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A Nutritive Evaluation of Over-Heated Fats

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According to the tests used, harmful substances do not occur in fried foods or in fats used in preparing foods. It is possible to obtain biologically undesirable materials by excessively heating and/or oxidizing fats in the laboratory, but the conditions required for the production of such materials differ greatly from those used in practical cooking or processing of foods. There appears to be no reason to believe that fats are nutritionally damaged when handled by normally-accepted good practice in present-day food preparation.

FATS ARE IMPORTANT and essential in the diet. They are not something we can take or leave alone; they provide energy, supply essential fatty acids, carry fat-soluble vitamins, improve flavors, modify textures, and add satiety values to meals.

Much of their utility depends upon their stability to heat. In frying operations they prevent sticking and transfer heat from hot surfaces to food. The stability of fats at high temperatures (up to 200°C. in some frying operations) invites repeated or continuous use, and questions have been raised concerning the nutritive value and wholesomeness of fats after long usage. Some data in the scientific literature show that undesirable changes occur in fats if they are heated in the laboratory to high temperatures for long periods or if they are subjected to severe oxidizing conditions. Other reports indicate that fats which have been used for prolonged commercial or home cooking retain their nutritive value and remain wholesome.

There are two major reasons why food technologists handling fats should be familiar with this subject. The nutritive values of fats at all stages of processing and use should be known, and there are significant and frequently adverse public relation aspects which must be handled. Publicity problems usually arise from misinterpretations or from unjustified extrapolations of laboratory findings. Even though the implied effects may not be true, headlines such as "The Carcinogenic Action of Heated Fats and Lipoids" (1) cannot be considered in the best interests of the fat industry. The facts must be known in order to understand the problems and to combat misleading reports or inferences.

It is not our intention to review this subject exhaustively. Instead we plan to consider published and unpublished research findings which indicate typical changes that can occur in food fats during laboratory treatments or cooking procedures and to contrast the findings with those obtained when food fats are tested. Noncritical review of the scientific literature relating to heated fats can lead to very erroneous conclusions since reports show that it is possible to mistreat fats experimentally with sufficient heat and/or oxygen to cause, when the abused fat is fed to test animals, retarded growth, poor feed efficiencies, rough, greasy matted coats, diarrhea, starvation, enlarged livers and kidneys, abnormal fat depots, impaired enzymatic functions, abnormal water metabolism, papillomas and other growth formations, and shortened life spans. In extreme cases animals may die in a few days after severely abused fats have been fed. There is thus no question whatsoever that fat can be damaged by purposeful abuse. The critical question is: "are fats damaged during processing or cooking operations?" To answer that question we must examine some of the conditions which produce the effects listed above. They may have been

deliberately designed to produce measurable damage rather than to simulate cooking procedures.

THERE IS NO EVIDENCE that food fats have carcinogenic properties unless they have been abused far beyond normal conditions. Nevertheless inferences that food fats may be involved in the disease are too frequent. One of the early suggestions that heated fats might cause cancer was made by A. H. Roffo (2), who claimed that sunflower and olive oils oxidized by heating to 250–350°C. (482–662°F.) had carcinogenic potencies when fed to rats. This should be recognized as very drastic treatment. Lane, Blickenstaff, and Ivy (3) however did not get tumors when lard "browned" at 350°C. (662°F.) for 30 min. was fed to rats from the colony of Dr. Roffo, but there were increased incidences of papillomas of the forestomach and ulcers in the glandular stomach. Peacock and Eck made similar claims for the feeding or injection of cottonseed oil heated to 350°C. (662°F.) for 4 hrs. (4, 5). He was unable to find any known carcinogen in the fat. Two mice out of 300 showed tumors after 15 months on the diets. Other investigators have had varying degrees of success in efforts to demonstrate carcinogenic properties in heated fats.

While there have been demonstrations that painting overheated fat repeatedly upon the skin, injecting it into the skin or muscle, or feeding it can result in conditions suggestive of the formation of cancer, it is perhaps significant that A. A. Newman, after going to considerable length to imply that epoxidized and heated fats are a nutritional hazard (6), states: ". . . the introduction of autoxidized and thermally-affected fats into test animals can produce pathological lesions ranging, according to conditions, from benign papillomas in the forestomach to malign neoplastic growth in the glandular region. While it must be emphasized that none of the latter type of lesions so far produced have satisfied the rigorous conditions of true carcinogenicity, in several instances the difference was not wide from a practical viewpoint." It might be added that the fats tested were not at all representative of cooking fats.

