THE DETRIMENTAL EFFECTS OF THERMAL TREATMENT ON THE NUTRITIVE VALUE OF LINSEED AND SOYBEAN OILS

by

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INTRODUCTION

Oil-seed crops are becoming increasingly important to Canadian economy. While the meal plays a significant role in livestock feeding, the oils pressed from seeds have been adapted for a variety of uses such as paint products, margarines and salad oils. Prior to World War II considerable quantities of olive, corn, peanut and other oils were imported. However with importation restricted during the war, a serious shortage resulted which spurred investigations on the use of domestic oils from crops such as linseed, soybean, rapeseed, mustard seed and sunflower seed. The recent sanctioning of margarine production has further stimulated the interest in these oils.

It was found that following hydrogenation, certain of the more highly unsaturated oils, such as linseed, soybean and rapeseed, developed unpleasant odours and flavours on exposure to moderate amounts of heat and light (Bailey 1946). This property, termed flavour reversion, prohibited their satisfactory use as edible shortenings. Evidence was obtained that linolenic acid was a precursor of the reversion compounds in linseed oil (Armstrong and McFarlane 1945). In 1947 Privett and his co-workers showed that linolenic acid could be removed from linseed oil by thermal polymerization followed by acetone segregation; these processes yielded a flavour stable product well suited for edible uses.

However, rat feeding trials conducted in these laboratories have shown that certain vegetable and fish oils, including linseed and soybean oils, when heat polymerized under the conditions outlined by Privett (1947), were inferior in nutritive value to unheated oils (Crampton et al 1951a). This inferiority was obvious regardless of whether or not the oils were hydrogenated; therefore the nutritive damage was assumed to occur during polymerization (Mills 1944).

Success of the polymerization process in assuring a palatable oil is apparent from the commercial sale of herring oil treated in this manner, in both Norway and the United States (Schwitzer 1948; Lassen et al 1949). Although thermal polymerization as such is not yet used to any large extent, the trend in current processing methods appears to be directed towards its use. The majority of edible oil industries employ thermal deodorization. While deodorization temperatures (240°C) do not quite approach those necessary for polymerization and such heating periods rarely exceed 30 minutes, nevertheless, higher temperatures and more deodorization periods are being found increasingly satisfactory in producing bland oil products well suited for human consumption (Grace 1951). Therefore, before heat polymerization is universally adopted for oil processing, the extent and cause of its damaging effect on vegetable oils must be determined. This would facilitate the revision of the heat polymerization process so that it would yield a wholesome as well as a palatable product.

REVIEW OF THE LITERATURE

The Importance of Dietary Fat.

A comprehensive study of the adverse physiological action of polymerized oils requires first an understanding of the nutritional importance of wholesome fat. Apart from its abilities to improve the flavour of many food products and to contribute calories, fat is now recognized as an essential diet constituent.

Its essentiality was first definitely established by Evans and Burr (1926), who demonstrated that the consistent exclusion of fat from the diet of rats resulted in a deficiency disease typified by the following symptoms: marked retardation of growth frequently culminating in death, interference in the reproductive processes, dermatitis, anemia, kidney lesions and hematuria (Burr 1942). The administration of small amounts of lard or cod liver oil cured these symptons. Because the therapeutic potency of different fats varied according to their content of the higher unsaturated fatty acids, it was postulated that certain of the unsaturates were the active curative agents (Burr and Burr 1930). Linoleic, linolenic, arachidonic, hexaenoic, and hexahydroxystearic acids all proved beneficial in the treatment of fat deficiency symptoms in rats (Burr 1942). The content of these acids in animal tissues appeared to be directly dependent on the dietary supply (Banks et al 1933); for that reason it was assumed that they were not synthesized by the body, hence they were termed the "essential fatty acids".

These fatty acids have not yet been proven essential for human nutrition, however eczematous patients have exhibited low levels of the unsaturates in the serum lipids. Supplementation with unsaturated fats was followed by a correction of the dermal disorder and a concomitant rise in serum linolenate (Finnerud et al 1941).

It has been widely demonstrated that dietary fat is necessary for optimum performances of the physiological processes over and above its function of supplying the essential unsaturates. Deuel and his co-workers (1947, 1950a) have shown that fat-free diets, even when supplemented with linoleic acid, were definitely inferior in supporting rat growth to diets containing fat. Furthermore it has been demonstrated by Scheer et al (1947a) that in rats subjected to a twelve-week period of undernutrition followed by ad libitum feeding, those animals receiving a diet containing 40% fat exhibited reproductive performances much superior to those of rats fed a fat-free diet containing methyl linoleate. The physical capacity for spontaneous or forced activity and the length of time of survival under fasting conditions has been shown by Samuels et al (1948) to be much higher in rats previously fed diets containing 80% of the calories as fat than in those fed diets containing 80% of the calories as either carbohydrate or protein. In addition it has been found by Ershoff (1949) that fats exerted a marked beneficial influence in partially checking the growth inhibition imposed by thyroid hyper-activity; he did not attribute this effect to the essential fatty acids so supplied as he found that a practically fat-free liver extract acted in a similar manner.

These benefits of fat cannot be ascribed to a greater intake of energy as Scheer and his co-workers (1947b) have obtained similar effects with iso-caloric feeding of fat-containing and fat-free diets. Rather they may be the consequences of the action of fat in sparing heat losses due to specific dynamic action, in conserving both protein and carbohydrate,and in reducing the requirements of certain of the B vitamins.

Forbes and Swift (1944) showed not only that fat had the least specific dynamic effect of the three foodstuffs but also that fat had an "associative dynamic effect" when combined with either carbohydrate or protein or both. That is, when fat wholly or partially replaced dietary carbohydrate , the subsequent reduction in the heat loss was greater than that accountable by the additive costs for specific dynamic action of the amounts of each foodstuff ingested. No such deviation from expected values was found where only carbohydrate and protein were combined. In later experiments (1946) these workers found that the efficiency of utilization of food energy was in the order of the increasing fat content of the diets. These diets supplied identical amounts of gross energy and protein. In addition, Forbes and Swift observed that fat improved nitrogen retention.

Other workers have also reported a sparing action of fat on protein catabolism which was particularly noticeable under conditions of caloric restriction (Hoover and Swanson 1950, Samuels et al 1948).

It has been established through the use of tracers that deposited fat exists in a dynamic state rather than in an inert form (Schoenheimer and Rittenberg 1936). Scheer and his co-workers (1947b) proposed that, in the absence of a readily available stock of dietary fat, essential fat substances would be formed largely from carbohydrate through a chain of reactions that would cause greater caloric expenditure than if fat were resynthesized from breakdown products of its own kind.

Therefore, where fat partly replaces dietary carbohydrate, both the amount of glycolysis and the extent of fat biosynthesis are decreased. As thiamine and nicotinic acid function coenzymatically in glycolysis, while pyridoxine, biotin and pantothenic acid serve in fat biosynthesis, the sparing action of fat on these water-soluble vitamins may be thus explained (Evans and Lepkovsky 1929; Salmon 1947; Sherman et al 1950; Rubin and Scheiner 1949; Stotz 1949).

As pyridoxine and biotin function respectively in the decarboxylation and deamination of the amino acids (Umbreit and Gunsalus 1945; Rubin and Scheiner 1949) the sparing action of fat on protein catabolism would further reduce their requirement. No complete explanation for the conservation of protein by fat has yet been found. Part of the effect may be attributable to the sparing action of fat on protein-derived coenzymes such as nicotinic acid which is formed from tryptophane (Salmon 1947).

It should be mentioned that part of the action of fat in decreasing some vitamin requirements may be due to the stimulatory effect of fat on microbial growth (Strong and Carpenter 1942).

Another important function of dietary fat is to aid the absorption of the fat-soluble vitamins, A, D, E and K. Although there is some mention in the literature of fat alone exhibiting vitamin D activity in preventing rickets (Booth et al 1942), it is doubtful that fat is concerned to any large extent with the utilization of any of these vitamins other than as a transport mechanism (Deuel and Greenberg 1950).

Comparative Value of Different Fat Sources.

As fats do appear to have a prime nutritional function, it is necessary to ascertain whether the need for them can be met equally well by all fat sources. From the standpoint of digestibility and absorption there appeared to be no obvious differences between a wide variety of animal and vegetable fats (Deuel and Greenberg 1950). However, attempts to determine a fat level for optimum rat growth have indicated that the specific composition of the oil may have a bearing on the desirable quantity. Barki and his co-workers (1950) found that at the 35% level, butterfat was superior to corn oil, while the reverse was true at the 10% level. These differences were not corrected by additions of the known essential fatty acids, or by intraperitoneal injections of liver extract. It has been postulated that the variation in the changing nutritive values of fat according to the level in the diet might be due to differences in their content of the fat-soluble vitamins, differences in the effect of fatty acids on the microflora of the intestine, or differences in the demands of various fatty acids for biological oxidation (Deuel and Greenberg 1950;

Barki et al 1950). Nevertheless, Deuel and his co-workers (1950b) have found that rats fed butterfat, corn, cottonseed, olive, peanut and soybean oils as approximately 9% of the diet responded equally well in weight increases, bone growth and reproductive performances throughout twenty-five generations. Other workers have shown that linseed oil too, supports normal growth and reproduction in laboratory animals (Molotkow 1932; Maynard 1942).

Flavour Reversion.

As vegetable and animal fats appear equally well utilized it would seem nutritionally justifiable to enhance the crop value of linseed and soybeans by using the oils pressed from them for edible purposes. However, a prerequisite for any food product is palatability. Among other vegetable oils, linseed and soybean have been found susceptible to "flavour reversion", a term used to describe the development of fishy or straw-like odours unlike those of the original oil (Bailey 1946). This phenomenon occurs on exposure to heat and light, and although oxidative in nature,it is not true rancidification. What causes the development of these undesirable characteristics has not yet been determined, however fats relatively free from linolenic acid are flavour stable (Bailey 1946). It has been postulated by Armstrong and McFarlane (1945) on the basis of considerable experimentation, that linolenic acid is a precursor of reversion compounds.

The economic advantage of using locally produced oils in shortenings and salad oils forced the investigation of methods to eliminate possible flavour instability. In 1945, Privett and his co-workers, found that by heating linseed oil at 275°C for 12 hours in the absence of oxygen, the linolenic acid, being highly unsaturated, conjugated and polymerized during the early stages of heating. Oil treated in this manner was flavour stable. The fraction of this polymerized oil that was soluble in acetone, and so presumably polymer free, was recommended as that most suited for edible purposes (Privett et al 1947).

The palatability of this heated oil fraction has been confirmed by Lips (1949). His taste panel studies showed that linseed oil should be heat polymerized at least four hours to ensure its acceptability in baked products while longer heating periods further improved the flavour of the oil.

Effects of Feeding Heated Oils.

Feeding trials conducted in these laboratories showed that, although heat polymerization might have improved the flavour stability of linseed oil, it severely damaged its nutritive value. Weanling rats fed the acetone soluble fraction of heated linseed, soybean, rape, peanut or corn oils or whole heated herring oil failed to grow as well as those fed the respective unheated oils (Crampton et al 1951a). Female rats in our laboratories fed whole polymerized linseed oil from the time of weaning were unable to reproduce normally. Of those bred,

there was a high incidence of resorption and hemorrhage prior to parturition; of the young born, none survived the nursing period (Siddall 1951).

