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Influence of Heat on Oxidative Stability and on Effectiveness of Metal-Inactivating Agents in Vegetable Oils[1]

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OUR STUDIES on lecithin as an edible oil stabilizer lead to the observation that acidic metal inactivators are not effective in undeodorized oils (4). Thus heating the oils may be necessary if an improvement in oxidative stability is to be obtained through the use of metal inactivators. The improvement in oxidative stability after heat treatments is observed in the processing of many foods. Most of the beneficial effects result from the destruction of enzymes, but heating at temperatures above those required for enzyme destruction give optimum stability.

The best example of oxidative stability resulting from heat treatments in food processing is the manufacture of dried milk (2, 6, 8, 13). Unshelled pecans (15) heated to 80°C. were found to be more stable to rancidity than the unheated controls. Walnut meats however, when blanched at 100°C., are reported to be considerably less stable to oxidation (17). Oil extracted from green coffee beans showed no improvement upon heating, but oil extracted from roasted coffee was much more stable (3). Butter showed a marked improvement upon heating to 300–400°F., but butter fat showed a decreased stability upon heating under the same condition (9). Lips (12) found that lard was not improved by heating unless certain additives, such as whey powder, were present.

The heat-imparted stability of fats is usually considered to be a direct result of peroxide destruction. We believe other factors are involved, but their elucidation is complicated. Studies on antioxidants and autoxidation are severely hampered by lack of adequate analytical methods and techniques. Baldwin (1), investigating the deodorization of corn oil, observed an optimum improvement in the stability with time of deodorization. Comparison between samples prepared by laboratory and plant deodorizations showed that temperature of about 195°C., not time, was the critical factor.

Fat peroxides are considered unstable, especially at temperatures above 100°C. Nevertheless some evaluation tests for shortening require holding the fat at 100°C. for more than 100 hrs. Other oxidative tests which depend on the development of a definite level of peroxides for the end point have used temperatures of 120° and 150°C. (11, 16). The rates of decomposition of fatty hydroperoxides have not been investigated at these higher temperatures. Our investigations on edible oils would indicate that, at a temperature of 185°C., the destruction of fatty hydroperoxides is accomplished within 30 min. Privett (20) studied destruction of hydroperoxides of lard at 100°C. under vacuum and found a 50% loss in about 14 hrs. Methyl linoleate hydroperoxide is reported to decompose at a rate of 1.6% per hour at 80°C. (7). The half-life for methyl linoleate hydroperoxide at 80°C. with an initial peroxide value of 1,222 is given as 28 hrs. (19).

Methods and Materials

Most of the oils investigated were commercially extracted, crude oils which were refined in the laboratory. A peanut oil was the only cold-pressed oil. The corn oil was hexane-extracted from wet, milled, whole corn germ in a special pilot-plant extraction, where care was taken to avoid temperatures above 95°C. during solvent stripping. The cottonseed oil was obtained as a straight, extracted crude oil, not as a mixture of prepressed and extracted oils. Commercial processors indicated that the crude oil samples had not been subjected to excessive temperatures at any time during processing. A sample of commercially refined and bleached soybean oil was also included in the study.

Oils were refined and bleached in accordance with A.O.C.S. methods. The oil samples were heated in individual, 1-liter deodorizers equipped with steam generators. Heating was done under vacuum (less 1 mm.), and agitation of the sample was accomplished by the water vapor supplied by the generator under the specified conditions of operation. No apparent change was observed in the color or condition of the oils submitted to the shorter heating times and lower temperatures. Higher temperatures and longer heating times, which approached deodorization conditions, gave the usual bleaching effect. Alcoholic solutions of the stabilizers were added to the oil after heating. In the A.O.M. stability determination the solvent was allowed to evaporate during the course of aeration. Oxidative stability data were obtained by subjecting the oil to the usual A.O.M. aeration conditions for 8 hrs. Values are reported as milliequivalents of peroxide per kilogram of oil.

Oxygen absorption studies were carried out on apparatus designed to yield samples of sufficient size (180 g.) for taste-panel evaluations. Samples were constantly shaken so that the oil was saturated with oxygen at all times; temperature was thermostatically controlled at 60°C. The oxygen absorption was calculated by the pressure drop, indicated by a manometer within the constant volume system. When stabilizer solutions were added, the solvents were removed from the oil by warming under reduced pressure before submitting the sample to an oxygen absorption test. Each 11.2 ml. of oxygen absorbed by a kilogram of fat is equal to one peroxide unit if only peroxide formation is assumed. The peroxide content of the oil and the ml. of oxygen absorbed

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agreed within 10% when the tests are conducted at 60°C.