Fats which have been heated to unrealistically high temperatures do not necessarily indicate intensification of cooking conditions. They may have undergone chemical changes quite different from those occurring under cooking conditions. Nevertheless inferences drawn from reports of exaggerated conditions can result in scare propaganda that is of no value to anyone. More research is needed to clarify the situation completely, and this research needs to be done with food sources of fat as well as with unrealistically-abused fats.

Feeding studies have been conducted by many different laboratories in studies of "abused" fats. No effort will be made to review all of the relevant reports. Only a few will be selected to illustrate the nature of the studies and typical findings. The treatments fall into three general categories: heating in the absence of air, heating in the presence of air, and oxidation at low temperatures.

ONE OF THE FIRST groups to study the biological effects of polymerization was that of E. W. Crampton and his co-workers (7). They blew carbon dioxide through various oils heated to 275°C. (527°F.) in all glass equipment for various intervals of time. Table I, adapted from their Table 2 (7), illustrates several things. The inclusion of severely heat-treated oils in rat diets at levels of 10 or 20% reduced rates of gain and decreased caloric efficiencies. The amounts of damage were proportional to the length of heat treatments. Different types of oils differ in response. Linseed, the most unsaturated, shows the most damage.

These workers observed that appetite was depressed when thermally-polymerized oils were fed and that the feces of the animals were dark and sticky. Hair coats were also oily and matted whereas the controls were sleek and clean.

TABLE I
Effects of Heat Treatment of Oils[a]

Test oil used	Duration of heating	Aver. daily gain	Aver. daily feed intake	Gain per 1,000 cal.
	hours	*g.*		
Linseed	0	3.9	9.6	91
	2	3.8	9.6	91
	4	3.4[b]	9.5	80
	8	2.5[b]	8.1	69
Corn	0	3.6	10.4	78
	15	2.8	8.7	71
	30	2.3[b]	8.0	64
Soybean	0	4.6	10.2	90
	3	4.0	9.4	85
	9	2.9[b]	7.7	76

[a] Adapted from the data of Crampton, Farmer, and Berryhill (7). Ten or 12 animals were used per group, and fats were fed at 10% of the diet except for 20% of soybean oil.
[b] Significantly lower than controls.

In contrast to these findings Lassen, Baron, and Dunn (8) report that adult rats fed edible, polymerized sardine oils at a level of 5% in the diet were healthy after short experiments and showed no significant changes in urine analyses. They demonstrated however that sardine oil which was 30% polymerized was only 85% digestible in comparison with the 98% digestible found for unpolymerized oils.

O. C. Johnson and his co-workers at the University of Illinois added the stress of oxidation to heat treatment by blowing 100 milliliters per minute of air through 1,500 g. of various oils heated to 200 ± 10°C. (392°F.) in stainless steel beakers for various periods up to 24 hrs. (9). This is a very rigorous treatment and results in increased acid values, increases in viscosity and color, and decreased iodine values. Rats fed thermally-oxidized butter oil gained as well as controls fed fresh butter oil; but rats fed thermally-oxidized corn oil gained poorly in comparison to their controls on fresh corn oil. Final weights after nine weeks on corn oil tests were 124 and 332 g., respectively. That these responses were not caused by differences in food intake was demonstrated by a paired feeding experiment, in which the thermally-oxidized corn oil gave significantly less growth (1% level) than the fresh oil. Margarine stock heated and oxidized under the same conditions depressed growth slightly when pair-fed in comparison with fresh oil.

Temperatures as high as 200°C. (392°F.) cause rapid destruction of fatty peroxides, and the course of polymerization at such severe conditions may be very different from what it is at lower temperatures. In this connection it is of importance to note that when Kaunitz and Slanetz (10) fed 15% cottonseed oil, which had been aerated at 95°C. (203°F.) for

200 hrs. (iodine No. 141), immediate diarrhea and weight loss occurred and about half of the animals died within three weeks. At a level of 10% peroxidized fat only a few rats died, and the others seemed to adjust to the diet, overcoming the diarrhea and gaining slightly in weight. The addition of fresh oil along with the heated oil seemed partially to overcome the effects of the peroxidized oil. The only symptom was slower gains. The addition of Vitamins E, A, and D did not result in a corresponding improvement.