Although polymerized oils are used for edible purposes in both Norway and the United States (Schwitzer 1948; Lassen et al 1949), few publications concerning their nutritive value are to be found. Harris (1947) reported that the feeding of polymerized herring oil inhibited rat growth and brought about a general appearance of ill health. Lassen et al (1949) working with mature rats noted a definite depression in the digestibility of sardine oil with increasing degrees of polymerization. These workers also found some indications of avitaminosis K.

In our laboratories Langerman (1949) found that the addition to the diet of either liver extract or feces from normal rats, improved the growth of rats fed heat polymerized linseed oil. From these results she theorised that some toxic substance within the heated oil inhibited the bacterial synthesis of an essential nutrient, either through interfering with enzyme systems of the microflora or through making the end-products of biosynthesis unavailable. Orally administered supplements of vitamins such as nicotinic acid, riboflavin, thiamine, folic acid, A, D, C or K failed to overcome the adverse effects of the heated oils.

The scientific literature contains a wealth of reports of malnutrition resulting from the feeding of fat oxidized by heating in air or by natural rancidification. The observed effects on rats

of oxidized dietary fat include growth inhibition, reproductive failure, anemia, dermatitis, paralysis and digestive disturbances (Quackenbush 1945a; Morris et al 1943). In 1946, Kennelly and Quackenbush found that when oxidized fat, preceded by a three-hour fast, was fed by dropper, neither decreased growth rates nor low fertility nor death resulted; whereas, such effects were produced when the oil was mixed into the diet. From this, they concluded that oxidized fat was harmful indirectly by destroying essential non-lipid components, possibly vitamins A and E which are known to be unstable in the presence of peroxides of linolenic acid (Quackenbush 1945b).

Although the biological manifestations of feeding oils heated in air (oxidized) appear similar in some respects to those of feeding oil heated in the absence of air (heat polymerized), it would be expected from the differences in the chemical nature of the oils that the sources of their adverse action would differ. Clark (1950) showed that autoxidation was not concerned with the deleterious action of polymerized oil. Furthermore, experiments to be reported in the following pages indicate that the deleterious action of polymerized oil was not the consequence of the destruction of non-lipid diet ingredients, but that some substance within polymerized oil was directly detrimental to the animal.

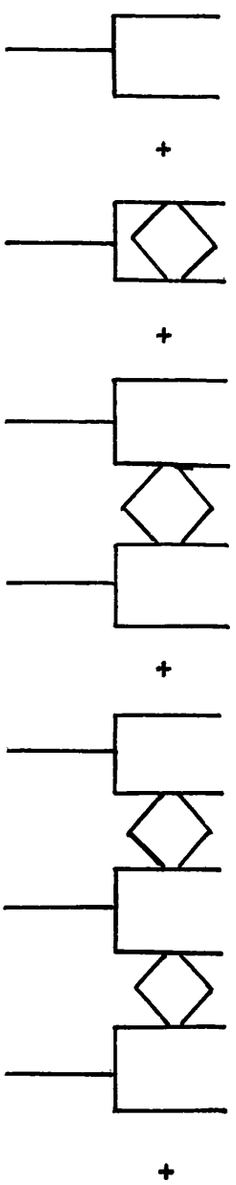
Products of the Heat Polymerization of Oil.

The extent to which a triglyceride molecule is polymerized is essentially a function of the unsaturated centres of its constituent fatty acids (Terril 1946). During severe heating and in the absence of oxygen, the double bonds shift along the fatty acid chain to give conjugated isomers of the original acids; when the percentage of these isomers has been built up to a certain point, cyclic dimerization is presumed to occur. Consequently the most highly unsaturated acids are polymerized most rapidly, followed by the lesser unsaturated acids in order of decreasing unsaturation (Privett et al 1947).

Although polymeric glyceride molecules may contain two, three, four or more glyceride structures, it is generally accepted that the fatty acid residues themselves do not proceed beyond the dimer stage (Kass 1947). Therefore it may be presumed that the polymeric glycerides are formed through the cyclic dimerization of adjacent fatty acid radicals. When this dimerization occurs between fatty acids of different glyceride molecules, an interpolymer is formed, whereas when cyclic union of fatty acids of the same glyceride molecule occurs, an intrapolymer is formed. The existence of intrapolymers has been a subject of considerable controversy. However, the failure of the molecular weight of polymerizing oil to increase as the unsaturation decreased, strongly implies the formation of such a compound (Adams and Powers 1944; Wiseblatt 1950).

Accordingly the structure of what are presumably the principal components of polymerized oil may be illustrated as in Figure 1 in which the "tuning fork" symbolizes the triglyceride molecule.

FIGURE 1. The principal components of polymerized oil.



True Monomeric Glycerides

+

Intrapolymeric Glycerides (Monomeric)

+

Interpolymeric Glycerides (Dimeric)

+

Interpolymeric Glycerides (Trimeric)

+

Higher Interpolymeric Glycerides

Wiseblatt (1950) showed, contrary to the opinions of Privett et al (1947), that the acetone soluble fraction of the oil did contain inter-polymeric material; such material was accordingly suspected of being injurious. However a fraction of the oil soluble in propanol, shown to be relatively free from any inter-polymeric material (Bernstein 1948; Wiseblatt 1950) still inhibited the growth of young rats (Clark 1950). This failure of solvents, which segregate chiefly on the basis of molecular weight, to isolate the toxic material implied that during polymerization some substance which was formed both intermolecularly and intramoleculary, was responsible for the deleterious effects.

In addition to those primary products of polymerization illustrated above, Kass (1947) states that up to 10% of the heated oil might consist of non-polymerizable products of reduced unsaturation but unchanged molecular weight. Such substances are thought to be formed chiefly from trienoic acids through cyclic isomerization. Also side reactions are known to yield free fatty acids, aldehydes, and hydrocarbons, as well as acids and esters of reduced molecular weight; these latter fragments sometimes polymerize among themselves (Kass 1947).

The Nutritive Quality of Fat Substances.

Although it is not yet known just what determines the nutritive quality of edible fats, it is becoming increasingly evident that certain structural characteristics of their fatty acids are of paramount importance. Mattil (1947) has shown that the amount of C_{18}

or higher saturated acids is the chief limiting factor in fat digestibility, while the degree of unsaturation determines an oil's ability to cure the symptoms of fat deficiency (Burr 1942). Although the essential fatty acids themselves show both qualitative and quantitative differences in their prophylactic effects on fat deficiency, they have in common the fact that they are all straight chain structures containing at least two non-conjugated double bonds of cis configuration (Daubert 1949). Isomerization of these unsaturates is known to lower their nutritive value. In 1948, Kummerow and his co-workers demonstrated that fats in which 70% of the double bonds were conjugated (eg. 10, 12 ethyl linoleate) aggravated a fat deficiency rather than having any therapeutic effect.

In respect to this influence of configuration on the nutritional quality of fats it is interesting to consider the recently reported growth-promoting factor in summer butter, vaccenic acid (Boer et al 1947). While the value of this acid as a superior growth stimulant has been virtually disproved (Euler et al 1948; Deuel et al 1948), it has not been shown harmful. Nevertheless in 1950, Morton and Todd found that the cis-form of vaccenic acid exhibited all the destructive properties of a natural hemolytic acid. It is significant that both these acids are but isomers of oleic acid, present in substantial amounts in both linseed and soybean oils (Hilditch 1949).

Structural changes other than configurational have also been shown to alter nutritive value. For instance, varying the number of hydroxy groups in stearic acid also produces substances of varying

biological value. Dihydroxystearic acid, when fed to rats as 8% of the diet predisposed avitaminosis K (Strong and Carpenter 1942) while hexahydroxystearic acid has been shown capable of partially curing the symptoms of essential fatty acid deficiency (Burr 1942). Thomas and Weitzel (1947) found that some branched chain fatty acids inhibited normal rat growth apparently through metabolic malfunction as indicated by the appearance of dicarboxyllic acids and their isomers in the urine.

Certain cyclic substances formed through heating are reportedly toxic in nature, for example tachysterol, a product intermediate in the conversion of ergosterol to calciferol by irradiation (Windauss et al 1932). Roffo (1944) reported the development of tumor-like lesions in rats fed heated oil. He believed these were caused by cyclic substances in the oil which contained the phenanthrene nucleus common to many carcinogens; however it should be pointed out that numerous workers have been unsuccessful in repeating Roffo's work (Anon. 1945).

From the literature reviewed herein it appears possible that the amounts of conjugation, isomerization, and cyclization that occur in the course of heat polymerization of oils, could cause the development of a number of substances which might be harmful in nature. In view of the high temperatures now used for the deodorization of oils in industrial production and the fact that longer heating periods yield more palatable oils, it is essential from both nutritional and economic

standpoints that the detrimental effects of heating oil be realized and any toxic materials be identified. The research reported in the succeeding pages has been directed toward these ends.

OBJECT OF RESEARCH

The primary aims of the research reported herein are to study more extensively the detrimental effects of feeding heat polymerized linseed and soybean oils to young animals and to determine the etiology of these ill effects.

EXPERIMENTAL PROCEDURE

General.

A series of trials was conducted in which young rats were fed linseed or soybean oils as either 10% or 20% by weight of identical diets. To study the nature and cause of the damaging effects of thermal polymerization on these oils, the duration of the polymerization period was varied, the amounts and methods of feeding the oils were varied, and different fractions of the heated oil constituents, separated through distillation, were fed. Linseed oil was used in the bulk of these experiments because of the facility with which it could be damaged by heating and because most of the previous work had been conducted with this oil. Soybean oil was later examined to compare the effects of polymerization on two oils of different composition.

Animals.

Albino rats of both sexes were used in these experiments. At the outset of each feeding period they ranged in age from 21-35 days; within each test the age range was not permitted to exceed 7 days. Animals were allotted at random within sex into lots of 6 to 18, according to the design of each particular experiment. Each trial within this series was an individual test based on either a simple randomized block or a factorial design.

Diets.

1. Composition.

In these experiments two basal diets were used which differed essentially in the fat component. Changes in the fat content of

the diet from 10% to 20% were made at the expense of the flour. Although such an alteration does decrease the proportion of calories derived from protein, it is presumed from the work of Millar (1947) that when protein contributes 20% of the calories the effects on rat growth of fat changes of this order are not significant. The fat component differed between and within tests in the oil source and in the processing prior to feeding. The composition of the basal diets fed is indicated in Table 1.

Table 1. Percentage composition of basal diets

Ingredients	Percentage by weight in the diet	
	10% fat diet	20% fat diet
White flour	54	44
Casein	11.5	11.5
Milk powder	19	19
Fat	10	20
Yeast[a]	3	3
Bone meal	2	2
Salt	0.5	0.5
% calories from protein	24.3	20.4

[a]In earlier trials, such as Test E, the following purified B vitamins were incorporated in the diet: thiamine 10 p.p.m., niacin 230 p.p.m. and riboflavin 5 p.p.m. Later, yeast was used instead as it was considered to be a more complete B vitamin supplement. It was included in the diet at the expense of the flour.