Discussion and Results

The inability of lecithin to exert a stabilizing effect on a commercially refined, bleached but undeodorized soybean oil is shown in Table I. As the concentration is increased, some evidence of stabilizing activity is observed. The activity however is almost negligible when compared to the results obtained with deodorized oil (3 hrs., 210°C.). Little difference in activity is shown when the oil and inactivators are deodorized together or when the oil is deodorized separately and the inactivator is added later. It is apparent that a change has occurred in the oil during deodorization. The stabilizers are active regardless of whether they have received any heat treatment with the oil. Only carboxymethylmercapto succinic acid (CMS acid) shows a slight activity in unheated oils. CMS also shows the highest activity in heated oils, followed by citric acid, lecithin, and sorbitol. This order of activity agrees with our previous experience with these metal inactivators (5).

TABLE I

The Effect of Deodorization on Stability of Soybean Oil and Metal Inactivation

Metal inactivator	Peroxide value A.O.M. conditions—8 hrs.		
	Deodorization treatment—3 hrs., 210°C.		
	None	Inactivator added after	Inactivator added before
Soybean oil control[a]	84	40	39
0.02% lecithin	80	17	18
0.05% lecithin	76	9	9
0.10% lecithin	68	6	7
0.01% sorbitol	86	16	13
0.01% citric acid	78	7	11
0.01% CMS[b] acid	56	4	—

[a] Peroxide value of oil before deodorization 7.6.

[b] Carboxymethylmercapto succinic acid.

TABLE II

Effect of Heat on the Oxidative Stability of Vegetable Oils and on the Activity of Metal Inactivators

Oil treatment		Peroxide value A.O.M. Conditions—8 hrs.			
		Control oil	Additives at 0.01% level		
Temp., °C.	Time, min.		CMS acid	Citric	Sorbitol
		Soybean oil[a]			
No heat		85	56	78	86
127	10	84	44	77	87
155	10	66	15	56	66
155	20	54	13	44	50
155	40	46	8.1	32	42
155	60	38	6.8	26	32
155	120	36	5.3	18	28
183	10	44	3.7	25	39
210	10	40	3.2	12	22
232	10	40	2.8	9.1	20
232	20	38	2.6	8.9	18
232	40	44	5.0	10	38
232	60	46	4.1	16	31
		Cottonseed oil[b]			
No heat		45	41	45	
127	10	43	38	41	
155	10	36	31	34	
183	10	17	14	19	
210	10	11	7.1	12	
232	10	8.6	6.6	11	
		Corn oil[c]			
No heat		395	165	225	
127	10	315	79	150	
155	10	215	31	67	
183	10	150	7.0	17	
210	10	72	7.4	10.4	
232	10	82	8.0	16	
		Peanut oil[d]			
No heat		24	18	19	
127	10	19	15	17	
155	10	17	13	14	
183	10	8.5	5.3	8.1	
210	10	11	4.2	5.5	
232	10	12	4.1	4.5	

[a, b, c, d] Initial peroxide values 7.6, 3.6, 15.0, and 6.9, respectively.

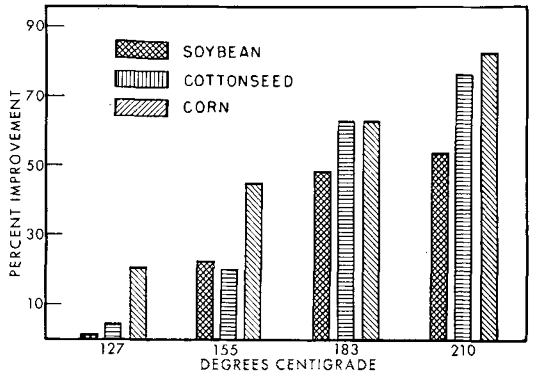


Fig. 1. Improvement in oxidative stability of vegetable oils subjected to a 10-min. heating.