Further heating of a cottonseed oil with peroxide value of 191 caused a reduction in peroxide value to 141 but an increase in severity of symptoms when fed, leading Kaunitz and co-workers to conclude that the amount of peroxide present was not related to the degree of toxicity. In addition to the growth-depressing effects Kaunitz has reported that the feeding of heated fats causes enlarged kidneys, livers, and adrenals, also small spleens and thymus glands. Water intake is also greatly increased (11).

Recently Andrews and co-workers (12) have published data to indicate that growth depression in rats fed oxidized soybean oil is proportional to the extent of oxidation from peroxide numbers of 100 to 1,200. Using cupric and ferric ions as catalysts, they oxidized soybean oil by aeration at 60°C. (140°F.) to peroxide numbers as high as 1,200. When fed as 20% of the diet, 1,200 peroxide number fat caused immediate, severe diarrhea and sustained losses in weight, also fatalities of all animals in three weeks. Dilution of this product with fresh soybean oil to give mixtures with peroxide numbers of 800 and 400 lessened the severity of the symptoms and prevented the fatalities.

THESE STUDIES and a number of others proved that fat can be damaged, but the conditions needed for damage were much different from home or commercial cooking operations. The need for severe laboratory treatment of highly unsaturated oils to get the marked changes suggested that sensitive methods might be essential if one were to study practical operating conditions. Furthermore rapid methods for measuring changes in nutritive value and wholesomeness are essential if many fats are to be examined. The tests commonly used required 8 to 12 weeks of test feeding of experimental animals.

With this in mind we undertook the development of methods which would quickly detect changes of fat quality. Perhaps the most successful of these has been a restricted-feeding technique, which permits exact comparison of control and experimental animals (13). This grew out of the postulate that fatty substances which had been damaged might not be available for energy. If this were true, under proper conditions the rate of gain of animals fed abused fats should be proportional to the amount of undamaged fat remaining. In practice weanling rats are fed 5-g. quantities of a basal ration containing only enough energy-containing substances to permit slight growth but formulated to supply an excess of the daily needs of all essential nutrients. Additions of energy-containing materials to such a diet permits growth in proportion to the amount of energy added. This is true whether the extra food is carbohydrate, fat, or protein in nature.

Table II illustrates the application of such a technique to laboratory-heated and/or oxidized fats. These are typical values, and it may be seen that the available energy is markedly reduced by severe heating or oxidation. A 7-day period is adequate for such determinations. The results are quite uniform and reproducible, permitting the use of small groups of animals. In addition to demonstrating reductions in energy value, animals fed fats which have been excessively heated or oxidized experimentally showed the organ enlargements that others have reported in longer feeding-studies. The increase in liver weight occurs very rapidly, being easily detectable in three days and maximal in five to seven days. These livers are not fatty; by analysis and by gross and limited microscopic inspection the tissue is normal.

TABLE II
Energy Values of Abused Fats

Substance added	Aver. gain in 7 days [c]	Available energy [d]	Liver sizes
	g.	%	*% body wgt.*
None	5.0	....	....
Fresh cottonseed oil	27.0	100	4.0
Severely heated CSO [a]	21.0	72	5.4
Severely oxidized CSO [b]	4.7	0	7.0

[a] 120 hrs. at 182°C. in household cooker.
[b] Air blown through oil held at 60°C. for 19 days.
[c] 1.2 g. of fat fed each day in addition to basal.
[d] In term of % of theoretical, based on fresh CSO as 100%.

This technique has been applied in a number of studies of factors which might damage fat. These will be reported elsewhere in more detail, but an outline of the findings will indicate the magnitude of the changes which may be expected with common food fats when heated or oxidized.

That these changes (Table II) are typical is shown by the decreases in available energy values listed in Table III for several samples of salad oil and shortenings. Values for any one sample are quite reproducible, but different lots of a single type of oil vary in response to heat as the two corn oils indicate.

