoxidize themselves. It was, therefore, tried to examine whether the autoxidized products show toxic effects, by the way of separating such products from other non-toxic ones.

In this case, it was presumed that all the autoxidized products of the unsaturated fatty acids, such as peroxides, conjugated acids, polymerized products, aldehydes, ketones, *etc.* might not be equally toxic but only one or some specific products might be highly toxic and the rest not being toxic or at the least toxic.

In order to verify this presumption, each product was separated and each of them was subjected to the animal test.

When autoxidized slightly, a greater part of unsaturated acids tend to remain for a while with the original structure, showing the most harmful results as stated in our previous report (*1*).

As regard to the method of concentrating the autoxidized products from autoxidized unsaturated acids, several papers have been reported. Privett *et al.* (*2*) separated autoxidized products, using Skellysolve F

and ethyl alcohol, the Changs (*3*) did it, using Skellysolve F and diethyl ether. Zilch (*4*), Fugger (*5*), Cannon (*6*), *etc.* separated by countercurrent extraction procedure and Atherton, Hilditch (*7*) did it by chromatographic method using silica gel. Furthermore, in these several years, Catravas (*8*), Coleman (*9*), Swern (*10*) have reported the method of fractionation by urea complexes.

Therefore, these methods were adopted to separate the autoxidized products. Firstly, adopting the Privett's method (*2*), petroleum ether and 85 per cent ethyl alcohol were used for separation. Most of the autoxidized products solved into alcohol and some remained in the part of the petroleum ether.

The both fractions were harmful for the rats.

The experiment means that, autoxidized products were condensed in this method, but the method was not effective to separate the wholly unharmful fraction out of the harmful one as desired by the authors.

The Atherton and Hilditch's chromatographic method (*7*) of using silica gel showed some toxic substances remaining in the fraction without being absorbed by the silica gel, and did not satisfy our object primarily. However, when chromatographic method is applied, using alminium oxide, the fraction not absorved to alminium oxide is totally harmless. But it is difficult to elute fully the absorbed products from the alminium oxide, endangering the materials to be autoxidized further in the course of experiments.

Having tried the separation by urea complexes, 75 per cent of ethyl esters of autoxidized linseed oil produced urea adduct, showing harmless, but the fraction which did not produce urea adduct was quite toxic.

The fraction which produced urea adduct contains unchanged fatty acids of linseed oil, slightly polymerized products, aldehydes, *etc.* and a very little of autoxidized products. On the other hand, the fraction which did not produce urea adduct contains a little aldehyde, showing a high peroxide value and also contains some conjugated fatty acids which were produced by autoxidation.

As a result of the above, aldehyde does not have any conspicuous toxic effect but the actual harmful products in autoxidized fatty acids are some products with branched-chain fatty acids, lactone, polymerized products, conjugated acids and peroxide.

When unsaturated fatty acids are polymerized, nature of the products varies according to the temperature. At the lower temperature

peroxide value goes higher, while at the higher temperature it does not go up, as well verified by Atherton and Hilditch as did by the authors.

With highly unsaturated acids polymerized at the higher temperature and decreased iodine value, no toxic effect appeared at the animal test and the rats grew up well. This means the polymerized products of highly unsaturated acids without oxidized product are harmless, and some substances which contain conjugated linkage often turns out to be toxic as has been known well for a long time. The unsaturated fatty acids which autoxidized at the lower temperature, according to our experiment, contains about 6 per cent of conjugated acids, equivalent to 0.03 g. of daily feed for rats. The test of feeding experimentally about 5 times of conjugated acids of tung oil show harmless result.

According to the results gained, conjugated fatty acids are not considered to be the prime cause of producing toxic effect in the autoxidized unsaturated acids.

From the above results, the most harmful fraction contained in autoxidized unsaturated acids must be branched-chain fatty acids, lactone or peroxide.

Judging from these results, the authors assumed that the toxic effects should certainly be attributable mainly to the formation of preoxide-structure.

Supposing the main toxicity is to be attributed to peroxide, autoxidized product, which were freed from peroxide, must be quite harmless or slightly toxic. Then, using the same method as applied to the Lea's peroxide determination method (*11*), potassium iodide and acetic acid were added to the autoxidized liquid esters of linseed oil and freed from peroxide structure, and then checked how harmful the feed was. We found out, as expected, the toxicity was quite weak and the rats did not die (rats had slightly loose bowels).

The same experiment was applied to the autoxidized highly unsaturated fatty acids and the result was also harmless.

In other words, it was confirmed from the above result that the most harmful factor among the autoxidized fatty acids was peroxide structure.