II. Preparation.

Diets were prepared immediately before the beginning of each test. In general, the dry ingredients with the exception of the yeast were mixed and the oil then incorporated through the action of a Hobart blender. In earlier trials (Tests C, D, E and G) the diets were baked for 20 minutes at 375°F to simulate conditions to which the oil would be exposed in ordinary food preparation. Following baking, the resulting biscuit was granulated and air-dried for 24 hours. The yeast (or purified B vitamins) was then added, the diets bottled and stored at room temperature.

In later experiments (Tests A, D and F) baking as a step in diet preparation was omitted as it was found to have no influence on the nutritiousness of diets containing polymerized oil (in triglyceride form), and it proved to be a time-consuming operation unnecessary in nutritional studies of this type (Berryhill 1950b).

In addition to the basal diet, each animal received weekly a single drop of corn oil containing 35 I.U. of vitamin D and 175 I.U. of vitamin A.

Variations in Diet Supplementation.

In Test B, half of the animals fed heated oil received weekly one dose of corn oil containing 7 mg. alpha tocopherol. These doses were administered two days following the routine dosing of vitamins A and D.

A list of the diets fed in this series of experiments is included in Table 2. Variations in the fat component, the method of feeding and the numbers of animals used are also cited.

Table 2. Allotment of Animals to Diet Treatments

Test No.	Diet No.	Oil Source	Heat treatment at polymerizing temperatures	Oil fraction fed	Variation in feeding methods	% Oil Fed	No. Animals Male	No. Animals Female
A	1[a]	Linseed	Nil	Whole		10	4	4
	2	Linseed	12 hours	Whole		10	4	4
	3	Linseed	Nil	Whole	Oil fed by dropper apart from other diet ingredients	10	4	4
	4	Linseed	12 hours	Whole		10	4	4
B	5	Linseed	12 hours	Whole	Vit.E supplementation	10	12	12
	6[b]	Linseed	12 hours	Whole		10	12	12
C[c]	7[a]	Linseed	Nil	Whole		20	9	9
	8	Linseed	Approx. 12 hrs.	Commercial preparation from acetone soluble fraction		20	9	9
	9	Linseed + Corn	Approx. 12 hrs.			5	9	9
			Nil	Whole		15		
D[c]	10[a]	Linseed	Nil	Whole		20	4	4
	11[a]	Linseed	Nil	Ethyl esters of whole oil		20	4	4
	12	Linseed	12 hours	Ethyl esters of acetone soluble fraction		20	4	4
	13	Linseed	12 hours	Distillate of Esters of acetone soluble fraction		20	4	4
	14	Linseed	12 hours	Residue of Esters of acetone soluble fraction		20	4	4

Table 2. (continued) Allotment of Animals to Diet Treatments

Test No.	Diet No.	Oil Source	Heat treatment at polymerizing temperatures	Oil fraction fed	Variation in feeding methods	% Oil Fed	No. Animals Male	No. Animals Female
E[cd]	15[a]	Linseed	Nil	Whole		10	10	
	16	Linseed	2 hours	Whole		10	10	
	17	Linseed	4 hours	Whole		10	10	
	18	Linseed	8 hours	Whole		10	10	
F	19[a]	Linseed	3 hours	Whole	% dimers in diet = 0.9	10	10	5
	20	Linseed	3 hours	Whole	1.8	20	10	5
	21	Linseed	10 hours	Whole	1.8	10	10	5
	22	Linseed	10 hours	Whole	3.6	20	10	5
	23[a]	Soybean	7 hours	Whole	0.9	10	10	5
	24	Soybean	7 hours	Whole	1.8	20	10	5
	25	Soybean	21 hours	Whole	1.8	10	10	5
	26	Soybean	21 hours	Whole	3.6	20	10	5
G[c]	27[a]	Soybean	Nil	Whole		20	10	
	28	Soybean	3 hours	Whole		20	10	
	29	Soybean	6 hours	Whole		20	10	
	30	Soybean	9 hours	Whole		20	10	

[a] Positive control
[b] Negative control
[c] All diets baked
[d] Pure vitamin additions rather than yeast.

Management.

The animals were housed in individual metal cages having wire bottoms, beneath which were fastened removable trays. The latter were covered with absorbent papers which were changed on alternate days. These facilities permitted the collection of feces and spilled feed which were subsequently separated through the use of a wire mesh strainer. Spilled feed so recovered was returned to its respective feeder. The approximate amount of feed required by each animal for the entire test was contained in individual light-repellent brown bottles from which the respective feeders were filled as required. Feed was supplied ad libitum in all cases except in Test A. In the latter, where the effect of polymerized oil on the intake and nutritive value of non-lipid diet ingredients was being investigated, access to the basal diet minus the fat was permitted for 16 hours daily. Following 3 hours fasting, an amount of oil equal to 10% of the fat-free diet consumed during the preceding feeding period, was force-fed by dropper. Access to the feed was again allowed following 5 hours fasting. Water was supplied ad libitum to all animals.

In the majority of cases, feces were collected for the last 7 days of the test period to permit the calculation of digestibility coefficients. Quantitative collections were attempted in earlier trials (Tests C, D, E and G). However, as intermittent diarrhea and coprophagy interfered with the accuracy of such collections, the index method of determining digestibility was later adopted. Where this technique was employed (Tests A, B and F), chromium oxide (sesqui)

served as the index and was added as 1% of the diet. Its dry weight in the feed and the feces, collected at random within lots, was determined by the method of Schürch et al (1950).

Records.

Where feces were collected quantitatively, the dry weight of the excreta for each lot of animals during the final week of the test was recorded.

The feed allotted to each animal was weighed in its container at the outset of the trial and on successive seventh days; the weekly intake was estimated by difference. In Test A, the daily feed intake was estimated in the same manner.

Each animal was weighed at the beginning of the test and on successive seventh days. Weekly gains in weight were determined by difference.

General Oil Processing.

Alkali-refined oils contained in an all-glass apparatus were heated in a salt bath at 275°C for the desired length of time. Carbon dioxide was blown through the oil continuously to exclude air and to remove volatile decomposition products thoroughly. Privett and his co-workers (1947) had recommended the acetone soluble fraction of linseed oil polymerized in this manner for 12 hours as that most suited for edible purposes. However as acetone segregation was found to have no significant effect on the nutritive value of the oil other than depressing feed intake (Langerman 1949), this process was not used in all the experiments reported herein.

Variations in Oil Processing.

In Test G, the soybean oil was heated at 280°C under nitrogen instead of carbon dioxide. In Test D, the linseed oils were esterified to reduce both intrapolymers and interpolymers to esters of their constituent monomeric and dimeric fatty acids. Separation of volatile from non-volatile material was effected through distillation. Oils were interesterified with anhydrous ethanol using concentrated H_2SO_4 as a catalyst. Distillation of esters was effected under vacuum (about 0.5 mm Hg absolute pressure) in standard distilling and receiving flasks suitable to the quantities of esters distilled. The distilling bath was filled with clean glass wool to minimize foaming and bumping. The distilling adapter was wrapped with glass wool to prevent excessive fluxing and to ensure the most rapid removal possible of distillable esters. These usually distilled entirely between 155 and 175°C. When vapour temperature reached a maximum and began to fall the heating bath was taken steadily up to 240°C. If no further rise in vapour temperature occurred the bath was removed and the residue allowed to cool rapidly in the bulb, without breaking the vacuum. Residues had to be recovered by washing out the glass in the bulb with ether and evaporating the ether in a stream of CO_2. This ensured complete recovery of the residue and accurate estimation of yields by collecting the ether washings in tared flasks.

In Test F, the accumulation of polymerization products was controlled by heating each oil until a specific content of dimeric fatty acid radicals was produced. Prior to the preparation of oils for feeding, the dimeric content of each oil heated a known length of

time at 275°C was determined by distillation. The refractive index of the oil was found to increase with increasing amounts of dimers. This relationship was plotted graphically; it was then possible to heat the oils to a specific refractive index which indicated the dimer content.

Criteria.

I. Qualitative.

The general appearance of health of all animals was observed, as was their activity. Post mortem examinations were conducted on all animals not surviving the test period and on representative samples of these that did. In certain instances (Tests C and D), survival time served as a criterion for the detrimental effects of polymerization on these oils.

II. Quantitative.

The nutritive value of linseed and soybean oils was assessed essentially by the intake and digestibility of diets containing these oils and by the growth of young rats fed such diets over a four-week period. Where the data permitted, statistical analyses of variance and covariance were applied; differences were judged significant at the 5% point.

Where feed intake and digestibility varied between diets, those effects on growth were removed by converting the ingested feed to digested calories in the following manner:

Grams feed ingested x calories/gm of feed x % digestibility = digested calories.

NOTE: It was found that the % digestibility of the dry matter coincided with the % digestibility of the calories ingested (see Appendix Table 2).

By calculating the regression of weight gain on the intake of digestible calories, it was then possible to adjust all mean gains to an intake of 1000 digested calories according to the following formula:

$$y - byx\ (x - 1000) = \hat{y}$$

where

y = mean gain
x = mean intake of digested calories
byx = regression of gain per unit intake of feed
$\hat{y}$ = mean gain adjusted for regression.

The adjustment of weight gain in this manner permitted comparison of the efficiency with which equal amounts of absorbed calories from various diets were used for weight increases. Where growth was still significantly inferior even when the effects of feed intake and digestibility were thus eliminated, the diet was considered to contain some toxic material.

Digestibility coefficients were calculated according to the following formula:

$$\frac{a - b}{a} \times 100 = \%\ \text{digestibility}$$

where

a = gms. dry matter ingested (Conventional Method)

or

gms. dry matter ingested per gm Cr_2O_3 (Index Method)

and b = gms. dry matter excreted (Conventional Method)

or

gms. dry matter excreted per gm. Cr_2O_3

Where digestibility coefficients could not be calculated owing to inaccuracies in the collection of feces imposed by their unsatisfactory consistency (Tests C, D and E), weight gains were adjusted through

regression coefficients to an intake of 1000 gross calories. The caloric values of these diets were assumed to be of the same order as other diets of identical fat content.

With the exception of Test G, the heats of combustion of all diets whose digestibility was calculated and of the feces from animals fed those diets were determined in a bomb calorimeter. In Test G, caloric determinations were not carried out. The caloric value of the diet was assumed to compare closely with that of other diets of similar fat content; it was also assumed that the caloric digestibility would not be different from the dry matter digestibility as this was found to be the case in Tests A, B and F (Appendix Table 2).

OBSERVATIONS AND RESULTS

Animals fed polymerized oils developed oily matted coats and excreted dark sticky feces. These conditions were more severe when the dietary oil was included at the higher level, when the oil was heated for a longer time, or when the dimeric content of the diet was high. They appeared to be the result of unabsorbed oil residues.

In numerous cases, rusty patches were observed on the hair coats; these were not of fecal origin as this condition was noted where the feces were green due to the presence of chromic oxide. A rust-coloured crust was also noticed about the nostrils of some animals fed heated oils.

