

# Nutritive Value of Methyl Linoleate and Its Thermal Decomposition Products

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Methyl linoleate was heated for 10 hrs. at 300°C. in the absence of air and fractionated by alembic distillation and urea adduct-formation.

Intestinal absorptions of the urea adduct-forming monomeric nonadduct-forming monomeric, and dimeric fractions were determined. It was found that dimers were half as well absorbed as the monomers.

When fed to rats, dimers were better accepted and exhibited some toxicity symptoms different from the nonadduct-forming monomers. The dimers caused diarrhea, irritability, and loss of hair during the early period of administration. The nonadduct-forming monomers were lethal and produced an increase in liver weight. Both fractions depressed growth.

**H**EATING of unsaturated oils in the absence of air is known to result in the formation of a mixture of monomeric and polymeric substances, both linear and cyclic. For reviews on the nutritional significance of such compounds the reader is referred to the papers of Brown (1) and of Rice *et al.* (2).

Crampton and co-workers (3,9) have been able to demonstrate that, when linseed, soybean, and sunflower oils are heated at approximately 275°C. for 12 to 26 hrs. in a CO<sub>2</sub> atmosphere, a polymeric fraction, which is very slightly absorbed from the intestine of the rat, and a nonurea adduct-forming monomeric fraction, which is harmful to the same animal, are formed. Comparison of the fatty acid composition of these oils with their deleterious action indicates that linolenic acid is the main precursor. Nevertheless some toxicity has been found to develop in heated oils containing relatively high proportions of linoleic acid and no linolenic acid.

More unsaturated oils, such as fish oils, seem able to develop toxic effects and form poorly absorbed substances if heated and administered under similar conditions (10-15).

Methyl and ethyl linoleate seem also capable of forming cyclic and polymeric substances by heating, as has been suggested by Paschke *et al.* (16,17).

In order better to characterize the toxic derivatives and their biological properties, relatively pure methyl linoleate was prepared, heated in the absence of air, then fractionated. The fractions were tested for intestinal absorption and deleterious action in the rat.

Recently Kaunitz *et al.* (18) have made a similar study on thermal oxidation products of the same ester.

## Experimental

*Preparation of the Fractions.* Methyl linoleate was prepared from the methyl esters of commercial sunflower seed oil<sup>2</sup> by the urea-adduct procedure (19). The esters were heated at 300°C. for 10 hrs. in the presence of CO<sub>2</sub> in an apparatus similar to that described by Bradley and Johnston (20). The heated product was fractionated by alembic distillation under reduced pressure into a distillable monomeric fraction and a residual polymeric fraction. The latter, as may be seen from the data in Table I, is essentially dimeric.

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<sup>2</sup> Generously supplied by Alba, S.A., Argentina.

TABLE I  
Typical Properties of Tested Products

Fraction	Iodine number <sup>a</sup>		Molecular weight <sup>b</sup>	
	Exptl.	Theor.	Exptl.	Theor.
Methyl linoleate.....	154.5	172.4	287 <sup>c</sup>	294.4
Heated methyl linoleate <sup>d</sup> .....	103.3	....	....	....
Distillable monomers <sup>d</sup> .....	111.1	....	290.5	294.4
Adduct-forming monomers <sup>d</sup> .....	114.9	172.4	....	294.4
Nonadduct-forming monomers <sup>d</sup> .....	95.2	85.6	....	294.4
Polymers <sup>d</sup> .....	92.2	D = 85.6 <sup>e</sup> T = 57.5	640	D = 588 T = 882

<sup>a</sup> Hanus, 30 min.

<sup>b</sup> Cryoscopy in benzene.

<sup>c</sup> From saponification value.

<sup>d</sup> Methyl linoleate was heated at 300°C. for 10 hrs. in the presence of CO<sub>2</sub> and fractionated by distillation into distillable monomers, and polymers. The distillable monomers were later separated into urea adduct-forming monomers and nonadduct-forming monomers.

<sup>e</sup> D = dimers; T = trimers.

The monomeric fraction was then separated by means of the procedure of Wells and Common (8) into urea adduct-forming monomers and nonurea adduct-forming monomers. Some of the chemical characteristics of the prepared methyl linoleate and its products of heating are shown in Table I. Iodine number was determined by the Hanus (30 min.) procedure. Mean molecular weights were obtained by means of cryoscopy in benzene.