Hence the values in Table III cannot be taken as an indication of the relative stabilities of the fats. Some of these tests were made before liver weights were routinely checked, but liver weights taken in other tests indicate increased size when various types of fats are excessively heated during experiments.

TABLE III
Available Energy of Various Fats After Heating at 182°C. for 120 Hours [a]

Type of oil heated	Available energy in heated fat as % of energy available from unheated product [b]
Cottonseed salad oil, Sample 1	72
Cottonseed salad oil, Sample 2	68
Peanut salad oil	67
Corn salad oil, Sample 1	86
Corn salad oil, Sample 2	75
Hydrogenated meat and vegetable fat shortening	81
Hydrogenated vegetable oil shortening	70
Hydrogenated vegetable oil shortening	84
Meat fat and vegetable fat shortening	76
Lard	66 [c]

[a] Approximately 3,000-g. quantities of fat were heated for 120 hrs. at 182°C. in a household deep-fat fryer. Four animals were fed at a level of 1.5 g. per day in addition to 5 g. of basal diet.
[b] These values are for samples of commercial products and do not necessarily reflect the relative merits of the type of product because there is variability from one supplier to another, as the two corn oil samples indicate.
[c] 168 hrs. of heating rather than 120.

Oxygen at moderate temperatures (60°C.) causes little or no decline in nutritive value until the lag phase is overcome; then available energy values go down, and liver sizes of test animals increase. The values in Table IV are for samples taken from cottonseed oil which has been stirred and blown with air.

TABLE IV
Influence of Oxidation Upon the Nutritive Value of Cottonseed Oil

Days of oxidation at 60°C.	I.P.V.	Available energy [a]	Liver size [a]
		%	% of body wt.
0	<1	100	4.0
16	310	100	3.9
19	1130	71	5.3
29	400	0	5.8

[a] Three rats per group, each rat getting 0.6 g. of test fat per day in addition to 5 g. of basal diet. At 1.2-g. test-fat levels rats receiving the fats oxidized for 19 days or more did very poorly and refused part of the diets.

TABLE V
Influence of Time Upon the Effect Which Heat Has Upon the Nutritive Value of Cottonseed Oil

Length of heating period [a]	Energy availability [b]	Liver size [b]
days	%	% of body wt.
0	100	5.0
1	95	5.7
2	95	6.1
3	93	6.4
4	72	6.9
5	75	7.1

[a] Heated in 3,000-g. quantities, not stirred, in household deep-fat fryer at 182°C.
[b] All animals fed 1.5-g. of test fat daily in addition to 5 g. of basal diet. Four rats per group.

Similarly heating fat in 3,000-g. quantities at 182°C. (360°F.) in a household deep-fat fryer causes gradual reduction of nutritive value (Table V). It should be noted that these are not applied conditions. No food was cooked, there was no addition of fresh fat, nor was there removal of volatile materials with steam. Even so, changes are slight during the early stages of heating.

THE INFLUENCE of varying the percentage of oil exposed to air at any given temperature is indicated in Table VI. In this case varying amounts of oil were heated in the same pan for the periods of time shown. In other tests constant amounts of oil were heated at different temperatures. It may be noted that a change in the amount of fat heated per unit of surface area has more influence than a change in temperature. The 50-g. quantities of oil rapidly became viscous, and the ones which had been heated for 6 hrs. had to be scraped from the pan with rubber spatulas. In general, samples which showed heat damage were quite viscous and certainly would not be suitable frying aids. Various fats respond differently although all tend to show the same changes.

TABLE VI
Influence of Surface Exposure Upon Changes in the Nutritive Values of Hot Cottonseed Oil

Heating period	Fat heated at 180°			Fat heated at 200°C.	Fat heated at 220°C.
Minutes	50 g.[b]	100 g.[b]	200 g.[b]	100 g.	100 g.
Energy Availabilities as Percentage of Control Oil [a]					
	%	%	%	%	%
0	100	100	100	100	100
30	102	106	108	102	112
120	98	90	94	94	90
360	50	80	98	77	69
Liver Weights as Percentage of Body Weights [a]					
0	4.4	4.4	4.4	4.4	4.4
30	4.6	4.4	4.0	4.2	4.5
120	5.4	5.3	4.5	5.0	5.4
360	6.8	6.3	5.4	6.2	6.7

[a] Samples fed at a level of 1.2 g. per animal, in addition to 5 g. of basal diet, to group of 4 animals on each test.
[b] The 50-, 100-, 200-g. quantities correspond to an oil depth of approximately 2, 4, and 8 mm., respectively, for oil heated in an 8½-in. diameter aluminum pan.