Post mortem examinations revealed a distinct absence of internal fat depots implying either an increased demand for calories or for fat substances not supplied in adequate amounts by the diet or a destructive action of some diet constituent on body fat. No other general internal abnormalities were observed.

The trials reported herein are not listed in chronological order but have been grouped to consolidate their implications and to provide a progressive picture of the elucidation of the problem. The figures cited in the succeeding tables are average values for all rats so treated. The feed efficiencies, or gains adjusted to equal caloric intake, have been calculated through regression coefficients as indicated in the preceding pages. The graphs presented illustrate linear relationships; the line of apparently best fit has been drawn in all cases. A summary of all data may be found in Tables 1 and 2 of the Appendix.

LINSEED OIL

I. The detrimental effects of feeding polymerized linseed oil to rats.

The inclusion of heat polymerized linseed oil in a diet for rats lowered feed intake and digestibility and inhibited normal growth. These facts are illustrated in the following table.

Table 3. The effects of heat polymerized linseed oil on intake, digestibility and utilization of feed (Test A).

Diet No.	Treatment of oil	Average gain[a] (gms)	Average feed intake (gms)	Digestion coefficient (%)	Average intake of digested calories[b] (cal)	Average gain[c] per 1000 digested calories (gms)
1	Unheated	118	348	96	1672	92
2	Heated	75	270	94	1270	65

[a]Necessary difference = 14.43 (5%)
[b]Necessary difference = 175.2 (5%)
[c]Necessary difference = 12.6 (5%)

However depression of appetite and digestibility did not entirely explain the poor growth of rats fed heated oil, as the digested calories from diets containing heated oil were less efficiently converted to weight increments than those from diets containing unheated oil.

II. The effect of polymerized linseed oil on non-lipid diet constituents.

Feeding the dietary oil by dropper, preceded and succeeded by periods of fasting prevented contact of the oil with non-lipid diet ingredients during diet storage, ingestion and digestion. When the heated oil was force-fed in this manner, the voluntary consumption

of the fat-free portion of the diet was the same as that of animals fed unheated oil, whereas feed intake dropped when heated oil was a diet component. This indicated that the inclusion of heated oil lowered the acceptability of the diet. However, as the utilization of digested calories was equally poor when the heated oil was fed either as a part of the diet or as a supplement, the inhibition of rat growth appeared to be the direct result of some defect within the polymerized oil rather than the consequence of the oil destroying some essential non-lipid diet constituents. Results of a feeding trial substantiating these statements are summarized in Table 4.

Table 4. The effects on growth, feed intake and feed utilization of polymerized linseed oil fed as a diet supplement rather than as a diet component (Test A).

Diet No.	Method of feeding oil	Treatment of oil	% Oil fed	Average gain[a] (gms)	Average feed intake (gms)	Average gain[b] per 1000 digested calories (gms)
1	Oil fed as a diet component	Unheated	10	118	348	92
2		Heated	10	75	270	65
3	Oil fed as a diet supplement (by dropper)	Unheated	10	116	350[c]	91
4		Heated	10	96	350[c]	71

[a]Necessary difference = 14.43 (5%)
[b]Necessary difference = 12.6 (5%)
[c]Feed intake adjusted to include oil supplement which equalled 10% of fat-free diet consumed.

III. The effect of polymerization on essential fat components.

The lethal effect of a commercial preparation from polymerized linseed oil was greatly relieved by substituting unheated corn oil for a large portion of the dietary fat. This effect is illustrated by the data in Table 5.

Table 5. The effect of diluting a lethal fraction of polymerized linseed oil with unheated corn oil (Test C).

Diet No.	Oil source	% unheated oil in diet	% heated oil in diet	Average[a] gain (gms)	Average feed intake (gms)
7	Linseed	20	-	67	174
8	Commercial preparation from acetone soluble fraction of heated linseed oil	-	20	-[b]	-
9	Commercial preparation + corn oil (1:4)	15	5	57	172

[a]Necessary difference: 7.94 (5%); 10.77 (1%).
[b]All animals died within 10 days; although feed intake was very low, a subsequent paired feeding trial showed death was not the consequence of starvation (Berryhill 1950a).

The difference between the gains of animals fed unheated linseed oil and those fed the oil mixture was barely significant at the 1% point as contrasted to the lethal effect of feeding the heated oil alone. Some of the toxic effects of the heated oil were probably relieved by decreasing the amount of heated oil in favour of the more acceptable and wholesome corn oil. However that so marked an improvement in growth resulted would imply that the corn oil contributed some essential fat substance which was not supplied in adequate amounts by the polymerized

oil, or whose requirement was increased in the presence of polymerized oil.

That the detrimental effects of polymerized oil were not evidences of the destruction of vitamin E was illustrated by the failure of vitamin E supplements to improve the nutritive value of heated oil diets. The latter effect is shown in Table 6.

Table 6. The effect of vitamin E supplementation to diets containing polymerized linseed oil (Test B).

Diet No.	Diet supplement	Average[a] gain (gms)	Average feed intake (gms)	Average gain[b] per 1000 digested calories (gms)
5	vitamin E	89.4	317	69
6	Nil	81.5	302	64

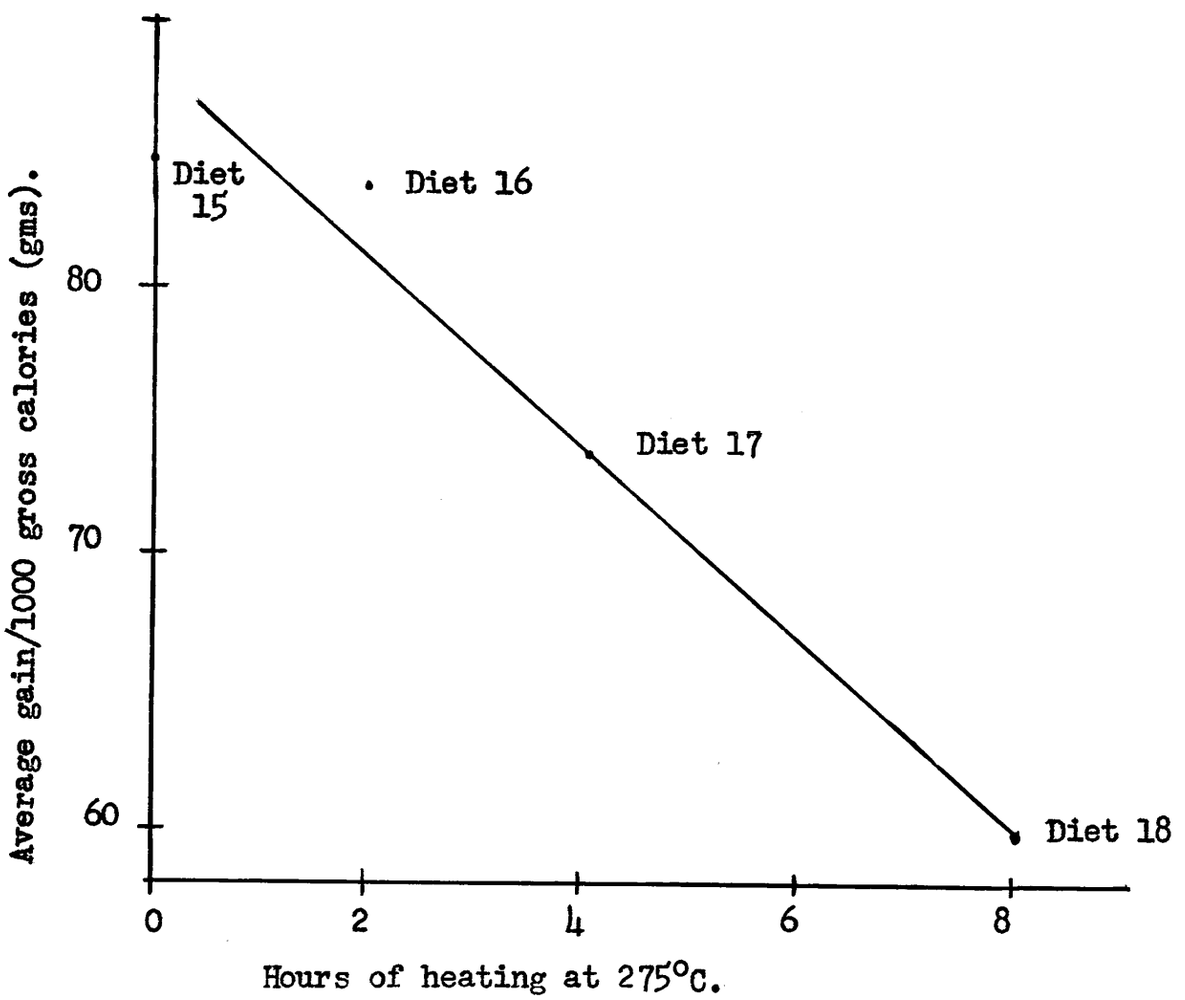
[a]Necessary difference = 16.77 (5%)
[b]Necessary difference = 11.88 (5%)

These data are supported by further experiments in this laboratory which showed vitamin E supplementation unable to correct the poor reproduction and lactation performances of rats fed polymerized linseed oil (Farmer et al 1951).

IV. Investigations concerning the identity of the deleterious material in polymerized linseed oil.

It had been previously established that increasing the dietary level of heated linseed oil intensified the inhibition of rat growth (Clark 1950). In this series it was found that prolonging the period of oil polymerization, thus increasing the extent of polymerization reactions, had a similar effect as is illustrated in Figure 2.

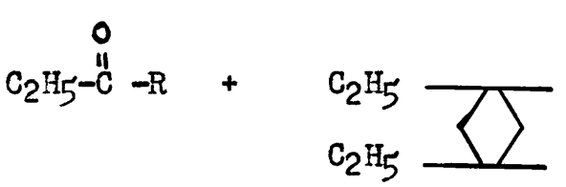
Figure 2. The effect on rat growth of increasing the polymerization period of linseed oil (Test E).



These findings could be interpreted to mean that some polymerization product in heated linseed oil was responsible for its toxic action. It had been found that oil fractions in which the principal products of polymerization were either intrapolymers or interpolymers were equally deleterious to rats (Crampton et al 1951c). Therefore it was supposed that the dimeric fatty acid radical common to both, was the toxic substance.

By esterification, it was possible to convert the heated triglyceride to a mixture of dimeric and monomeric material the theoretical structure of which may be represented as in Figure 3.

Figure 3. Principal components of the ethyl esters of polymerized linseed oil.



Monomeric Esters Dimeric Esters

As the dimeric esters were non-volatile, they were separated from the monomeric esters by distillation. These oil fractions were then fed to rats as 20% of the diet; however neither appeared free from toxic substances. The majority of the rats fed the non-volatile residue died within 7 days while the distillate, although presumably of the same structural character as the esters of unheated oil, was as poor in nutritive value as the esters of heated oil (acetone soluble fraction). Data supporting these statements are summarized in Table 7.

Table 7. Effect on rat growth of feeding distillation fractions of esterified polymerized linseed oil (Test D).