The relationship of temperature of the various heat treatments to the stability of refined and bleached soybean oil as well as the simultaneous improvement in activity of three different metal inactivators is given in Table II. Also shown are the results obtained upon heating cottonseed oil, corn oil, and peanut oil. The improvement in oxidative stability with each increment of heat is similar in each of the four types of vegetable oils. The magnitude of improvement might depend a great deal on the initial peroxide value of the oil, especially if it is high. Sufficient data are not available to ascertain the form of this relationship, but a high degree of correlation might be expected. One sample of cottonseed oil showed only slight improvement in stability on heating; however the effect of heat on the activity of the metal inactivators was similar to the other oils.

Little or no activity has been shown by the different metal inactivators in all types of unheated vegetable oils. Activity of the metal inactivator increases rapidly with the heating of the undeodorized oils. Metal inactivators do not show their highest activity until the oils have been heated for at least 10 min. at approximately 180°C. This response in increased activity (Figure 3) corresponds closely to the temperature of rapid peroxide destruction.

Table II shows how the duration of heating at 155° and 232°C. influences the oxidative stability of soybean oil, and the activity shown by three metal inactivators. Even after a 2-hr. heating at 155°C. (longest time employed) both the stability of the oil and the activity of the stabilizers were improving. If the heat is increased to 232°C., the optimum stability of the oil and the highest activity of the stabilizers are attained after 20 min. of heating. Heating for longer times at this temperature causes a decrease in oil stability.

TABLE III

Iron Contents and Initial Peroxide Values of Refined, Bleached, Undeodorized Vegetable Oils

Oil	Iron content in p.p.m.	Initial peroxide value milleqv./kgr.
Soybean	0.15	7.6
Cottonseed	0.13	3.6
Corn	0.38	15.0
Peanut	0.10	6.9

TABLE IV

Effect of Heat on the Oxidative Stability, the Activity of Pro-oxidant Metals and Metal Inactivators in Vegetable Oils

Oil Treatment		Peroxide values A.O.M. conditions—8 hrs.								
		None			0.3 p.p.m. Fe			0.1 p.p.m. Cu		
Temp., °C.	Time, min.	Control	CMS 0.01%	Citric 0.01%	Control	CMS 0.01%	Citric 0.01%	Control	CMS 0.01%	Citric 0.01%
Soybean oil										
Unheated		85	56	78	340	67	92	380	77	96
155	10	63	15	57	345	49	76	250	47	74
210	10	39	3.3	13	190	17	32	130	9.6	20
Cottonseed oil										
Unheated		46	42	45	160	44	46	280	61	56
155	10	36	31	34	210	36	39	340	50	50
210	10	11	7	12	380	14	16	440	38	40

Improvement in oxidative stability by heating three different vegetable oils is expressed in Figure 1 as the percentage reduction in the amount of peroxides formed in 8 hrs. under A.O.M. conditions. Table III lists the iron contents and the initial peroxide values of the various oils. The association between the metal contents and peroxide contents with the improvement in oxidation upon heating can be seen by comparing these analytical data with the stability results shown in Figure 1. Since heat has such a marked effect on stability, it was of interest to determine the effect of heating upon catalytic effect of added trace metals. Table IV gives stability values of heated soybean oil obtained in the presence of added metals. The oils were heated to 155° and 210°C. for 10 min. under high vacuum and were cooled before the addition of 0.3 p.p.m. of iron and 0.1 p.p.m. of copper. Metal inactivators were added after the addition of the metals but before the samples were submitted to any aeration under A.O.M. conditions. In unheated soybean oil, iron was active as an oxidation catalyst; however, after heating, the activity was less. The activity of the CMS acid and citric acid exhibits the same trend as illustrated in the previous tables in that greater activity is experienced with greater heat treatment. This trend holds in the presence of added metals. A study of metallic catalysts in the A.O.M. test has been reported (16). The use of such catalysts in the A.O.M. test had to be discarded because of erratic and nonreproducible results. At the concentrations studied the activity of copper as an oxidation catalyst is no greater than iron in unheated soybean oil. It is also surprising to find that the catalytic activity is progressively lessened as heating is increased.