The lethal dose for mice of the peroxide in autoxidized fatty acids was confirmed by the fact that about 278 mg. of total peroxide oxygen per kg. of mice caused the death of the half of the mice tested.

Now, the remaining question is that by what process and mechanism

the peroxide in autoxidized acids gives toxicity to the rats.

As the means of inquiring into this question, the authors asked Mr. Saito and Mr. Ushiki of Tokyo University, Medical Department to make sample tips of the internal organs of the rats which were fed with autoxidized acids and about to die. They have found that the tubuli uriniferi of kidney was extended at the border of medulla and cortical layers, and the cell-infiltration was observed at the mucous membrane of small intestine.

Moreover, the mitochondria were isolated from the rats liver with 0.25 *M* saccharose solution, and when one drop of autoxidized highly unsaturated acids added to half of this solution, the number of mitochondria decreased quickly. On the other hand, when the peroxide-free acids were added to another half, the mitochondria did not decrease. And we observed that the peroxide was found from fats of liver and muscle of rats when autoxidized unsaturated acids were fed.

Having concluded from the above results obtained, peroxide in the autoxidized fatty acids might directly affect the digestive organs of rats, or indirectly might be absorved, through the rats digestive organs, into the rats organs and give toxic effects to them by the peroxide itself or its secondary disintergrated product.

EXPERIMENTAL

Feeding Procedures—Weanling male and female rats obtained from our stock colony were depleted by feeding them with 9.5 g. of the fat-deficient diet per day per rat. When their body weight remained almost constant, they were separated into different groups.

TABLE I

Composition of Diets

Ingredients	Amount (%)	Ingredients	Amount (mg./100 g. diets)
Polished rice powder	83	Vitamin A	80 (U.S.P.U.)
Casein (ether extracted)	10	Tocopherol	3
McCollum salts mixture	3	Thiamine	0.125
Sample lipides	2.5	Riveflavin	0.1
		Pyridoxine	0.1
		Pantothenic acid	1
		Niacin	1.5
		Choline	100
		Inositol	200
		p-Aminobenzoic acid	0.5
		Vitamin B_{12}	0.0015

0.2–0.5 g. of sample lipides were daily administered per rat over a 30-day period to observe growth and other physiological conditions of these animals.

Experiment I

Preparation of Ethyl Ester of Liquid Acids of Linseed Oil—The mixed fatty acids of linseed oil were dissolved in aceton, fractionally crystallized in cold storage and separated the solid acids portion. The aceton of filtrate was distilled under vacuum. The obtained liquid acids were changed into ethyl ester in the presence of HCl-catalyst, and subjected to the vacuum distillation. Then the sample was purified by chromatography of alminium oxide.

The sample of autoxidized acids was prepared by leaving a 4 mm. layer of genuine liquid acids of linseed oil in an open basin at 30°, 50 hours, and 100 g. of this autoxidizes liquid ester was added to a hot solution of 700 g. urea in 1000 ml. of methanol. A precipitate was formed immediately, but the mixture was heated to effect complete solution, and then it was allowed to cool to the room temperature. The well-defined needles of urea complexes were filtered with suction (Fr. 1), and the filtrate was then dissolved to 65 per cent methanol solution and separated the petroleum ether soluble fraction (Fr. 2). Moreover, the petroleum insoluble fraction was extracted with ether (Fr. 3).

TABLE II

Diagram showing Scope of Fractionation of Autoxidized Liquid Acids of Linseed Oil by Urea Adduct Formation

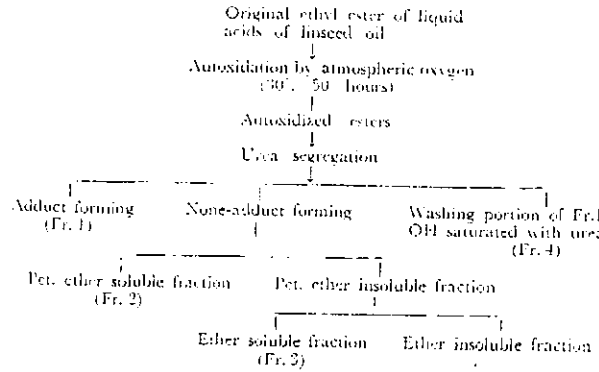

TABLE III

Analysis of the Samples Used in the Experiments

Sample ester	Yield (%)	Acid value	Sap. value	Iodine value	Peroxide value (S.C./kg.)	Kreis test	Conjugated acid (%) Diene	Triene	Tetraene
Original ethyl ester of liquid acids of linseed oil		0	176.01	168.28	0	—	4.97	0	—
Autoxidized esters of above acids		6.4	184.85	137.45	409	⊕	6.07	0.03	0
Fr. 1	74.9	3.5	177.30	143.44	33	⊕	1.01	0	—
Fr. 2	6.1	14.8	213.20	116.43	1216	⊕	30.15	0	0
Fr. 3	11.9	3.8	242.97	77.53	1585	⊖	17.63	0.69	—
Fr. 4	3.7		223.43	106.91	1203	—			—
Total	96.6								