Diet No.	Oil fraction fed	Principal components of oil fraction	Average gain[a] (gms)	Average feed intake (gms)	Average gain[b] per 1000 gross calories (gms)
10	Whole unheated oil	Monomeric glycerides	119	338	64
11	Esters of whole unheated oil	Monomeric esters	80	263	52
12	Esters of acetone soluble fraction of heated oil	Monomeric + dimeric esters	29	203	23
13	Distillable esters of acetone soluble fraction	Monomeric esters	23	156	33
14	Undistillable esters of acetone soluble fraction	Dimeric esters	-[c]	-	-

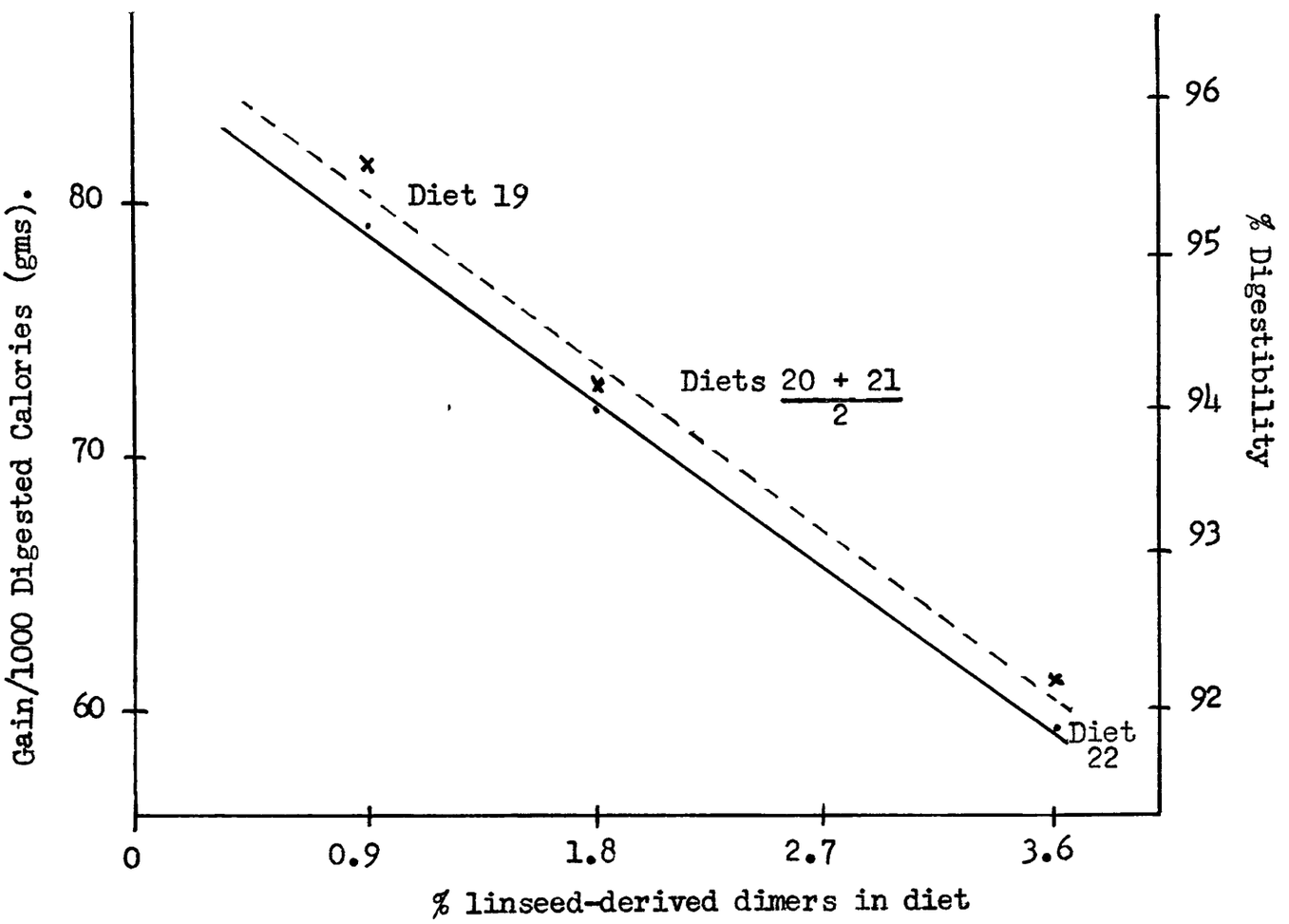
[a]Necessary difference = 13.71 (5%)
[b]Necessary difference = 11.49 (5%)
[c]Six out of 8 animals died within the first week. Their average daily feed intake = 2 gms.

NOTE: It is apparent that the esters of unheated oil were inferior in nutritive value to the unheated triglyceride. However it is presumed that the use of esters in this feeding trial did not mask the effects of polymerization. The difference in nutritional value between the heated and unheated esters is of the same order as that observed in other experiments between heated and unheated triglycerides. In addition, the general inferiority of esters for feeding purposes is reportedly due to their susceptibility to autoxidation (Quackenbush et al 1942a); it has been established that autoxidation does not increase the deleterious nature of the toxic factor in polymerized linseed oil (Crampton et al 1951b).

It was observed in this and in other experiments that animals fed whole heated oil or its acetone soluble fraction (i.e. a mixture of monomeric plus dimeric material) developed pily matted coats and excreted dark sticky feces. These conditions were evident to an extreme degree in animals fed the dimeric residue, while they were completely absent from animals fed the monomeric distillate. Unfortunately the digestibility coefficients of these diets were not calculated as the consistency of the feces prevented quantitative collections from all groups of animals and the index method of determining digestibility was not in use at that time.

As the former trial proved inconclusive in determining the role of dimeric and monomeric substances in the deleterious effects of heated linseed oil, a subsequent experiment was conducted in which diets containing specific amounts of dimeric material in whole heated linseed oil were fed. It was found therein that although the efficient use of the feed for growth was depressed according to the dimer content of the diet, so also was the diet digestibility (Figure 4). The quantity of the undigested calories was so closely related to the quantity of calories fed as dimers that it appeared that the dimers might not have been absorbed. Calculations which gave rise to this statement are presented in Table 8, while the relationship between increases in dimer-derived calories and undigested calories of the diets is illustrated graphically in Figure 5.

Figure 4. The effects on the efficient use of digested calories for weight gain and on diet digestibility of increasing the linseed-derived dimeric material in the diet (Test F).



——————— denotes weight gain:dimer relationship

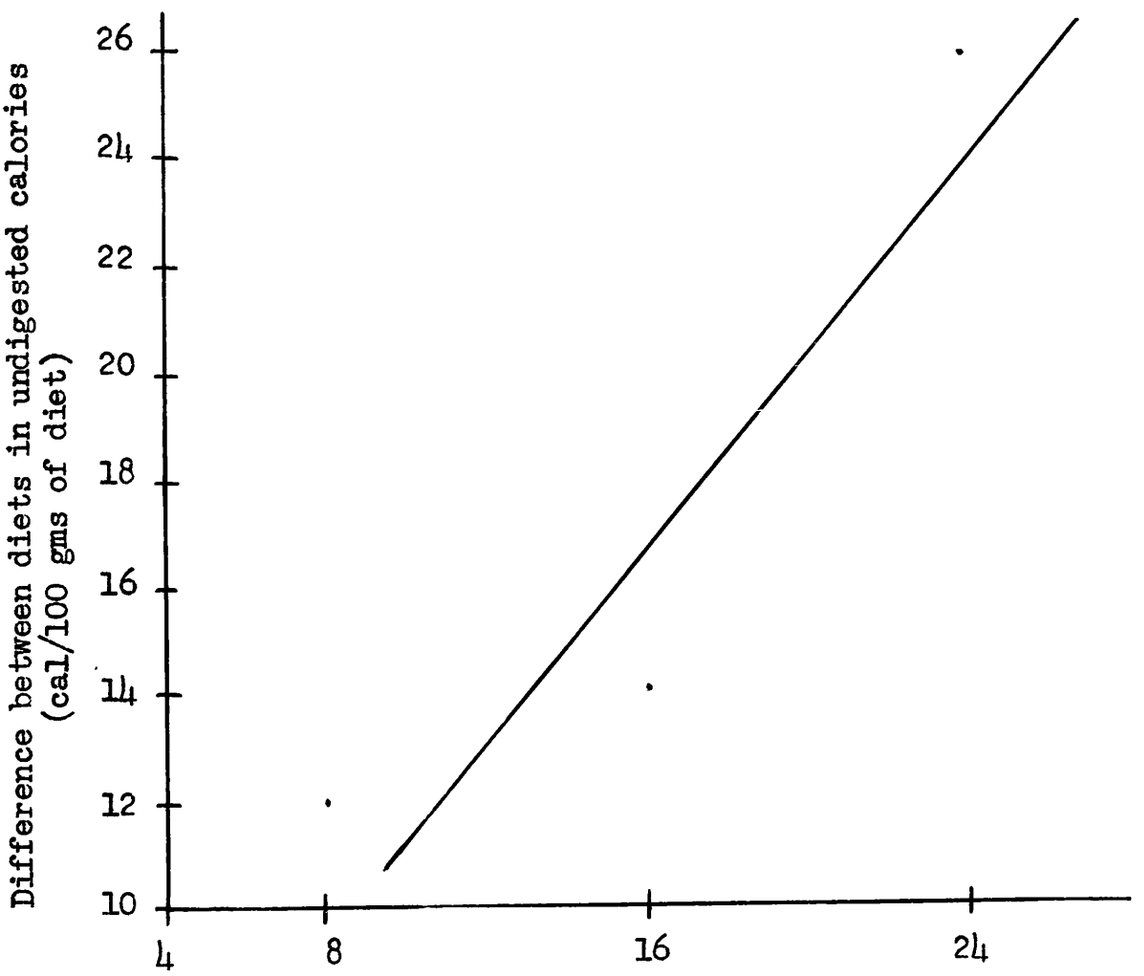
- - - - - - - - denotes digestibility:dimer relationship.

Table 8. Quantitative changes in undigested calories with increases in diet content of linseed-derived dimers (Test F).

Approximate Dimer Content of Diets (gms/100 gms feed)			Difference in Dimer content between diets (gms)	Calculated difference in Calories derived from dimers (9 cal/gm)	Difference between diets in undigested Calories[a] per 100 gms of feed
Diet 19	Diets $\frac{20 + 21}{2}$	Diet 22			
0.9	1.8	-	0.9	8	12
-	1.8	3.6	1.8	16	14
0.9	-	3.6	2.7	24	26

[a]Undigested calories from 100 gms. of each diet cited in Appendix Table 2.

Figure 5. Quantitative changes in undigested calories with increases in dietary content of linseed-derived dimers (Test F).



As cyclic dimerization is a function of polymer formation, the dimeric content of an oil is merely an expression of the extent of polymerization that has occurred. Therefore although only the dimers were measured quantitatively in the preceding experiment, it may be assumed that all products of polymerization increased proportionally with increases in the dimer content.

Table 9 is a summary of the data which accrued from that trial. These data illustrate conclusively that some product of polymerization was toxic, as the depression of the weight gain from digested calories was the same when the amounts of polymerization products were established either by increasing the extent of polymerization or by increasing the amount of heated oil in the diet (Diets 20 and 21).