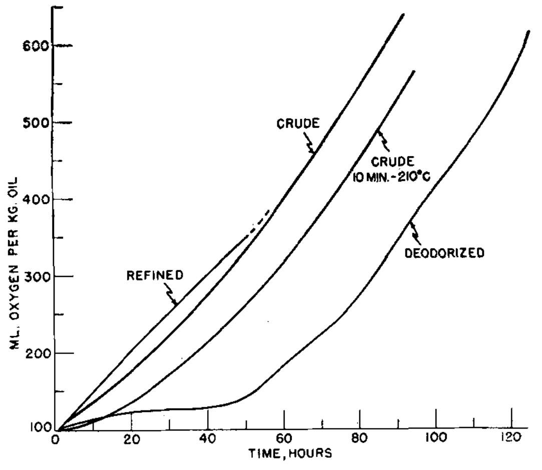


FIG. 2. Rates of oxygen absorption at 60°C. for crude, refined, and deodorized soybean oil.

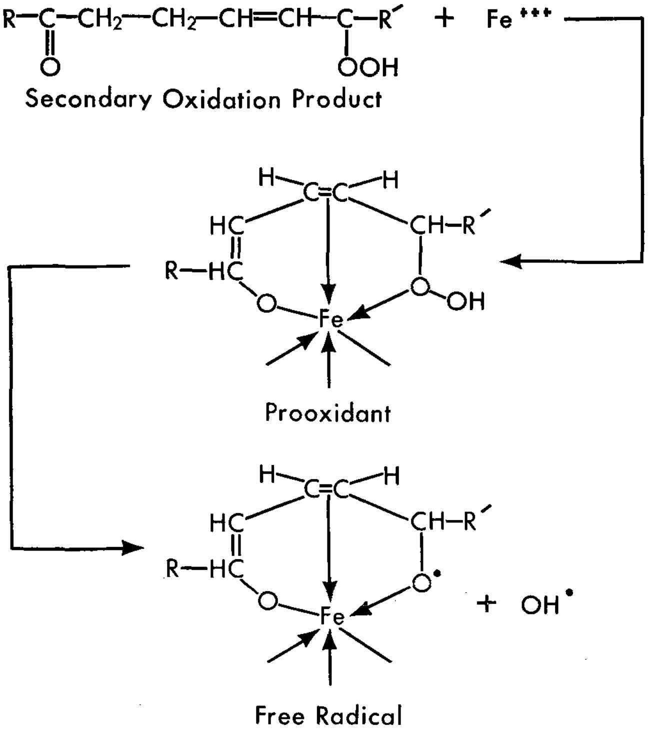
In cottonseed oil both copper and iron were potent oxidative catalysts. In the absence of metal inactivators a tremendous increase in the rate of peroxide development occurred in the heated oil. The applying of heat appears to increase the catalytic effect of the pro-oxidant metals. This effect is opposite to that observed with soybean oil and also opposite to that obtained in the absence of added metals. The effect of added pro-oxidant metals to heat-treated oils cannot be generalized with any degree of certainty at this time. Because of the erratic behavior of the catalysts and the labile nature of hydroperoxides, further work is needed and suitable oxygen uptake studies may be necessary on these oil systems.

Figure 2 shows the rate of oxidation as measured by actual oxygen uptake at 60°C. for a crude soybean oil; the same crude soybean oil heated for 10 min. at 210°C.; the refined soybean oil; and the refined, bleached, and deodorized soybean oil. These curves are from data obtained from one lot of solvent-extracted soybean oil which was refined, bleached, and deodorized in the laboratory. These curves substantiate the previous A.O.M. data on the stabilizing effect of heat. Rapid oxidation and the absence of any induction period are noted for the crude oil and the refined oil. When the crude oil was heated at 210°C. for 10 min., an appreciable induction period was obtained. Although this induction period does not equal the 45-hr. induction period of the deodorized oil, longer heating would be expected to give a more pronounced break in the curve and a longer induction period. The refined deodorized oil (210°C., 3 hrs.) gives the typical flat induction period observed in stabilized and refined fats and oils.

Studies of oxygen absorption in a refined and bleached cottonseed oil revealed no induction period, and the sample absorbed oxygen at a constant rate. Thus either the presence of an optimum amount of pro-oxidant catalyst is present or the absence of any inhibitor or antioxidant is indicated. Upon deodorization this same oil gave an induction period of approximately 16 hrs. before oxygen was absorbed. The difference between the refined and deodorized oils may be only the result of peroxide destruction, but it appears likely that several factors are involved.