TABLE IV

Feeding Records of Autoxidized Esters of Linseed Oil

(Period: From Mar. 16 to April 5, 1954)

Type of fat fed	Brood series	Sex	Body weight (g.) Initial	Last day	State of mortality
Autoxidized esters of linseed oil, 0.5 g.	C	♀	49	40	Died on 4th day
	C	♀	41	30	" 3rd "
	A	♀	39	44	" 3rd "
	A	♂	38	37	" 7th "
Fr. 1, 0.4 g.	C	♀	46	56	Survived over 20 days
	C	♀	48	64	" " "
	A	♂	51	79	" " "
Fr. 2, 0.2 g.	C	♀	46	46	Died on 2nd day
	C	♀	39	39	" " "
	A	♂	53	53	" " "
Fr. 3, 0.2 g	C	♀	48	48	Died on 2nd day
	C	♀	46	46	" " "
	A	♀	48	43	" 6th "
	A	♂	52	52	" 2nd "

From these results, it was made clear that the original autoxidized ester and Fr. 2 or Fr. 3 which did not form urea adduct showed the toxic effects upon the growth of rats, and they all died in 7 days. However, Fr. 1 (consisting mainly of un-autoxidized acids) did not give any retarding action to the rats.

Experiment II

Polymerized Esters of Highly Unsaturated Fatty Acids—Genuine ethyl ester of highly unsaturated acids are polymerized in the presence of Japanese acid clay at 120°, 6 hours in carbone dioxide. The properties of the samples were as follows:

TABLE V

Analysis of the Samples Used in the Experiments

	N_D^{20}	Iodine value	Sap. value	Peroxide value (m.m./kg.)
Ethyl ester of oleic acid (control)	1.4440	82.70	184.25	
Original ester of highly unsaturated fatty acids	1.4846	348.79	158.14	11
Polymerized ester of above acids	1.4854	310.62	156.23	62

TABLE VI

Feeding Record of Polymerized Ester of Highly Unsaturated Acids

(Period: April 16 to May 1, 1954)

Type of fat fed	Sex	Body weight gained in 15 days	Mean weight gained
None (Fat-free basal)	♀	8	8
	♀	8	
Ethyl ester of oleic acid (Control), 0.5 g.	♀	16	13
	♀	10	
Original ester of highly unsaturated acid, 0.5 g.	♀	19	17.5
	♂	17	
	♀	16	
	♀	18	
Polymerized ester of above acids, 0.5 g.	♀	20	18.7
	♀	19	
	♀	19	

Polymerized highly unsaturated acids show the nutritive effects to the rats and no retarding effects are noticed.

Experiment III

Nutritive Value of Peroxide-free Products of Autoxidized Unsaturated Fatty Acids—Autoxidized esters of unsaturated fatty acids are weighed into test tube, and the same weight of powdered potassium iodide is added. After adding glacial acetic acid and chloroform (2:1 by volume) 20 times as much as the quantity of the sample ester, carbon dioxide passed into the air space above the liquid to displace most of air, and the tube is heated. The peroxide-free products thus obtained are washed with sodium thiosulfate solution and water, then chloroform is distillated away *in vacuo*.

TABLE VII

Properties of Peroxide-free Products from Autoxidized Liquid Acids of Linseed Oil or Autoxidized highly Unsaturated Fatty Acids

	Iodine value	Sap. value	Peroxide value (S.C./kg.)
Original autoxidized liquid ester of linseed oil	137.45	184.85	415
Peroxide-free products of above acids	136.28		45
Original autoxidized ester of highly unsaturated acids	291.00	179.87	485
Peroxide-free products of above acids	301.04	170.64	33

TABLE VIII

Feeding Records of Peroxide-free Products of Autoxidized Linseed Ester

(Period: Mar. 26 to April 15, 1954)

Type of fat fed	Sex	Body weight		State of mortality
		Initial	Last day	
Autoxidized liquid ester of linseed oil, 0.5 g.	♀	50 g.	50 g.	Died on 2nd day
	♂	61	61	" 3rd "
	♀	82	94	" 11th "
Peroxidized-free products of above acids, 0.5 g.	♂	53	55	Survived over 3 weeks
	♀	50	50	" "
	♀	91	100	" "