Table 9. The utilization for weight gain of diets containing specific amounts of the products of polymerization of linseed oil, these amounts determined by the dimer content of the oil (Test F).

Diet No.	Approx.duration of heating period of oil at 275°C.	Dimers in oil (%)	Oil in diet (%)	Dimers in diet (%)	Average gain[a] (gms)	Average feed intake (gms)	Average gain[b] per 1000 digested calories (gms)
19	3 hours	9	10	0.9	104	287	79
20	3 hours	9	20	1.8	91	250	73
21	10 hours	18	10	1.8	91	273	71
22	10 hours	18	20	3.6	58	196	59

[a]Necessary difference = 12.8 (5%)
[b]Necessary difference = 9.9 (5%)

It is possible that the lethal effect of the residue from the distilled esters of heated oil (Test D, Table 7) was the result of the unpalatability and poor digestibility of the diet. The average daily feed intake of animals that died within 7 days was approximately 2 grams. If the 20% fat was totally indigestible, being mainly dimeric in nature, the absorbed feed could at the maximum be but 1.6 grams. It has been found in this laboratory that animals restricted to 1.5 gms of the same basal diet but containing unheated oil, failed to live more than 4 days (Berryhill 1950a). The unabsorbability of the dimeric portion of heated oils would account for the oily coats and sticky feces observed where animals were fed heated oils or the dimeric esters of heated oils, and for the absence of these conditions where animals were fed the monomeric esters of heated oils.

SOYBEAN OIL VS LINSEED OIL

Heat polymerization also damages the nutritive value of soybean oil. The inclusion of polymerized soybean oil in a diet lowered its palatability, digestibility and utilization for growth of rats. These facts are illustrated by the data in Table 10.

Table 10. The effects of heat polymerized soybean oil on intake, digestibility and utilization of feed (Test G).

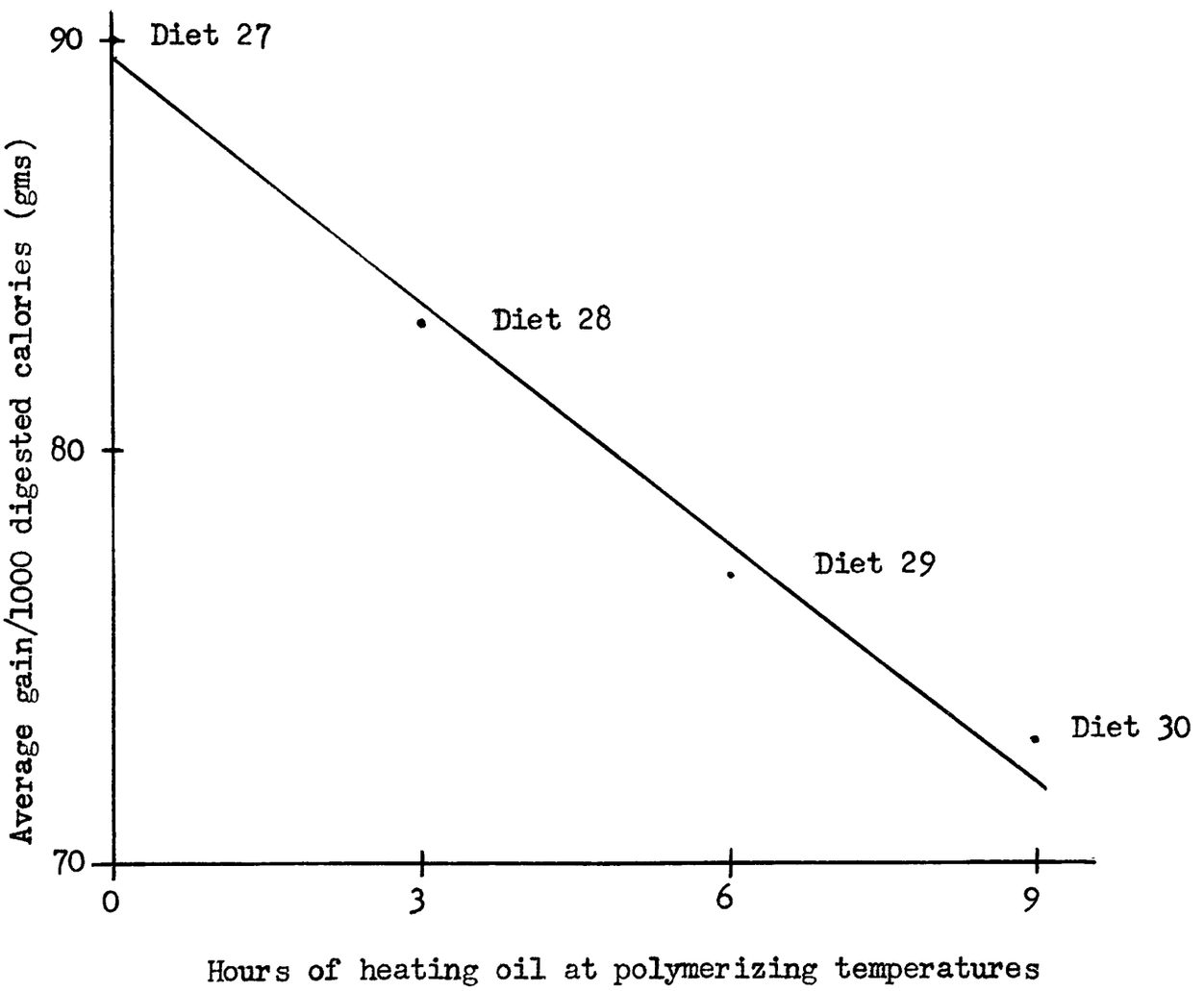
Diet No.	Treatment of oil	Average gain[a] (gms)	Average feed intake (gms)	Digestion coefficient (%)	Average intake of digested calories[b]	Average gain[c] adjusted to intake of 1000 digested calories (gms)
27	Unheated	130	285	98	1509	90
30	Heated	81	216	95	1106	73

[a]Necessary difference = 18.27
[b]Necessary difference = 169.8
[c]Necessary difference = 12.76

It is apparent from these data that the ill effects of the oil on feed intake and digestibility are not of sufficient magnitude to account for the defective growth of animals fed the polymerized oil. Equal amounts of digested calories were used less efficiently by these animals than by those fed unheated oil.

Increasing the length of time of heating soybean oil (i.e. increasing the extent of polymerization) was found to intensify the deleterious effect of the oil on rat growth as is illustrated in Figure 6.

Figure 6. Effect on rat growth of increasing the polymerization period of soybean oil (Test G).



As this relationship between heating time and nutritive value was exhibited by linseed oil, it was supposed that the detrimental material in those two polymerized oils was also similar. To check this supposition rats were fed diets containing linseed or soybean oils with identical amounts of polymerization products, as determined by the dimeric fatty acid content. The results of the linseed oil diets have been reported in the preceding pages. It was found that with soybean oil, as with linseed, the digestibility of the diet was depressed according to the dimer content of the diet as is illustrated in Figure 7. The data in Table 11 show the similarity between quantitative caloric changes in those two factors which imply that the dimeric material was not absorbed. This relationship between dimers and undigested residue is shown graphically in Figure 8.

Figure 7. The effect on diet digestibility of increasing the soybean-derived dimeric material in the diet (Test F).

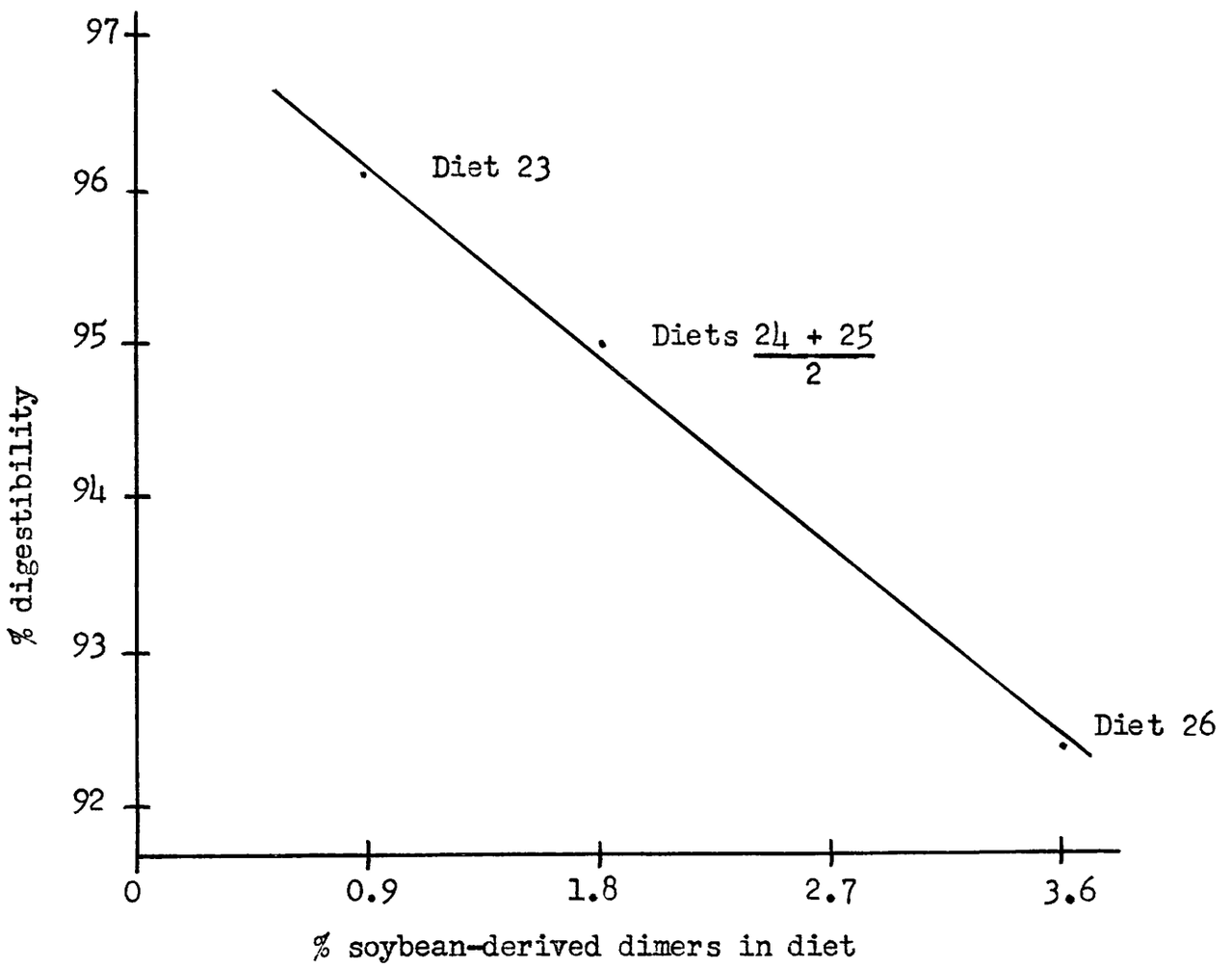
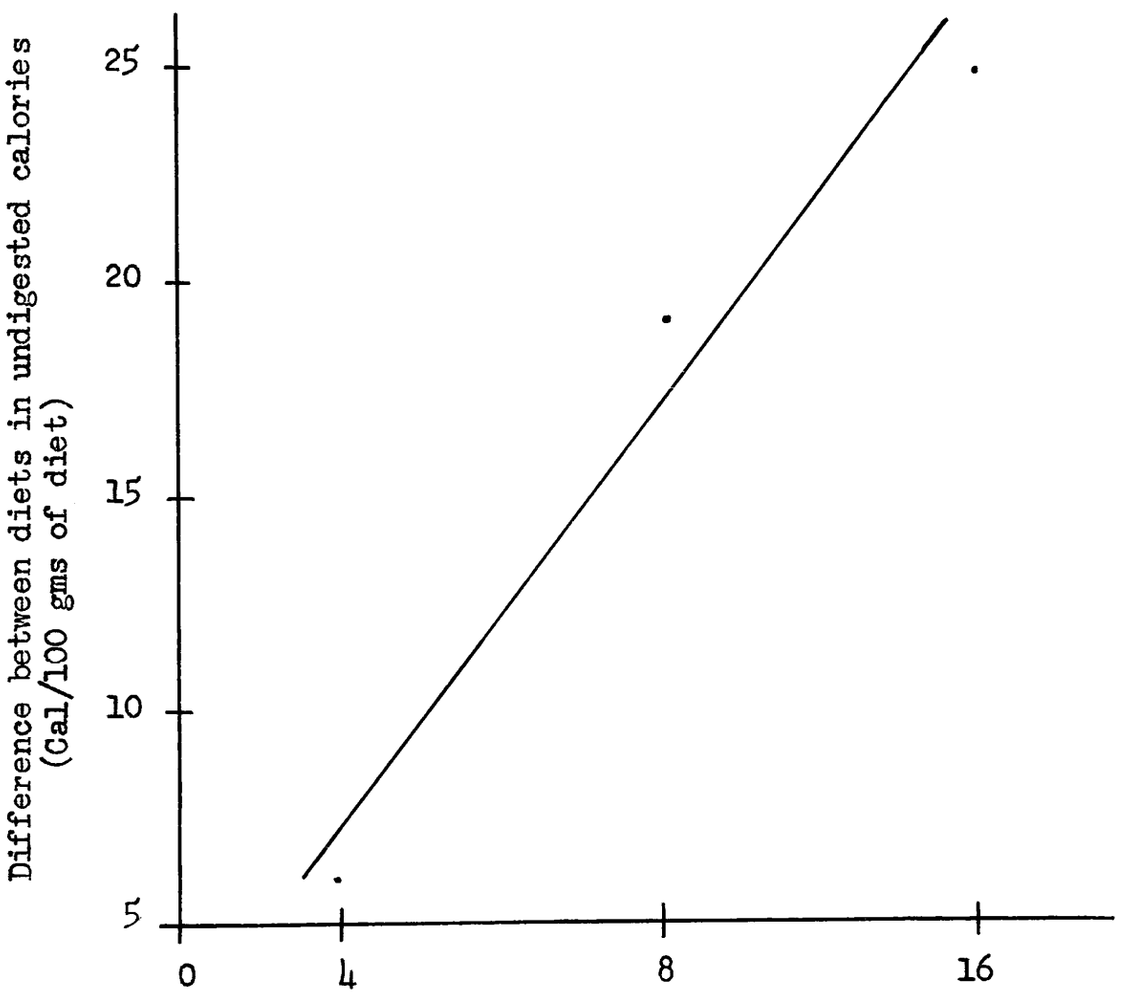


