THE EFFECT OF HEAT ON THE NUTRITIVE VALUE OF SOME VEGETABLE OILS.

by

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ABSTRACT

Linseed, corn, peanut, soybean and rape-seed oils, heated at 275°C. were incorporated in the basal diets of rats at levels of 10, 20 or 36% by weight. Linseed oil was rendered toxic to young rats by treatment for 12 hours, but 30 hours heating was required to reduce the nutritive value of the other oils. Toxicity symptoms were non-specific but in severe cases included darkened adrenals, weight losses, diarrhea and death within 14 days. Acetone segregation partially concentrated the toxic factor in the soluble fraction of the oil, suggesting a specific polymer might be responsible.

Of the various supplements tested, only rat feces and liver extract were effective in preventing toxicosis, therefore it was concluded that heated oils caused a deficiency of some essential nutrient. It is not clear how the toxic factor exerted its effect, but evidence suggests that bacterial synthesis might in some way be involved.

INTRODUCTION

In the past, fat in the human diet has been considered a luxury and a nutrient without which good health could still be maintained (Osborne and Mendel 1920). Recent work, however, has conclusively shown that fat is nutritionally essential and required in the diet of the adult at a level of approximately one-third of the total calories (Deuel 1946). Anderson and Williams (1937) maintain that apart from any nutritional rôle, fats and oils have a unique place in the diet because of their ability to impart a richness of flavour and texture to foods which increase our enjoyment of them. In addition, they retard the emptying time of the stomach and thus exert a "staying power" which is valuable in preventing between meal hunger and associated fatigue.

Butter has long been placed above other edible fats and oils in acceptability. The unavailability of this commodity, however, excludes it from the daily diet of a large percentage of this country's population, and attention has therefore been focused on vegetable oil substitutes. According to Bloor (1944), "vegetable oils can generally be supplied at a lower cost than animal fats," and they have the additional advantage of "being easily modified and blended to provide products of almost any desired properties." With the possible exception of olive oil, the majority of vegetable oils possess relatively strong odours, flavours and colours which have to be

removed by extensive processing before they are acceptable for human consumption. Before the war of 1939-45, Canada was able to import all the oils she required for human consumption (Canada Year Book 1937). The bulk of this supply was olive oil which was produced in abundance in the Mediterranean countries and in Southern California (Woodman 1941). During this war, however, a fat shortage existed throughout the world, with a result that many countries were forced to turn to their own natural resources for a supply. In Norway, for example, a heat polymerized herring oil product was developed and widely used in place of olive, cotton seed and peanut oils. It was pale yellow, free from any fishy taste or odour and was found to be suitable for cooking, for the canning of fish and other foods, and to some extent for use as a table and salad oil (Schwitzer 1948).

Rape-seed oil was used extensively in Germany for the manufacture of their wartime margarines, while other erucic acid oils have been used for a number of years in India and in China (Grace 1948). German technologists also developed a synthetic fat, which, they claimed, was tasteless, odourless, well-digested and nutritious. In appearance, this product was described as "an intermediate between pig and goose fat" (Flössner 1943). In Switzerland tobacco seed and grape seed oils were used (Bernhard 1944), while more recently, the British government has inaugurated an extensive peanut growing scheme in Africa for the production of oil to be used in the manufacture of edible shortenings.

The chief Canadian oil bearing crops include linseed, rape-seed, sunflower seed and soybean (Grace and Zuckerman 1947). In North America their use for human consumption has not, until recently, received serious consideration, due probably to anticipated difficulties regarding effective colour, flavour and odour removal. If these handicaps could be overcome without detrimental effect to the nutritive value of the oils, Canada could be ensured of a supply of fat, independent of imports and regardless of a continued or a future world shortage.

REVIEW OF LITERATURE

The existence of a world shortage of edible fats during the past few years has promoted extensive research concerning the nutritional significance of this component in the diet.

Anderson and Williams (1937) state that the human alimentary canal has been developed so as to cope with a diet containing 20-25% of the energy as fat. To get the same energy from carbohydrate, the alimentary canal would have to be much longer. These authors claim that the ordinary individual deprived of fat consumes less food and therefore exists on a lower metabolic level.

Working with young rats, Deuel et al (1947) found that a diet containing 10-50% fat promoted better growth rate, physical capacity, reproduction and lactation and an earlier attainment of sexual maturity, than did a fat-free diet. In these tests, optimum growth was observed on diets containing 20-40% fat. Similar evidence was furnished by Scheer et al (1947:1947a) who subjected rats to severe caloric restrictions on diets varying in fat content. It was found that weight-loss was less rapid and mortality lower among the animals receiving liberal amounts of fat, while during the recovery period, weight was more rapidly regained if fat was present in the diet. It appears that the growth promoting effect of fat is not solely due to the deposition of body fat (Deuel 1947) or to an increased caloric intake, as restricted iso-caloric feeding gave similar results (Scheer 1947:1947a). In addition, there is no evidence to support