TABLE IX

Feeding Records of Peroxide-free Products of Autoxidized Highly Unsaturated Fatty Acids

(Period: May 24 to June 11, 1954)

Type of fat fed	Sex	Body weight		State of mortality
		Initial	Last days	
Non (Fat-free basal)	♂	76 g.	92 g.	Survived over 18 days
	♀	67	77	" "
Autoxidized ester of highly unsaturated acids 0.5 g.	♂	72	72	Died on 7th day
	♀	73	70	" 4th "
	♀	71	61	" 3rd "
Peroxide-free products of above acids 0.5 g.	♂	77	96	Survived over 18 days
	♀	71	84	" "
	♂	75	84	" "

As it is obvious from Tables VIII and IX, peroxide-free products from autoxidized unsaturated fatty acids become non-toxic, and showed nutritive effects upon the growth of rats.

Moreover, linoleic acid was isolated from mixed fatty acids of soybean oil by urea complexes. (The yield of linoleic acid was 87.5 per cent.) Then we carried out the same experiment on the autoxidized liquid acids of linseed oil or highly unsaturated acids, and the same result was obtained.

From these results it has come to the conclusion that the most toxic structure in autoxidized fatty acids is peroxide.

Experiment IV

Lethal Dose of Peroxide Oxygen in Autoxidized Unsaturated fatty Acids to Mice—In order to survey the degree of the toxicity of peroxide produced in unsaturated fatty acids, 0.15–0.5 g. of autoxidized highly unsaturated acids (peroxide value 448 S.C./kg.) was given to mice and estimated the lethal dose of peroxide oxygen against mice.

TABLE X

Lethal Dose of Peroxide Oxygen in Autoxidized Highly Unsaturated Acids Against Mice

Total peroxide oxygen in samples fed to mice *mg.*	1.79	3.58	5.017	7.16
Mortality	0/12	2/12	6/12	10/12

From these results, the lethal dose was not estimated accurately, however, if calculated with 5.017 mg. peroxide oxygen, the LD_{50} is 278 mg. of total peroxide oxygen per kg. of mice.

Experiment V

Peroxide Content of Fats Separated from Liver and Muscle of Rats which Were Fed with Autoxidized Unsaturated Fatty Acids—In order to investigate the origin of toxicity of autoxidized unsaturated acids, the authors determined the peroxide contents of fats of liver and muscle of rats when the autoxidized acids were fed.

The rats which received 0.5 g. of autoxidized fatty acids per day per rat were killed when the retarding action was noticed. The liver and muscle of rats were separated from rats, treated with Na_2SO_4 and the fats were extracted with diethyl ether.

TABLE XI

Peroxide Value of Fats Separated from Liver and Muscle of Rats which Were Fed with Autoxidized Unsaturated Fatty Acids

Type of sample oil	Peroxide value of sample oil (S.C./kg.)	Peroxide value (S.C./kg.)	
		Liver oil	Muscle oil
None (Fat-free basal)			3
Ethyl ester of oleic acid (control)	0		2
Original ester of highly unsaturated acids	40		14
Autoxidized products of above acids	1405		37
Slightly autoxidized ester of liquid acids of linseed oil	409	20	
Autoxidized ester of liquid acids of linseed oil	3170	89	

No peroxide was found from tissues of the controlled rats which received unautoxidized oleic acid, however, rats fed with autoxidized unsaturated fatty acids contained a large quantity of peroxide in liver and muscle.

DISCUSSION

The survey of the above results leads us to assume that both autoxidized highly unsaturated fatty acids and autoxidized unsaturated fatty acids are all harmful for the rats, and we confirmed that the toxicity is actually caused by the peroxide product.

According to the Duboulozes (*12*), the half of the peroxide was found in the digestive tract of the rats 1.5 hours after the feeding was given to the rats through their mouths.

The authors as well confirmed that considerable volume of peroxide was in the muscle of the rats after autoxidized acids were fed to them.

Based upon these results, it might possibly be considered that the toxicity caused on the rats could be sorted into the direct action which the peroxide gives to the rat's digestive tract or their other internal systems and the indirect one which the absorbed peroxide itself or its secondary disintegrates give toxic effects to tissues.