Table 11. Quantitative changes in diet digestibility with increases in the diet content of soybean-derived dimers (Test F).

Approximate dimer content of diets (gms/100 gms feed)			Difference in dimer content between diets (gms)	Calculated difference in calories derived from dimers (9 cal/gm)	Experimental difference between diets in undigested calories[a] per 100 gms feed eaten
Diet 23	Diets $\frac{24 + 25}{2}$	Diet 26			
0.9	1.8	-	0.9	8	6
-	1.8	3.6	1.8	16	19
0.9	-	3.6	2.7	24	25

[a]Undigested calories from 100 gms. of each diet cited in Appendix Table 2.

Figure 8. Quantitative changes in undigested calories with increases in dietary content of soybean-derived dimers (Test F).



However, contrary to the results with linseed oil, when heated soybean oil was fed, the efficient use of digested calories for weight gain was in no way related to the diet content of polymerization products, nor did increasing the level of heated soybean oil further intensify growth inhibition. These observations are apparent from the data summarized in Table 12. The depression of growth would appear to be chiefly a reflection of the length of time the soybean oil was heated.

Table 12. The utilization for weight gain of diets containing specific amounts of the products of polymerization of soybean oil, these amounts established either by varying the heating period of the oil or by varying the oil level (Test F).

Diet No.	Approximate duration of heating oil at 275°C.	Dimers in oil (%)	Oil in diet (%)	Dimers in diet (%)	Average gain[a] (gms)	Average feed intake (gms)	Average gain[b] per 1000 digested calories (gms)
23	7 hours	9	10	0.9	118	306	89
24	7 hours	9	20	1.8	114	268	91
25	21 hours	18	10	1.8	107	303	80
26	21 hours	18	20	3.6	94	254	77

[a]Necessary difference = 12.8 (5%)
[b]Necessary difference = 9.9 (5%)

NOTE: It should be pointed out that the results concerning the effects of different levels of heated soybean oil are somewhat contradictory. What are presumed to be the most valid have been reported herein. However, it had been observed previously that soybean oil heated 9 hours was as nutritious as unheated oil when fed as 10% of the diet (Appendix Table 3)

but when fed as 20%, in a subsequent test, it was definitely inferior in nutritive value (Table 10). Further experimentation must be conducted to clarify this point.

It was found that much longer heating at 275°C was required to produce the same degree of polymerization in soybean oil as in linseed oil. Nevertheless as it may be seen in Table 13, although the diets were digested to the same extent, the soybean oil diet supported significantly greater weight gain than linseed. Although unheated oils were not fed in this particular experiment, data from other tests would not imply a marked difference in the nutritive quality of these two oils. It would, therefore, appear probable that heating at polymerizing temperatures affects these oils differently.

Table 13. The effects on rat growth of linseed and soybean oils containing equal amounts of the products of polymerization (Test F).

Diet No.	Oil source	Approximate duration of heating period of oil at 275°C	Dimers in oils (%)	Oil in diet (%)	Dimers in diet (%)	Average gain[a] (gms)	Average feed intake (gms)	Dig. Coeff. (%)	Average gain[b] per 1000 Dig. Calories (gms)
22	Linseed	10 hours	18	20	3.6	58	196	92.2	59
26	Soybean	21 hours	18	20	3.6	94	254	92.4	77

[a]Necessary difference = 12.8 (5%)
[b]Necessary difference = 9.9 (5%)

DISCUSSION

Identity of the Toxic Materials Produced by Heating Oils at 275°C.

Although the majority of the experimental work reported herein has been carried out with linseed oil, it has been shown that heating at polymerizing temperatures progressively damages the nutritive value of soybean oil as well. However, whether or not the toxic materials formed in these oils on heating are similar, is doubtful. It was observed that, while increasing the dietary level of heated linseed oil intensified inhibition of rat growth, increasing the dietary level of soybean oil had no such effect. This would infer that in linseed oil, longer heating increased the quantity of toxic material, while in soybean oil, it intensified the toxic quality of certain substances.

Considering the different fatty acid compositions of the two oils (Table 14) it is not unlikely that the reactions occurring during polymerization, and the resulting products, would differ.

Table 14. Unsaturated fatty acid composition of linseed and soybean oils (Hilditch 1949)

Oil	Oleic (% by wt.)	Linoleic (% by wt.)	Linolenic (% by wt.)
Linseed	20	17	53
Soybean	23	55	7

As the ability of an oil to polymerize is determined by its unsaturation, (Terril 1946) it is understandable that linseed oil would polymerize more rapidly than soybean oil. Also, the polymerization products of linseed oil would be derived primarily from linolenic acid while those of soybean oil would arise chiefly from linoleic acid (Kass 1947).

It does not appear that any direct product of polymerization is responsible for the growth inhibiting action of heated soybean oil. However, it is known that conjugation is a preliminary step in polymerization (Kass 1947). It is unlikely that during heat polymerization any of the linoleic acid in soybean oil would escape conjugation, although all of it would not form polymers under the duration of the heating periods used in these experiments. It has been reported by Kummerow et al (1948) that the nutritive value of linoleic acid decreases with increasing degrees of conjugation and that very highly conjugated linoleic acid (i.e. 70% conjugated) is biologically harmful. It is possible that such conjugated compounds are responsible for the lowering of the nutritive value of soybean oil on heating. The deleterious material in polymerized soybean oil has been shown to be much less harmful than that in polymerized linseed oil and also it requires much longer heating periods to become evident. It is feasible that the multiplication of low quality fat substances through increasing the dietary level of the oil would not cause the diet to become more harmful when increased damage to the oil is achieved only through longer heating. However, in that relatively few experiments have been conducted with soybean oil, and considering that some of these data are contradictory, the nature of

the deleterious effects of thermal polymerization on soybean oil must remain purely speculative at the present.

However with regard to the ill effects of feeding heated linseed oil it is apparent that some product of polymerization within it is toxic. Although its identity has not yet been established it would seem that, because little, if any of the dimeric material in heated oils appeared to be absorbed, the previous theory that some polymeric substance was harmful, is false. Experiments now in progress in these laboratories tend to confirm the indigestibility of dimeric material while a similar report from fat balance studies has been issued by Lassen and his co-workers (1949) regarding polymerized sardine oil.

If the dimeric substances are not at fault, then the deleterious substance must be monomeric in nature. Such an hypothesis is substantiated by the failure of the presumably monomeric esters of heated linseed oil to be as nutritious as the monomeric esters of unheated linseed oil. Kass (1947) has pointed out that up to 10% of heat polymerized oil might consist of monomeric products reduced in unsaturation by cyclic isomerization, particularly in oils largely made up of trienoic acids (e.g. linseed oil). Several cases have been cited in the literature review in which branched or cyclic compounds proved biologically deleterious (Windauss et al 1932; Roffo 1944; Thomas and Weitzel 1947). The possibility that such substances were responsible for the adverse effects of polymerized linseed oil is now being investigated by fractionating the oil with urea which

separates branched from straight chain material (Schlenk and Holman 1950).

Mechanism of the Toxic Action.

The mode of action of the harmful substances remains as much a matter for conjecture as their identity. It is apparent that the digested calories from heated oil diets are not efficiently converted to weight gains. Therefore it may be presumed that either the heated fat requires extra energy for its catabolism or it is not fully degraded. In the former case, the heat lost through specific dynamic action would increase while in the latter, the calorie content of the urine would rise due to the excretion of intermediate metabolites. Both these possibilities would merit further investigation to elucidate the mechanism through which polymerized oil is toxic.