*Determination of the Absorption Coefficient.* The technique described by Irwin *et al.* (21) and by Deuel *et al.* (22) for the determination of intestinal absorption was followed with minor modifications. Adult female albino rats belonging to the strain of the Institute and weighing between 165 and 300 g. (average 233 g.) were fasted for 48 hrs. They were fed by stomach tube approximately 300 mg. of accurately weighted ester per dm<sup>2</sup> of body surface. After 4 hrs. the rats were sacrificed with ether, and the gastrointestinal tract was flushed with 150-ml. portions each of physiological saline, petroleum ether, and saline in that order. The aqueous-etheral extract was acidified, and the fat in the ether phase was determined gravimetrically. For control the fat was extracted from the intestinal contents of similarly conditioned animals not given the test material. The absorption coefficient was calculated from the difference between the fat content of the control and test animals. The method was tested by giving separately adduct-forming monomers and dimers to rats in identical fasting conditions and by immediately washing the digestive tract. The absorption coefficients were expressed as mg. of fat absorbed per dm<sup>2</sup> of body surface per hour. The percentage of fat recovered was also determined. The data from all animals which exhibited diarrhea were discarded (Table II).

*Test of Biological Action.* Adult male albino rats weighing between 144 and 233 g. (average 186 g.) were distributed in groups of 10 animals each. One group was made up of only six rats because of the small amount of nonadduct-forming monomers available. During periods of 25 to 36 days the group 1 animals were fed a fat-free diet and the other groups a diet containing 10% by weight of the following lipids: group 2, methyl linoleate; group 3, adduct-forming monomers; group 4, dimers; group 5, non-

TABLE II

Absorption Coefficients of Thermal Decomposition Products of Methyl Linoleate

Fraction	No. of expts.	Avg. weight of rats (g.)	Absorp. period (hrs.)	Absorp. coeff. <sup>b</sup> (mg./dm <sup>2</sup> /h)	Ester absorbed (%)
Adduct-forming monomers.....	12	204	4	43.3 ± 5.5 <sup>c</sup>	55.6
	3	266	0		89.5
Nonadduct-forming monomers.....	6	203	4	45.8 ± 9.6	62.4
Dimers.....	7	232	4	25.5 ± 5.0	31.7
	9	246	0		76.4
None.....	10	241	0	Average recovery 26.4 ± 18.1 mg./rat	

<sup>a</sup> Methyl linoleate was heated at 300°C. for 10 hrs. in the presence of CO<sub>2</sub> and fractionated by distillation into distillable monomers, and polymers. The distillable monomers were later separated into urea adduct-forming monomers and nonadduct-forming monomers.

<sup>b</sup> Determined by the procedure of Irwin *et al.* (21) and Deuel *et al.* (22).

<sup>c</sup> Standard deviation.

adduct-forming monomers; group 6, corn oil (Mazola); group 7, dimers plus corn oil (1 + 1). The composition of the diets is presented in Table III. Rations were prepared daily in order to prevent alteration of the fat and were placed in the cages in metal boxes with lids. A circular hole in the lid allowed easy access to feed and prevented losses. Feed was given *ad libitum* to all groups except group 6, which received 5 g. of feed per day per rat. The animals were weighed daily. Groups 5 and 6 were kept in individual cages because otherwise the weaker or dead animals were eaten by the others. It was also necessary to isolate the rats of group 4 because their diarrheic feces acquired a varnish consistency which kept the animals stuck to each other and to the floor. At the end of the experimental period the rats were sacrificed with gas, and their livers were weighed and histologically studied (Table V).

### Results and Discussion

**Degree of Absorption.** The amount of fat found in the intestine of fasted rats (Table II), averaging 26.4 mg. per rat, agrees with previously reported values (22). The recovery of adduct forming monomers (about 90%) indicates the acceptable capability of the procedure for washing the digestive tract. Published recoveries obtained with different oils and procedures are of the same order of magnitude (22). On the other hand, the amount of fat obtained from the intestine by washing immediately after giving dimers was lower than after other fractions even though the dimers are less well absorbed than monomers, as shown by the data from the 4-hr. absorption period. No correction for this anomaly was applied. The recorded values show no differences in the absorption coefficients of the adduct-forming and nonadduct-forming monomeric substances tested but do show differences between the monomers and dimers.