In other words, just as reported in the previous paper that the rats which were contacted with the autoxidized acids exhibited a depilation around their mouths and legs and also some inflammation, the autoxidized acids are considered to affect the rat's internal systems, attesting it by the fact that the small intestines were injured. Autoxidized acids accumulated in the rat's liver and muscle, which as previously verified, tend to decrease mitochondria in the livers, are assumed to work upon various enzymes, resulting the decrease of enzyme function. However, further research should be made in this respect.

SUMMARY

In the previous paper it has been established that the nutritive value of highly unsaturated fatty acids is not much lower than that of oleic acid, and that generally-believed toxicity of highly unsaturated acids is not produced by the acids themselves but actually by the formation of autoxidized matters.

The observation led us to an assumption that natural unsaturated fatty acids, even if different in the autoxidation degree, would always become less nutritious by some degree of autoxidation.

The assumption was confirmed by the toxic effect on rats of the autoxidized product which was prepared from the ethyl ester of liquid acids of linseed oil, as the rats all died in a few days after the feeding.

On the basis of these findings, our efforts have been extended to throw light on the nature of the above-mentioned toxic product. The method used for and the result obtained from the present test are as follows:

1. The autoxidized products were prepared by leaving the original

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 270 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.

REFERENCES

(1) Kaneda, T., and Ishii S., *J. Biochem.*, **41**, 327 (1954)
(2) Privett, O. S., Lundberg, W.O., and Nickell, C., *J. Am. Oil Chem. Soc.*, **30**, 17 (1953)
(3) Chang S. S., and Kummerow F. A., *J. Am. Oil Chem. Soc.*, **30**, 403 (1953)
(4) Zilch K. T., *Anal. Chem.*, **23**, 775 (1951)
(5) Fugger, J., Cannon, J.A., Zilch, K.T., and Dutton, H.J., *J. Am. Oil Chem. Soc.*, **28**, 285 (1951)
(6) Cannon J. A., *J. Am. Oil Chem. Soc.*, **29**, 452 (1952)
(7) Atherton D., and Hilditch T. P., *J. Chem. Soc.*, 105 (1944)
(8) Catravas, G. N., and Knafo, G., *Bull. mens. inform. ITERG*, **6**, 34 (1952); *Oleagineux*, **8**, 79 (1953)
(9) Coleman, J. E., Knight, H.B., and Swern, D., *J. Am. Chem. Soc.*, **74**, 4886 (1952)
(10) Swern D., *J. Am. Chem. Soc.*, **75**, 3135 (1953)
(11) Lea C. H., Rancidity in edible fats (1953)
(12) Dubouloz P., Fondarai J., and Lagarde C., *Acta Biochem. et Biophys.*, **3**, 371 (1949)

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.

LITERATURE CITED

GYORGY, P. 1950 Vitamin Methods. I. Academic Press, Inc., New York. Section on microchemical methods by O. A. Bessey. Pages 292–293, 305–308.

NORTHEAST REGION 1951 Cooperative nutritional status studies in the Northeast Region. I. Techniques. Northeast Reg. Pub. no. 5. Memoir 307. Cornell Univ. Agr. Exp. Sta.

MORSE, E. H., M. POTGIETER AND G. R. WALKER 1956 Ascorbic acid utilization by women. Response of blood serum and white blood cells to increasing levels of intake. J. Nutrition, *58:* 291.

ROE, J. H., AND C. A. KUETHER 1943 The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem., *147:* 399.

STEELE, B. F., R. L. LINER, Z. H. PIERCE AND H. H. WILLIAMS 1955 Ascorbic acid nutriture in the human. II. Content of ascrobic acid in the white cells and sera of subjects receiving controlled low intakes of the vitamin. J. Nutrition, *57:* 361.

WATT, B. K., AND A. L. MERRILL 1950 Composition of Foods — Raw, Processed, Prepared. U. S. D. A. Agriculture Handbook no. 8.

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

HANS KAUNITZ, C. A. SLANETZ, R. E. JOHNSON AND J. GUILMAIN
College of Physicians and Surgeons, Columbia University, New York, N. Y.[2]

H. B. KNIGHT, D. H. SAUNDERS AND DANIEL SWERN
Eastern Regional Research Laboratory, Philadelphia, Pa.[3]

(Received for publication May 21, 1956)

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.

[2] From the Department of Pathology and the Institute of Research in Animal Diseases, Columbia University, New York, N. Y.

[3] A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

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 [4] 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.

[4] 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, was supplied by the Sterling-Winthrop Research Institute, Rensselaer, N. Y., and the crystalline beta-carotene, by the Barnett Laboratories, Long Beach, California.