Clinical symptoms of malnutrition that developed in animals fed polymerized oils resemble those apparent in rats deficient in the essential fatty acids or certain of the B vitamins. For instance, the rusty coloration of the hair coat and about the nostrils suggests the porphyrin deposits typical of a pantothenic acid deficiency (Unna and Richards 1942). The prolonged period of labour and failure to produce viable young observed in female rats fed polymerized linseed oil from the time of weaning (Siddall 1951) are reproductive disorders also exhibited by animals depleted in pyridoxine or fat (Richards 1949; Quackenbush et al 1942b).

It is quite possible that polymerized oils would be lacking in available essential fatty acids. During heating, the majority of

the higher unsaturates would dimerize and hence become indigestible, while those that did not, would become conjugated and hence lower in nutritive value (Burr 1942). Nevertheless it is doubtful that, in four weeks, a simple lack of essential fatty acids could have a very noticeable effect on rats fed wholesome diets prior to the test period, as customarily a depletion period of approximately eight weeks is required to develop fat deficiency symptoms (Quackenbush et al 1942b). Also, it has been illustrated that although corn oil did improve the nutritive value of a diet containing a commercial preparation from polymerized linseed oil, it did not counteract the ill effects entirely.

However, it has been postulated by Kummerow et al (1948) that undesirable fats have a priority claim on tissue pyridoxine. Biotin and pantothenic acid are also intimately concerned with fat metabolism (Williams et al 1950; Sherman et al 1950; Rubin and Scheiner 1949). If certain substances in heated oils increase the requirements of these B vitamins and at the same time these oils are not supplying adequate amounts of available essential fatty acids, it is quite possible that deficiency symptoms of both these groups of nutrients would develop, for it is known that they exert a mutual supplementary and sparing action in regulating dermal conditions, growth and reproduction (Jurgens et al 1945). It is true that no marked dermal disorders have been noted in rats fed heated oils, but it is unlikely that they would develop under the conditions of high humidity common to this area (Burr 1942). Such action of heated oils might explain the beneficial effect on growth observed by Langerman (1949) of

feeding feces from normal rats to those fed polymerized oils, as certain of the B vitamins, for example biotin, are customarily obtained by the rat through copraphagy (Anon. 1949).

The Value of Polymerization for Processing Edible Oils.

Although the heat polymerization process recommended by Privett et al (1947) for producing edible oils may render linseed and soybean oils flavour stable, it produces biologically harmful substances which are not removable by any of the common solvent segregation processes. The four hours heating shown to be the minimum requirement to assure the palatability of linseed oil in baked products (Lips 1949) caused obvious nutritive damage (Figure 2). Therefore until the toxic material so produced is identified and a process devised whereby it may be easily removed, heat polymerization should not be used for the processing of linseed oil for human consumption.

However, soybean oil, containing much less linolenic acid than linseed oil would presumably require much less heating than linseed to assure its flavour stability, assuming that linolenic acid is responsible for flavour reversion. As much longer heating periods were required to damage the nutritive quality of soybean oil as compared to linseed, and as increasing the dietary level of soybean oil may prove to have no further deleterious effects, it would seem probable that heat polymerization may, in the near future, be recommended as a convenient and harmless method for producing edible soybean oil.

CONCLUSIONS

In light of the preceding results the following conclusions are warranted.

1. Thermal polymerization damages the nutritive value of both linseed and soybean oils.
2. The inclusion of thermally polymerized oils in rat diets, depressed their palatability and digestibility. The efficient use of those diets for rat growth was reduced more than was accountable by low intake and poor digestibility, implying the presence of some toxic substance in the heated oils.
3. The deleterious effects of both heated linseed and heated soybean oils are increased as the duration of their heating periods is increased.
4. The dimeric material in polymerized oils appears to be unabsorbed and thence not responsible for their growth inhibiting action.
5. The toxic material in polymerized linseed oil appears to be some product of polymerization that is monomeric in structure.
6. Thermally polymerized linseed oil does not destroy non-lipid diet ingredients.
7. Feeding polymerized linseed oil does not produce a vitamin E deficiency.
8. The adverse effects of a lethal fraction of polymerized linseed oil were partly alleviated by diluting it with unheated corn oil.
9. The author chooses to refrain from drawing any conclusions as to the identity or nature of the deleterious material in polymerized soybean oil until further experiments have been carried out.

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APPENDIX

APPENDIX TABLE 1

Summary of Data from Feeding Trials

Test No.	Diet No.	Oil source L=linseed S=soybean	Heat treatment of oil at polymerizing temperature (hrs)	Individual diet variation	% oil fed	Av. gain (gms)	Av.feed intake (gms)	% Dig.	Av. caloric intake	Av.gain adjusted to intake of 1000 calories	r_{xy}	b_{yx}^{k}
A						[a]			(dig.[b] cal.)	(dig.[c] cal.)		
	1	L	Nil		10	118	348	95.6	1672	92		
	2	L	12		10	75	270	93.8	1270	65	0.5	0.039
	3	L	Nil	Oil fed by dropper	10	116	350	95.6	1673	91		
	4	L	12		10	96	350	93.8	1637	71		
B	5	L	12	Vit.E supplements	10	89	317	93.8	1487	69	0.6	0.042
	6	L	12		10	82	302	93.8	1487	64		
C	7	L	Nil	Commercial prep. from the Acet.Sol.Fract. of heated linseed oil	20	67	174		(gross cal.) 940	(gross cal.) 71		
	8	L	Approx.12		20	- [b]	-		-	-		
	9	L (1 prt.) Corn 4 parts	Approx.12 Nil		5 15	57	172		929	61		

[a]Nec.diff. = 14.43 at P = 0.05
[b]Nec.diff. =175.2 at P = 0.05
[c]Nec.diff. = 12.6 at P = 0.05

[a]Nec.diff. = 16.77 at P = 0.05
[b]Nec.diff. = 11.88 at P = 0.05

[a]Nec.diff.=7.94 at P = 0.05; 10.77 at P = 0.01
[b]All animals died within 10 days.

Appendix Table 1 (continued) Summary of Data from Feeding Trials

Test No.	Diet No.	Oil source L=linseed S=soybean	Heat treatment of oil at polymerizing temperature (hrs)	Individual diet variation	% oil fed	Av. gain (gms)	Av.feed intake (gms)	% Dig.	Av. caloric intake	Av.gain adjusted to intake of 1000 calories	r_{xy}	b_{yx}
				Fraction of oil fed		a			(gross cals)	(gross cals) [c]		[k]
D	10	L	Nil	Whole oil	20	119	338		1824	64		
	11	L	Nil	Esters of whole oil	20	80	263		1417	52	0.72	0.067
	12	L	12	Esters of Acet. Sol.fraction	20	29	203		1091	23		
	13	L	12	Dist.esters of Acet.Sol.fract.	20	23	156		851	33		
	14	L	12	Undist.esters of Acet.Sol.fract.	20	- [b]	-		-	-		

a Nec.diff. = 13.71 at P = 0.05
b 6 out of 8 animals died within 7 days. Their av.daily feed intake = 2 gms.
c Nec.diff. = 11.49 at P = 0.05

						a			(gross cals)	(gross cals) [b]		
E	15	L	Nil		10	108	269		1345	85		
	16	L	2		10	106	268		1340	84	0.654	0.064
	17	L	4		10	96	266		1330	74		
	18	L	8		10	71	226		1130	60		

a Nec.diff = 13.13 at P = 0.05
b Nec.diff = 10.08 at P = 0.05

Appendix Table 1 (continued) Summary of Data from Feeding Trials

Test No.	Diet No.	Oil source L=linseed S=soybean	Heat treatment of oil at polymerizing temperature (hrs)	Individual diet variation	% oil fed	Av. gain (gms)	Av.feed intake (gms)	% Dig.	Av. caloric intake	Av.gain adjusted to intake of 1000 calories	r_{xy}	b_{yx}^{k}
				% dimers in diet		a			(Dig. cals)	(Dig. [b] cals)		
F	19	L	3	0.9	10	104	287	95.6	1405	79		
	20	L	3	1.8	20	91	250	94.2	1282	73		
	21	L	10	1.8	10	91	273	94.1	1309	71	0.624	0.0626
	22	L	10	3.6	20	58	196	92.2	973	59		
	23	S	7	0.9	10	118	306	96.1	1474	89		
	24	S	7	1.8	20	114	268	95.5	1364	91		
	25	S	21	1.8	10	107	303	94.5	1425	80		
	26	S	21	3.6	20	94	254	92.4	1272	77		

[a]Nec.diff. = 12.8 at P = 0.05
[b]Nec.diff. = 9.9 at P = 0.05

						a			(dig. [b] cals)	(Dig. [c] cals)		
G	27	S	Nil		20	130	285	98	1509	90		
	28	S	3		20	112	264	97	1382	83		
	29	S	6		20	94	233	96	1207	77	0.728	0.078
	30	S	9		20	81	216	95	1106	73		

[a]Nec.diff. = 18.27 at P = 0.05
[b]Nec.diff. =169.8 at P = 0.05
[c]Nec.diff. = 12.76 at P = 0.05

k. 'byx' represents the change in y for every unit change in x, where y = calories ingested (gross or digested as indicated), and x = weight gain.

APPENDIX TABLE 2

Heats of Combustion of Dry Matter Ingested and Excreted and the % Digestibilities of Dry Matter and Calories

Column No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Diet No.	% Digestibility of dry matter	Dry Matter Ingested		Dry Matter Excreted			Calories Digested		% digestibility of calories $\frac{(8)}{(3)} \times 100$
		Cal/gm	Cal/100 gms	Gms/100 gms ingested dry matter	Cal/gm	Calories excreted per 100 gms ingested dry matter	(3) x (1)	(3) - (6)	
1	96.4	4.890	489	3.6	4.142	15	471	474	96.9
2	93.8	4.871	487	6.2	5.421	34	457	453	93.0
3, 4, 5, 6 [a]									
19	95.6	5.116	512	4.4	4.595	20	489	492	96.1
20	94.2	5.439	544	5.8	4.989	29	512	515	94.6
21	94.1	5.084	508	5.9	5.117	30	478	478	94.1
22	92.2	5.303	530	7.8	5.445	42	489	488	92.1
23	96.1	5.044	504	3.9	4.725	18	484	485	96.4
24	95.5	5.287	529	4.5	5.041	23	505	506	95.7
25	94.5	5.037	504	5.5	5.245	29	476	475	94.2
26	92.4	5.381	538	7.6	5.516	42	497	496	92.2

[a] As diets 1-6 were prepared and fed coincidentally, and had identical fat contents it was assumed that the caloric digestibilities of diets containing the same type of fat treated in the same manner were identical.

APPENDIX TABLE 3

The Effect on Rat Growth of Polymerized Soybean Oil fed as 10% of the Diet.

Length of time of heating oil at polymerizing temp. (hrs)	% Oil fed	No. Animals	Average gain[a] (gms)	Average feed intake (gms)
Nil	10	10	104	298
3	10	10	111	302
6	10	10	98	292
9	10	10	102	308

[a]Necessary difference = 15.33 (5%)