From these data it must also be concluded that, before heating, the natural antioxidants present in either soybean or cottonseed oil are essentially ineffective in preventing oxidations carried out at 60°C. The amount of antioxidant present in these vegetable oils was not sufficient to exhibit either an induction period or to reduce the initial rate of oxidation. The induction part of the oxygen absorption curve is usually attributed to the activity of the antioxidant. Although antioxidant activity is high in these oils after heating, no inhibiting action was observed prior to heating, which would indicate that the antioxidants were not destroyed but, by some means or other, were rendered inactive. It is suggested that a short induction period is the time required for formation of the necessary level of hydroperoxide-metal catalyst. Thus the induction period can be extended either by antioxidants, which presumedly function in preventing hydroperoxide formation, or by the metal inactivation in preventing hydroperoxide formation of the metal-hydroperoxide complex. Once the critical level of metal-hydroperoxide complex is formed, neither the antioxidants nor metal inactivators are effective in preventing oxidation. Such a concept of the initiation of fat oxidation would explain the observed experimental facts. Kern and Willersinn (10) found that metals activate only hydroperoxide, not the dialkyl or diacylperoxides in the autoxidation of metal linoleate. Tappel (21) proposed that in aqueous systems a hemin-linoleate hydroperoxide complex functions as a catalyst for the generation of free radicals. Uri. (22, 23) attributes the initial production of free radicals in unsaturated fat oxidation to heavy metal catalysts, which function in the form of a solvent-coordinated, heavy-metal complex.

We believe the catalytic effect of iron results from the formation of a coordination complex between iron and secondary oxidation products. This complex might be formed between an unsaturated carbonyl compound containing a hydroperoxide as follows:



This complex could generate free radicals, as suggested by Tappel, and accelerate oxidation, without releasing iron in the presence of an added, metal inactivating agent.

Figure 3 illustrates the rate of breakdown at different temperatures for peroxides in soybean oil starting with an initial peroxide value of 98. A deodorized soybean oil was oxidized in the dark at 60°C. by bubbling oxygen through it until the desired peroxide value was attained. The rate of hydroperoxide destruction at elevated temperatures was not influenced by the addition of metal inactivator or by the addition of iron. Temperature and the time of holding at the elevated temperature were the determining factors. During the 10 min. required to raise the temperature to 180°C. about 80% of the peroxides were destroyed; after 10 min. of holding, 95% of peroxides were destroyed; and after 30 min. practically all were destroyed. At 100°C. only about 6% of the peroxides were lost in 1 hr., and the rate of destruction appears to be a linear function of time. However, as the temperature is increased, a greater percentage of the destruction occurs in the first stages of heating. In Figure 3 the percentage improvement in A.O.M. stability is plotted and was based on the reduction in peroxide development in 8 hrs. when a refined but undeodorized soybean oil is heated at 155°C. As the heating time increases, improvement in stability follows very closely the destruction of the peroxides until about 85% were destroyed. After 15 min. at 155°C. about one-half of the peroxides are destroyed, and about one-half of the maximum stability is obtained. Identical rates of peroxide destruction were obtained at 150°C., regardless of the initial peroxide value of the oil.

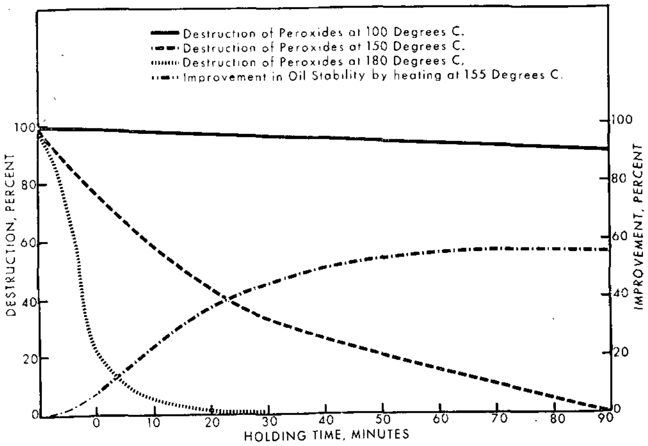


Fig. 3. Rate of peroxide destruction in soybean oil at 100°, 150°, and 180°C., and the rate of improvement in oxidative stability.