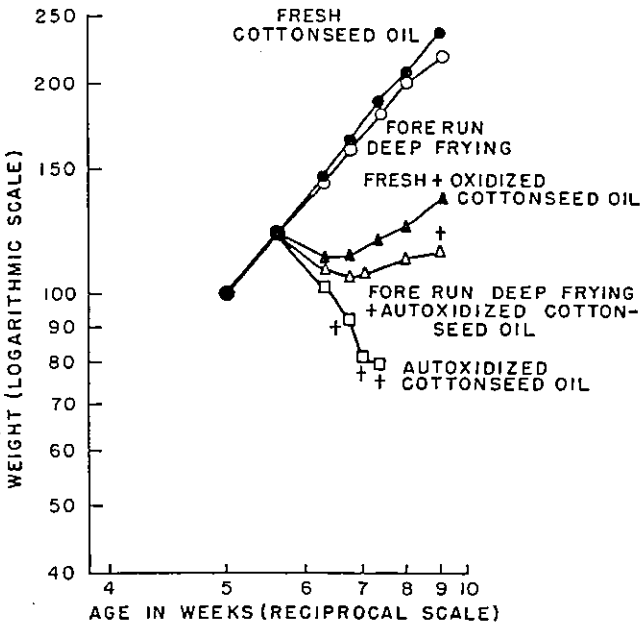

Fig. 1 Influence of the distillate from the molecular distillation of a hydrogenated vegetable oil after its use for deep frying for 80 hours. Each curve is based on the average of 8 well-matched male rats. After the third week of the experiment, the difference in weight of the groups fed oxidized plus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

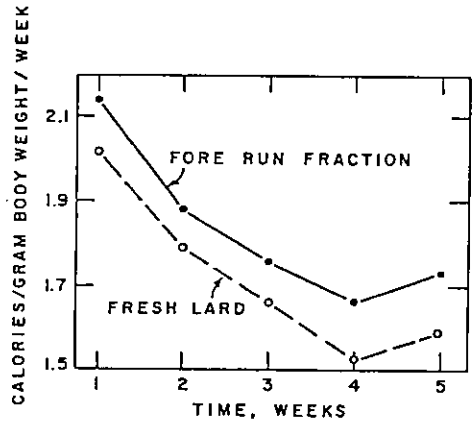

Fig. 2 Influence of fresh lard and the distillate from the molecular distillation of a hydrogenated vegetable oil previously used for deep frying for 80 hours upon the caloric requirements of matching male rats maintained at constant weight. Each curve is based on the average of 8 animals.

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

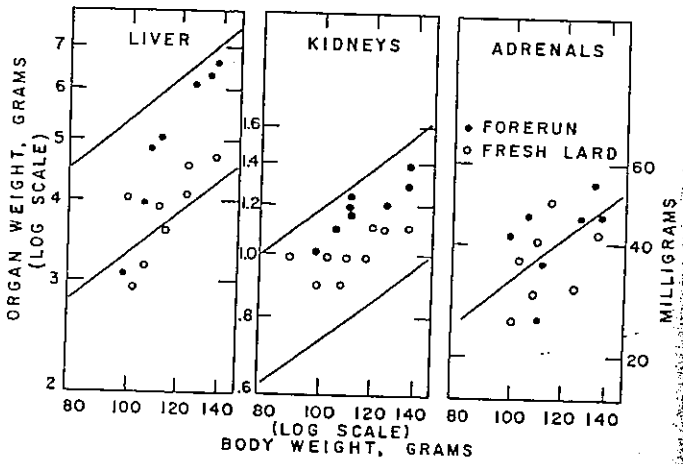

Fig. 3 Influence of fresh lard and distillate from the molecular distillation of a hydrogenated vegetable oil previously used for deep frying for 80 hours upon the organ weight-body weight relationships of male rats kept at constant weight by restricted feeding for 5 weeks. On the log — log plot, the 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.

LITERATURE CITED

KAUNITZ, H. 1953 Studien über die Ernährung von Ratten mit hoch-oxydierten Fetten. Arch. exper. Path. u. Pharmakol., *220:* 16.

KAUNITZ, H., C. A. SLANETZ AND R. E. JOHNSON 1955 Antagonism of fresh fat to the toxicity of heated and aerated cottonseed oil. J. Nutrition, *55:* 577.

KAUNITZ, H., C. A. SLANETZ, R. E. JOHNSON, H. B. KNIGHT, D. H. SAUNDERS AND DANIEL SWERN 1956 Biological effects of the polymeric residues isolated from autoxidized fats. J. Am. Oil Chemists' Soc. In press.

——— 1955 Nutritional effects of fractions of heated and autoxidized lard and cottonseed oil. Federation Proc., *14:* 408.