In the present tests none of the fats changed rapidly enough to give cause for concern. The treatments in which changes were detectable were more severe than conditions encountered in reasonable home or commercial cooking. In fact, undesirable increases in viscosity, color, and flavor precede the detectable biological effects even in those relatively sensitive tests where the treated fat is the sole additive to the diet. Hence, while these data suggest that slight changes may occur during normal usage, such changes will be minimal and by no means as severe as those reported in the scientific literature on abused fats.

A different type of response to a substance in fat was noted in 1957 when a heavy incidence of an edematous condition in the broiler type of chickens appeared in flocks fed specific lots of fat, which were later shown to include residues from fat-processing operations (14). When the contaminated fats were fed, fluid accumulated in the heart sac and/or in the abdominal cavity, sometimes in spectacular quantities. Severely afflicted birds developed distended abdomens, resulting in the designation "water belly" in trade areas. Pathological examination also revealed gross liver and kidney damage. Extensive studies by the regulatory officials and in the laboratories of many feed manufacturers and feed-ingredient suppliers proved that feed-grade fats were harmless if they did not include a particular type of residue from one type of fat processing.

The toxic material could be concentrated in the unsaponifiable fraction of fats, and extensive studies of the chemical and physical properties of concentrates have been made (14, 15, 16, 17). So far, these have not led to identification of the toxic material or to rapid tests for it although Harman *et al.* (17) have recently reported crystallization of about a milligram of material which they believe to be the toxic substance. This crystalline material is reported to be effective at a level of 0.1 mg. per kg. of feed or at a concentration of one-tenth part per million in the diet. There are no indications that this material is related in any way to the factors which are produced when food fats are heated.

SINCE THE ORIGINAL OUTBREAK of poultry disease, various types of food and feed-grade fatty materials have been examined. The application of sensitive tests by Food and Drug Administration officials led to the detection, in some shipments of oleic acid, of traces of materials which gave chickens mild symptoms of hydropericardial disease. On the basis of these findings officials of the Food and Drug Administration are requiring all producers of oleic and stearic acids to chick-test products intended for food use. A detailed method for this purpose has been distributed (18). This involves measurements of the volume of pericardial fluid in chickens after they have been fed test materials at a 16% level for three weeks. Normally chickens have almost no fluid in

TABLE VII
Potato Chip Frying

Treatment of cottonseed oil	Calorie Availability[a] %	Liver size		14-Day gains		Liver size	
		% of Body wt.	% of Control	Actual	Adjusted to aver. food intake	% of Body wt.	% of Control
1. 28 lb. CSO heated to 182°C.—5 lb. potato chip fried immediately	100	4.42	100	74.3	65.6	6.2	100
2. Heated oil at 182°C. for 24 hrs.—5 lb. chips fried	89	5.16	116	70.0	63.6	6.7	104
3. Heated oil at 182°C. for 48 hrs.—5 lb. chips fried[b]	89	5.58	128	63.3	64.3	6.5	102
4. Heated oil at 182°C. for 72 hrs.—5 lb. chips fried[b]	95	5.41	121	53.5	57.3	7.1	107
5. Heated oil at 182°C. for 96 hrs.—5 lb. chips fried[b]	91	6.23	138	54.5	59.7	7.2	108
6. Heated oil at 182°C. for 120 hrs.—5 lb. chips fried[b]	86	5.98	125	53.3	58.0	6.7	104
7. Heated oil at 182°C. for 120 hrs.—no frying	72	7.14	159	......	......	....	......
8. Heated oil at 182°C. for 120 hrs.—then 5 lb. chips fried[b]	63	6.80	139	40.0	54.4	6.6	103
9. Commercial chips	....			80.0	72.1	6.1	99

[a] Based on an arbitrary value of 100 for the fresh, heated oil.
[b] Oil foamed violently when potato slices were placed in it.

the heart sac, and the presence of as little as 0.2 ml. is considered by Food and Drug officials as a positive symptom of the condition. Others who have used the method or variants of it do not agree that such a low volume necessarily indicates the presence of toxic materials.