**Biological Action.** The growth curve (Fig. 1) of the group fed adduct-forming monomers [consisting, as found by Paschke *et al.* (16,17) of a main portion of normal methyl linoleate and about 14% of its conjugated isomer] shows no striking difference as compared with the controls fed methyl linoleate (group 2) or the fat-free diet (group 1). The growth curves of groups 4 and 5, which received dimers and nonadduct-forming monomers, respectively, are remarkably abnormal. After only two days on experiment the average weight of group 4 was less than that at the beginning, even with good acceptance of feed. At the third day the animals had marked diarrhea, accompanied by colorless fluid feces at the moment of

TABLE III  
Composition of the Diets

Component	Percentage in the diet	
	Fat-free	With fat
Sucrose.....	72	62
Casein.....	20	20
Ester or oil-tested.....	0	10
Yeast (dry).....	4	4
Salts (McCallum-Davis).....	4	4
Supplement:		
vitamin A, 200 i.u./100 g.		
vitamin D, 20 i.u./100 g.		

excretion, which became darker, semisolid, and sticky in the contact with the air and acquired a varnish aspect. After five days the rats showed pronounced irritability, being almost constantly in a fighting position, one in front of the other. Hair became yellow and absent in small but increasing areas. Both diarrhea and the loss of weight and hair continued until around the 15th day, after which slight improvement in the general aspect was noted with diminishing diarrhea and loss of hair, and an increase in weight. The growth curve of this group during the first two weeks coincides remarkably with that of group 6, which was restrictively fed. After two weeks the curves separate; the weight of group 4 increases slightly and that of group 6 decreases steadily. Feed consumption of group 4 (Table IV) averaged 10.0 g./day/rat during the first two weeks and 14.6 g./day/rat for the whole experimental period, indicating that loss of weight cannot be explained as resulting from the rejection of feed by the animals. The results could be due to an adverse effect of the dimers on the utilization of the other nutrients, as suggested by Raulin and co-workers (23). As can be seen in Table V, rats receiving the dimer diet for 25 days showed a smaller ratio of body to liver weight than animals fed identical fat for 36 days. The values in the latter test were at the level of the controls. The number of dead animals in

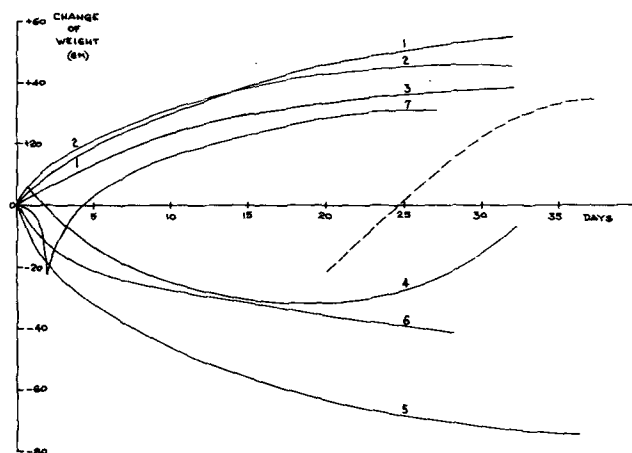


FIG. 1. Change, with time, of the weights of rats fed methyl linoleate and its thermal decomposition products.

Methyl linoleate was heated at 300°C. for 10 hrs. in the presence of CO<sub>2</sub> and fractionated by distillation into distillable monomers, and polymers. The distillable monomers were later separated into urea adduct-forming monomers and nonadduct-forming monomers.

Curve No. 1 corresponds to a group fed a fat-free diet. The other curves correspond to groups fed diets containing 10% by weight of the following lipids: No. 2, methyl linoleate; No. 3, adduct-forming monomers; No. 4, dimers; No. 5, nonadduct-forming monomers; No. 6, corn oil; No. 7, dimers plus corn oil (1 + 1). Feeding was *ad libitum* in all groups except in group 6, which received only 5 g. of feed per day per rat. The dotted line represents the change of weight of a group fed dimers (group 4) until the 20th day, and corn oil afterwards.