KAUNITZ, H., H. WIESINGER, F. C. BLODI, R. E. JOHNSON AND C. A. SLANETZ 1954 Relation of protein and fat intake to growth and corneal vascularization in galactoflavin-produced-ariboflavinosis. J. Nutrition, *52:* 467.

QUIMBY, F. H. 1948 Food and water economy of the young rat during chronic starvation and recovery. Ibid., *36:* 177.

ZUCKER, L. M., AND T. F. ZUCKER 1942 A simple time-weight relation observed in well nourished rats. J. Gen. Physiol., *25:* 445.

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

ROBERT A. MACLEOD AND J. F. MURRAY

The Fisheries Research Board of Canada, Technological Station, Vancouver, B. C., Canada

(Received for publication June 4, 1956)

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.)	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	'Benadryl' (50 mgm.) by mouth	4	8·5	37·3
Dummy by mouth	7	10·9	43·8	'Phenergan' (50 mgm.) by mouth	7	3·9	23·6
Iontophoresis of 0·9 per cent saline	3	—	46·9	Iontophoresis of 2·0 per cent 'Phenergan'	3	—	37·5

time and the results expressed (1) as the mean area enclosed by this curve[1], and (2) as the latent period of the response[2]. 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 anæsthetic action of promothazine hydrochloride[3,4]. 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[5,7], 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.*[4].

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

G. C. Whirrow

Department of Physiology,
University of Malaya,
Singapore.
June 2.

[1] Lewis, T., *Heart*, 15, 177 (1930).
[2] Wolff, H. H., and Pochin, E. E., *Clin. Sci.*, 8, 145 (1949).
[3] Duff, F., Greenfield, A. D. M., Shepherd, J. T., Thompson, I. D., and Whelan, R. F., *J. Physiol.*, 121, 46 (1953).
[4] Halpern, B. N., Perrin, G., and Dews, P., *C.R. Soc. Biol.*, 141, 1125 (1947).
[5] Greenfield, A. D. M., Shepherd J. T., and Whelan, R. F., *J. App. Physiol.*, 4, 785 (1952).
[6] Winter, C. A., *J. Pharmacol.*, 91, 7 (1948).
[7] Glaser, E. M., *Brit. J. Pharmacol.*, 8, 137 (1953).

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. If 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

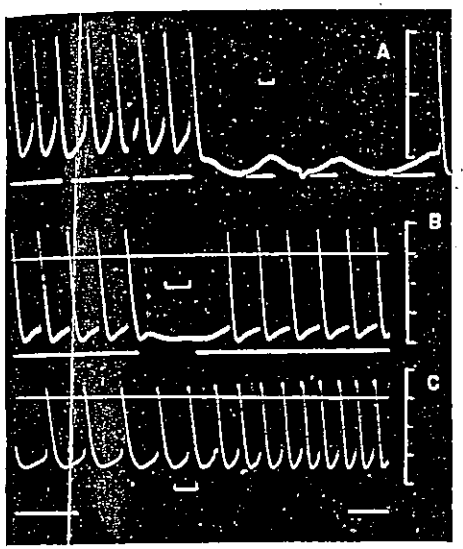

Fig. 1. Intracellular records from the sinus venosus of a frog's heart. Vagal stimulation indicated by breaks in the lower beam. (*A*) Vagi stimulated at 10 per sec. during the repolarization of the third action potential. During the eighth action potential, vagal stimulation starts again (see text). 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.

O. F. Hutter
W. Trautwein

Wilmer Institute,
The Johns Hopkins Hospital and University,
Baltimore, Md. April 20.

[1] Burgen, A. S. V., and Terroux, Kathleen G., *J. Physiol.*, 120, 410 (1953); Hoffmann, B. F., and Suckling, E. E., *Amer. J. Physiol.*, 173, 312 (1953).
[2] Weidmann, S., *J. Physiol.*, 115, 227 (1951). Trautwein, W., and Zink, E., *Pflügers Arch.*, 256, 68 (1952).
[3] Gaskell, W. H., *J. Physiol.*, 8, 404 (1887).
[4] Howell, W. H., and Duke, W. W., *Amer. J. Physiol.*, 21, 51 (1908).
[5] Lehnartz, E., *J. Physiol.*, 86, 37 P (1936).

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	0·67	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.

N. V. Raju
R. Rajagopalan

Department of Biochemistry,
Indian Institute of Science,
Bangalore 3.
Nov. 23.