Except for the work with fats producing chick edema, most of the reports of biological damage have resulted from the feeding of fat damaged by severe laboratory treatments. In order to obtain more data on cooking fats a series of samples was obtained from various commercial operations: potato chip or doughnut fryers, restaurants, grills, and so forth. These were obtained at the time of maximum heat treatment, often actually after the user had discarded the product as unsuitable for further cooking. When tested by our rat-feeding procedure, none of these showed marked changes from unheated fats, as has been reported elsewhere (19, 20).

In a study of oils from 89 potato chip manufacturers, Melnick (21) found insignificant changes in iodine values during processing and claimed on this basis that the products have not been changed in any significant amount. This same conclusion was reached a number of years ago by Deuel and his co-workers (22), who fed oils obtained from potato chip preparation to rats and were unable to detect changes caused by the processing.

Other studies in our own laboratories have led to substantially the same conclusion. In these studies two commercial types of 28-lb. deep-fat fryers were filled with refined cottonseed oil and heated to 182°C. (360°F.). Sliced potatoes to yield five pounds of potato chips were fried twice daily in one of the fryers. After the second frying each day sufficient fresh oil was added to restore the original volume. Except for sampling, oil in the other fryer was undisturbed until the end of the experiment. Samples of potato chips and of oil in each fryer were taken after each 24-hr. interval.

While the cooking of two 5-lb. quantities of potato chips twice daily did not provide the intensive usage experienced by fats in the vats of a grill or a commercial potato chipper, it did cause vigorous agitation and introduced food particles and steam. In addition, each day about 10% of heated fat was removed on the potato chips and had to be replaced. After only two days of use the oil foamed violently during cooking and could be kept in the kettle only by immersing small lots of sliced potatoes. Commercial usage would have been impossible. It is interesting to note that changes in biological quality appeared about the same time that foaming made use impractical.

As a source of energy Oil No. 7, which had been heated without use for cooking, was less effective than Oil No. 6, in which chips had been periodically cooked. Livers of rats fed Oil No. 7 also were heavier than livers of the No. 6 group. It must be remembered however that fresh make-up fat had been added periodically to No. 6 to keep the volume constant.

Chips produced during the experiment were mixed into diets in amount sufficient to supply 20% of fat. The rations provided generously for all nutrient needs of weanling rats. Previous 12-week studies had indicated that maximum effects on growth would occur in two weeks (19, 20). After that, rats fed severely heated and unheated fats grew at about the same rate. Hence the growth studies were restricted to a two-week period.

The gains of the groups fed fat heated for several days were smaller than when fresh fat was fed. However much of this decline in weight probably resulted from the decreased palatability of the diet since statistical adjustment of body weights to a common level of food intake evened out the gains. Certainly there is no evidence of decreased nutritive value on the basis of the adjusted weight gains or on liver sizes for animals fed products from the first three treatments. Livers of animals that were fed oil heated 48 hrs. were heavier than those of the first two groups, but at this time the oil was already foaming when used. Further evidence that the 48-hr. heated oil was beyond practical usage was provided by Schaal accelerated stability tests of the potato chips produced in it. These chips gave a stability of only two days as compared to 19 days for chips fried in fresh oil.

Studies of fats extracted from fried, broiled, or roasted meats have similarly shown no decrease in energy availability or in fractions which cause increase in liver weights (23).

SO FAR, most attention has been given to conditions which produce substances that cause diarrhea, retard growth, and alter organ size, and little mention has been made of the nature of the substances formed or their mode of action. Actually little is known in either of these areas. Almost all of the research workers who have produced damage in their samples have been able to show that the active substances are in the unsaponifiable material or in a fraction that is not adducted by urea. Attempts to obtain pure substances have been somewhat unsuccessful.

Kaunitz and others (24, 25) have reported that simple oxidation products, such as monohydroxy stearate, 9-10 dihydroxy stearate, *cis*-epoxy stearate, or oleate peroxide, do not produce symptoms of tox-

icity and are at least not the principal toxic agents. Andrews *et al.* (12) however showed that t-butyl hydroperoxide depressed growth when fed. Polymeric residues obtained after molecular distillations were more toxic than distillable factions, leading to the conclusion by Kaunitz that polymers of some type were responsible for the physiological effects.