TABLE IV  
Feed Consumption

Group No.	Fat in the diet	Duration of the experiment (days)	Consumption (g./day/rat)
1.....	None	25	15.8
2.....	Methyl linoleate	25	12.0
3.....	Adduct-forming monomers <sup>a</sup>	32	11.7
4.....	Dimers <sup>a</sup>	36	14.6
5.....	Nonadduct-forming monomers <sup>a</sup>	36	6.2
6.....	Corn oil	25	5.0 <sup>b</sup>
7.....	Dimers + corn oil (1+1)	25	12.8

<sup>a</sup> Methyl linoleate was heated at 300°C. for 10 hrs. in the presence of CO<sub>2</sub> and fractionated by distillation into distillable monomers, and polymers. The distillable monomers were later separated into urea adduct-forming monomers and nonadduct-forming monomers.

<sup>b</sup> Restricted.

group 4 was one to ten, similar to the ratio of some of the control groups accordingly considered normal.

In a separate experiment, rats given a diet of 10% dimers for 20 days were then fed the same proportions of corn oil. After 12 days improvement in the general condition was observed, including an increase of weight to normal levels (Fig. 1), growth of hair, and disappearance of diarrhea. The growth curve corresponding to group 7, which was fed a mixture of equal parts of dimers and corn oil, runs slightly under the control curves, but the difference seems to be insignificant. The aspect of the animals was completely normal, indicating either a dilution of dimers to nontoxic levels or a protection by the nonheated oil, as previously pointed out by others (24).

The above evidences suggest that the dimeric fraction, although absorbed in relatively small degree, has a weak toxic effect to which the rat appears to develop a tolerance. The toxic effect also disappears as soon as other fat is substituted for dimers in the diet or fresh nontoxic fat is added to the diet.

The group fed nonadduct-forming monomers (group 5) showed rather different characteristics. Loss of weight was much more marked than in groups fed dimers and corn oil. Hair became yellowish after five days but did not fall out. At the end of the 32-day period the only surviving rat looked meager and inactive. There was one death on the 14th day, two on the 15th, and one each on the 18th and 23rd days. The daily average feed consumption of 6.2 g./day/rat was small as compared with that of 11.7 to 15.8 g./day/rat of the control groups (Table IV). However it was larger than the 5 g./day/rat of the corn oil-fed group. The loss of weight was also much more remarkable than in this last group, thus indicating a deleterious effect. The ratio of body weight to liver weight (Table VI) was notably diminished in the only animal of this group that was autopsied.

It is thus evident that nonadduct-forming monomers from methyl linoleate possess a lethal effect although to a lesser degree than similar fractions from heated linolenic acid-rich oils (6). This weaker toxic activity, as well as the very small amounts of this

TABLE V  
Ratio of Body to Liver Weight<sup>a</sup>

Group No.	No. of rats	Fat in the diet	Avg. body weight (g.)	Avg. liver weight (g.)	Body weight/ liver weight (g./g.)
1.....	5	None	243	10.2	23.8
2.....	5	Methyl linoleate	260	12.4	21.0
3.....	5	Adduct-forming monomers <sup>b</sup>	233	10.2	22.8
4 <sup>c</sup> .....	2	Dimers <sup>b</sup>	202	12.7	15.9
4 <sup>d</sup> .....	7	Dimers <sup>b</sup>	171	7.8	21.9
5.....	1	Nonadduct-forming monomers <sup>b</sup>	108	9.4	11.5

<sup>a</sup> Liver histology was normal in all animals.

<sup>b</sup> Methyl linoleate was heated at 300°C. for 10 hrs. in the presence of CO<sub>2</sub> and fractionated by distillation into distillable monomers, and polymers. The distillable monomers were later separated into urea adduct-forming monomers and nonadduct-forming monomers.

<sup>c</sup> Sacrificed after 25 days of feeding.

<sup>d</sup> Sacrificed after 36 days of feeding.

fraction formed on heating linoleic acid-rich oils, may explain the lack of harmful effects found when sunflower seed and soybean oils were tested by Crampton.

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