[1] Roy, A., *Ann. Biochem. and Exp. Med. (India)*, 4, 17 (1944).
[2] Roffo, A. H., *Biol. Abst.*, 19, 922 (1945). Beck, S., and Peacock, P. R., *Brit. Med. J.*, 1, 81 (1941).
[3] Morris, H. P., and Larsen, C. D., *J. Nat. Cancer Inst.*, 4, 235 (1943).
[4] Crompton, E. W., Farmer, F. A., and Berryhill, F. M., *J. Nutrition*, 43, 431 (1951). Crompton, E. W., Common, R. H., Farmer, F. A., Berryhill, F. M., and Wisrblatt, L., *ibid.*, 43, 533 (1951); 44, 177 (1951). Crompton, E. W., Common, R. H., Farmer, F. A., Wells, A. F., and Crawford, D., *ibid.*, 49, 333 (1953).
[5] Kaunitz, H., *Arch. Exp. Path. Pharmakol.*, 220, 16 (1953).
[6] Chalmers, J. G., *Biochem. J.*, 56, 487 (1954).

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].

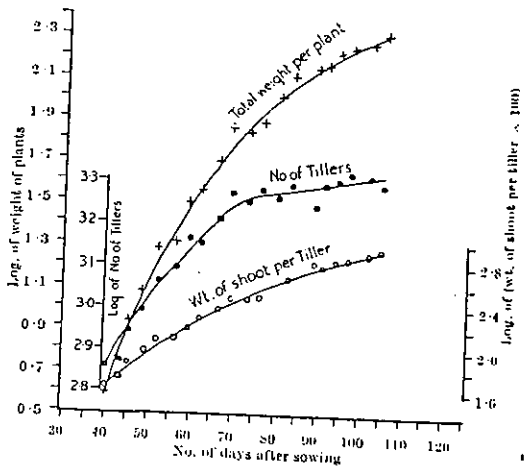

Fig. 1

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.

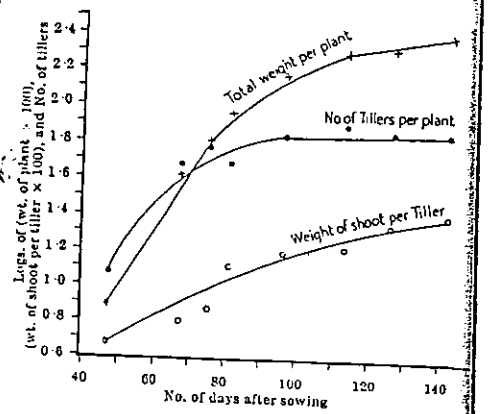

Fig. 2

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.

Arthur Troughton

Welsh Plant Breeding Station,
University College of Wales,
Aberystwyth.
March 11.

[1] Cooper, J. P., *Nature*, 161, 894 (1948).
[2] Troughton, Arthur, *J. Auric. Soc. Univ. Coll. Wales*, 35, 29 (1954).
[3] Cooper, J. P., *J. Ecol.*, 39, 228 (1951).

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].

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.

P. R. Wilkinson

Veterinary Parasitology Laboratory,
Division of Entomology,
Commonwealth Scientific and
Industrial Research Organization,
Yeerongpilly, Queensland.

[1] Hitchcock, L. F., *Aust. J. Agric. Res.*, 4, 360 (1953).
[2] Legg, J., C.S.I.R. Aust. Pamphlet No. 56 (Melbourne, 1935).

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.

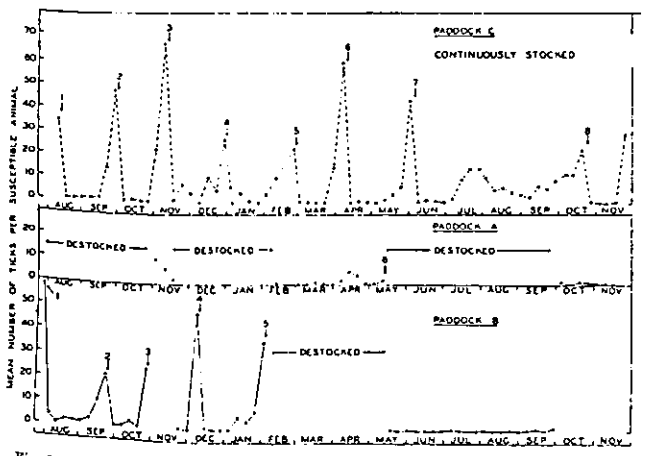

Fig. 1. Comparison of infestations of cattle on a continuously stocked and a pasture spelling paddock. Counts of female ticks, 0·6 cm. and upwards in length, on the right side of the cattle

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,