Crampton (7), considering the relative potencies of fractions separated from thermally-polymerized linseed oil by distillation and urea segregation, concluded that at least two factors were involved: acyl radicals whose esters could be easily distilled and which would not form urea adducts, and dimeric or polymeric factions which neither distilled nor formed adducts. The monomeric substances were digestible, *i.e.*, they disappeared from the gastrointestinal tract, and they depressed growth, but the polymeric materials were neither digestible nor toxic (26). Perkins and Kummerow (27, 28) also demonstrated that the urea nonadducts obtained from oxidized fats were the most potent factors.

We also have been able to concentrate the biologically effective materials in unsaponifiable or nonadductible fractions. In one series of studies the nonadduct-forming material (NAF) from cottonseed oil which had been heated to 360°F. for 120 hrs. was serially extracted three times with Skellysolve B (Fraction 1), three times with 30% ethyl ether in Skellysolve B (Fraction 2), three times with 60% ethyl ether in Skellysolve B (Fraction 3), and with pure ethyl ether (Fraction 4). Insofar as material was available, 0.3 g. of each of these fractions was fed with 5 g. of basal diet to rats to determine biological responses. The molecular weights of the fractions were estimated by the technique of Donnelly (29). Results of the study in Table VIII show the fraction soluble in pure Skellysolve B to be as active as an equivalent amount of the unfractionated NAF, and the fractions extracted with ethyl ether to be more potent. Not enough material was obtained for feeding after the extraction with 60% ether.

TABLE VIII
Influence of Fractions of Heated Cottonseed Oil Upon the Growth and Liver Sizes of Rats

Diet	Gain	Liver size	Average molecular wt.
Basal	7	4.1	
Basal + 0.3 g. CSO	15	3.7	
Basal + 0.3 g. heated CSO[a]	13[b]	4.0[b]	
Basal + 0.3 g. NAF of heated CSO	11	5.0	
Basal + 0.3 g. NAF—Fraction 1	11	4.8	673
Basal + 0.3 g. NAF—Fraction 2	10	5.3	752
Basal + 0.3 g. NAF—Fraction 3	6	5.5	1320
NAF—Fraction 4	..	....	1420
NAF—Residue	..	....	very large

[a] 182°C. for 120 hrs.
[b] Interpolated from standard curve.

In another study cottonseed oil heated for 120 hrs. at 182°C. (360°F.) was treated with propanol. Two layers formed the upper or more soluble layer containing 86% of the original oil. When fed in the energy-restriction technique at a 1.5-g. level per day (in addition to 5 g. basal), the energy of the insoluble fraction proved to be only 17% available in contrast to 67% for the soluble fraction and 65% for the unfractionated heated oil. Livers from the corresponding animals averaged 5.5, 7.9, and 7.5% of the body weights. This suggests, as does Crampton's work, that a relatively indigestible but harmless fraction and a readily digestible but harmful fraction exist.

EFFORTS TO PURIFY these materials further have been frustrating, partially because of a lack of test methods applicable to small quantities. Even a 7-day rat test, using 0.3 g. per day for four rats requires 8.4 g. of product. Some urea nonadductable materials from heated fats have been separated into major fractions chromatographically, using silica gel and alumina preparations. There are some differences in the spectral characteristics of such fractions, but so far they have not been tested adequately on a biological basis to permit definition of the active fractions. The very limited work done indicates that several chromatographically distinct fractions have activity. This suggests that a family of compounds of similar physical properties and perhaps similar structures may be involved rather than one or two substances. Such a development would not be unexpected in view of the variety of structures available in unsaturated fatty acids, especially in partially hydrogenated fats where isomerization occurs.

It seems very probable that separations achieved by use of the various types of distillation, chromatography, solvent distributions, molecular sieves, etc., will soon result in much better understanding of the chemical nature of the substances responsible for the several biological effects that have been noted. This will permit more exact study of the amounts of these substances in experimental and food fats and will aid in evaluating the acceptability of heated food fats.