These data indicate that the minute amounts of trace metals dissolved in the oil are complexed to some active functional groups. These pro-oxidant metals are not available in the unheated oil to strong chelating agents, such as citric acid. The metal becomes available for chelation after heating, indicating a dissociation or breakdown of some existing complex. The release of the metal follows closely the breakdown of the fat hydroperoxide. This relationship would indicate that the metals and the hydroperoxide might be associated in some manner to form a hydroperoxide-metal complex. Since hydroperoxide groups are *alpha* to an unsaturated linkage, these complexes may or may not include an association of the metal to the double bonds of the fatty acid chain. Heavy metals are known to coordinate with unsaturated linkages; thus this concept does have some fundamental basis for support.

Regardless of the structure of such a metal complex it is very potent as an oxidative catalyst. Rapid absorption of oxygen occurs in unheated oils without showing the usual induction period even though the presence of antioxidants may be easily demonstrated. It may be that the fatty hydroperoxide-metal complex is part of the mechanism through which oxidation of the fat proceeds. The studies by Martell, Calvin, and others (14) as well as Myers and Zittlemoyer (18) on the reactions and properties of oxygen-carrying metal chelates offer some possibilities for explaining fat-oxidation catalysts.

Summary

Metal-inactivating agents, such as citric acid, sorbitol, lecithin, and carboxymethylmercapto succinic acid, are not active in unheated vegetable oils. Apparently trace metals present in normal glyceride oils are held within a complex of unknown structure. After heating an oil, the metals can be complexed by metal-inactivating agents, such as citric acid. The release of metals appears to be associated closely with the breakdown of the fatty acid hydroperoxides. Formation of some association or complex between the metal and the hydroperoxide group or between the metal and the unsaturated linkage of the fatty hydroperoxide is suggested. The metals are held very tenaciously within this unknown structure. Although the metal is not available as an uncomplexed metallic ion, it does behave as a very strong pro-oxidant catalyst. The application of heat releases the metal so it can be complexed by added metal inactivators.

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The Effect of Mono-enoic Fatty Acid Esters on the Growth and Fecal Lipides of Rats

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THERE IS NOW EVIDENCE that when erucic acid or its ester is fed to rats, their growth is less than when shorter chain fatty acids are fed. Thomasson and Boldingh (9) observed a consistent retardation of growth on feeding rapeseed oil and showed that this was due to the erucate content of the oil. Carroll and Noble (2) also noted growth retardation by erucic acid and methyl erucate as compared to oleic acid or methyl oleate. They further noted that erucic acid and ester increased the fecal excretion of cholesterol.

Less is known concerning eicosenoic acid. Carroll and Noble (2) fed eicosenoic acid to a small number of rats and observed a retardation of growth and an increase in fecal cholesterol of about the same order as that caused by erucic acid.

In the course of an investigation into the deposition of various fatty acids in the body fat of rats by Hopkins *et al.* (5) the opportunity was afforded of comparing the effect of corn oil, methyl oleate, methyl 11-eicosenoate, and methyl erucate on the growth of rats, and on the excretion of fecal lipides. The following is an account of these investigations.

Experimental

The rats were fed a purified diet which contained 5% corn oil, or the pure methyl ester of oleic, eicosenoic, or erucic acids. Four groups of 5 males and 5 females were fed each diet and a fifth group was fed a similar diet in which the fat was replaced by an equal weight of corn starch. The methyl esters were prepared in small amounts as previously described (5) and were used promptly to reduce the danger of oxidation. Details of the diets, method of feeding, and handling of the rats have been published previously (5). At weekly intervals the rats were weighed and examined for symptoms of essential fatty acid deficiency. Feces were collected during the second, third, fourth, fifth, and twelfth weeks of the experiment and were kept frozen until analyzed.

At the end of 12 weeks the males were killed by decapitation, examined for gross pathological changes, and frozen for later analyses. Sections of the lung, heart, arteries, liver, kidney, and bladder were taken for histological study. The females were bred to normal males and were fed the same diets until their litters were weaned, a further eight weeks.

Feces were dried, ground, and allowed to stand in hexane (petroleum ether) containing hydrochloric acid to convert soaps to free fatty acids. Extraction was carried out in Soxhlet extractors with the same hexane-acid mixture. The extracted lipide was weighed and saponified with 10% alcoholic KOH; the unsaponifiable fraction was extracted with hexane. The soaps were acidified, and the acidic material was taken up in ethyl ether. The residue from the evaporation of the ether was dissolved in hexane, and the soluble portion was collected.