To date little is known of the biological causes of the effects observed. Under selected conditions it is possible to cause diarrhea, rough fur, decreased growth, and even death, but we do not know exactly why. There have been demonstrations that abused fats are less digestible than fresh fats (8, 20, 24) and that organs of animals fed the abused fats have altered sizes and enzyme activities (9, 12, 23, 24), that some fractions may accelerate the formation of certain types of abnormal tissues (1, 2, 3, 4, 5), and that oxidized fatty acids alter enzyme activity *in vitro* (30, 31), but the surface of this type of problem has scarcely been scratched. We can be certain that there will be much more work of this nature in the future.

We hope that, in interpreting the data, investigators will remember that alterations in rates of growth or organ size or in enzyme activity are not necessarily indicative of undesirable changes. They may be be beneficial. For example, moderate repeated exercise develops (enlarges) muscles although sustained violent exercise leads to sore, exhausted muscles. Furthermore many changes in quantity or quality of diet result in changes in organ size and composition. Before any substance can be considered harmful, the biological changes induced by its ingestion must be proved to be detrimental to temporary or long-term health. It must be borne in mind however that pathologists are suspicious of any change from the accepted normal.

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Newer Analytical Methods for the Fat and Oil Industry

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THE NEWER METHODS for the analysis of fats and oils fall for the most part into two groups, that is, those relating to physical characteristics and those used for the estimation of composition. This is altogether fitting because certainly the functional properties of fats and oils are intimately related to their composition and to their physical properties. Major advances in analysis in the last several years have occurred in the following specific areas: chromatography, dilatometry, nuclear magnetic resonance, urea fractionation, and spectroscopy—with a few miscellaneous methods in other areas.

Chromatography certainly heads the list of important advances in fat analysis. It has probably been the single most active area of exploration in the field in the last several years and still remains very active. The word chromatography which identifies this technique stems from the work of Tswett and probably is not a good choice since it does not denote the action or the process. However we do not need to be concerned here with nomenclature.

Chromatography does provide a means of obtaining quantitative separations of certain mixtures, and if you will permit a prediction, the applications will undoubtedly be greatly expanded in years to come.

Chromatography can for our purpose be very simply defined as a technique which utilizes such phenomena as surface adsorption, partition between solvents, and ion exchange to bring about separations of simple and complex mixtures into their various components. It is recognized that the definition may not be altogether adequate or complete but it will furnish a basis for our discussion.

The materials commonly involved in chromatography as applied to the analysis of fats include a) a solid support, such as diatomaceous earth, silicic acid, various chemical salts, paper, and other substances, b) a liquid stationary phase, usually adsorbed to the surface of the support, of varying composition depending upon the specific application, and c) a mobile phase which passes over or by the stationary phase. The mobile phase may be a liquid or a gas and in the case of the former may be designated as solvent, eluant, etc.

A good though elementary example of column adsorption chromatography is the A.O.C.S. method for the estimation of total neutral oil. Briefly this procedure involves pouring the sample, dissolved in a solvent, onto a column of aluminum oxide, and allowing the solution to percolate through the column. The eluate, *i.e.* the portion that passes through the column, is collected and the solvent is evaporated. The weighed residue represents neutral triglyceride.

The reason for being able to separate neutral oil from free fatty acids under the prescribed conditions for this method is that the less strongly held neutral triglycerides pass through the column with the solvent and the more polar, free fatty acids are adsorbed on the surface of the aluminum oxide and thus do not pass through the column.

In the case of partition chromatography separation is attained by distribution of the components of the mixture between the mobile and stationary phases based on partition coefficients.

Broadly speaking then, the fact that the different components of a mixture can be retained on or can be made to pass over or through a column at different rates by suitably adjusting the conditions and by properly selecting the solvents and other materials is the basis for the technique of chromatography. The separation may involve adsorption as in the procedure just mentioned, or partition between liquids as is applied to the fractionation of fatty acids. Ion exchange is not to my knowledge applied in many areas of fat analysis.

Separations employing column adsorption or partition chromatography have been successfully applied to the fractionation of fatty acids, to the determination of individual fatty acids such as butyric acid in butterfat and others, and to the determination of saturated fatty acids. It obviously is a good technique but when the mixture becomes complex the labor involved is not inconsiderable. Therefore in such instances paper chromatography and gas chromatography are more practical.