the view that the effects of fat in the diet on resistance to under-nutrition and on physical capacity are the results of differences in gross body composition (Scheer 1947:1947a). Forbes and Swift (1944: 1946: 1947)found that the partial replacement of carbohydrate by fat in a mixed diet increased the metabolic efficiency of food utilization, and resulted in a sparing of energy which otherwise would have been wasted. This effect which fat has in lowering specific dynamic action, is probably due to a decrease in the amount of conversion of carbohydrate to fat taking place in the body. In addition, increasing the amount of fat in the diet from 2% to 30%, was found by Forbes and Swift (1946), and French, Black and Swift (1948), to improve nitrogen digestibility and to increase its retention in the growing rat. This means that fat supplies the animal with extra calories for growth, work or storage which could not be supplied as efficiently by an iso-caloric mixture of carbohydrate and protein. It is evident, therefore, that body fat can no longer be considered an inert storage material derived from excess diet, but a material which takes an active part in a variety of fundamental metabolic processes underlying the phenomena of growth, physical capacity, resistance to malnutrition, reproduction and lactation.

Certain members of the vitamin family are present in animal, vegetable and marine oils. It is not surprising, therefore, to discover a non-caloric function of fat which is closely associated with these food adjuncts. In 1943, Burr and Barnes found that dietary

fat was necessary for the normal absorption of vitamin A, because amounts of fat up to 10% of the total weight of the diet increased the apparent absorption of this vitamin. Fat acts as a transport medium for these vitamins and facilitates their entry into the blood stream. It appears evident, however, that some other mechanism might also be involved. Sherman (1941), for example, fed standard beta carotene in conjunction with cotton seed, linseed, corn or wheat germ oils, to vitamin A depleted rats, and found the growth response to be better than when butter fat or cocoanut oil were used. These differences, he stated, could not be explained on the basis of varying efficiency in carotene absorption. Fat has also been attributed with an anti-rachitic property when it is included at a 10% level in the diet. This percentage was found by Burr and Barnes (1943) to decrease the pH of the lower portion of the small intestine, thus probably allowing the maximum absorption of vitamin D. As a result of experiments conducted at the University of Wisconsin, Bunkfeldt and Steenbock (1943) concluded that the anti-rachitic potency of fat was conditioned by the presence of phosphorus in the diet, and that with a low phosphorus intake, the addition of fat to the diet resulted in a decrease in calcification. Only slightly beneficial effects were demonstrated when the phosphorus intake was high, while the maximum beneficial effect was obtained when the phosphorus level was optional and the diet of these rats contained no more than 5% fat. No explanation was offered, but it was evident that as the

calcium-phosphorus ratio increased, the anti-rachitic potency of fat decreased. Claims have been made by various authors that fat has a sparing action on the anti-neuritic vitamin (Evans and Lepkovsky 1928: 1932: 1932a) (Salmon and Goodman 1937) (Anderson and Williams 1937). It was found that less thiamine was required to establish a definite level of growth or frequency of ovulation in rats receiving fat in their diet, while animals near death from beri-beri resumed growth after only three doses of rice polishings if fat were introduced into the diet. The obvious reason for this phenomenon is based on a function of fat already discussed; i.e. that fat is able to partially replace carbohydrate, thus removing the need for thiamine which constitutes an active part of the enzyme systems involved in carbohydrate metabolism (Stirn, Arnold and Elvehjem 1939).

Natural fats are not simple compounds, but are substances built up of various fatty acids attached to a glyceride-radicle. While some fatty acids are saturated, others, to which considerable nutritional importance has been attached, are unsaturated. In 1943, Burr and Barnes found that the exclusion of fat from the diet of rats led to the development of scaly skin, caudal necrosis, retardation of growth, kidney lesions and hematuria, early death, sterility in males, and the production of poor litters or complete reproductive failure in females. Collectively, these pathological symptoms have been referred to as the "fat deficiency syndrome", and were caused, not by the absence of fat as such, but by the absence of certain

essential unsaturated fatty acids which it carried. The term "essential" when applied to fatty acids, as when applied to amino acids, implies that they are required preformed in the diet as their bio-synthesis does not take place, or does not occur rapidly enough to meet the animal's demand.

It has been postulated that the more unsaturated, liquid fatty acids provide better growth in rats, and lead to a greater storage of carotene than the solid, saturated acids. It is also believed that the saturated fatty acids may cause a greater deposition of fat in the liver and thus contribute towards fatty liver disorders (Anon. 1947). Work done by Channon and Wilkinson (1936) substantiates this opinion, and presents evidence to show that the degree of liver fat infiltration is directly related to the proportion of C_{14} - C_{18} saturated fatty acids ingested. This condition, however, was only observed when a high fat, low choline diet was fed to the test animals, suggesting that other substances required for the transport of fat may also be involved in this pathological condition.

There has been much controversial evidence as to the identity of the fatty acids responsible for inhibiting the "fat deficiency syndrome" and for promoting growth in rats. Burr et al (1932) showed that the saturated fatty acids of hydrogenated cocoanut oil and oleic acid were ineffective, while methyl oleate, linolenic and linoleic acids possessed therapeutic activity. In view of this and many similar experiments, it was considered that the curative property of fat lay in its fatty acids which were more unsaturated than oleic acid

(Burr and Burr 1930). As a result of extensive work carried out by many investigators it has now been established that those fatty acids capable of preventing or curing the "fat deficiency syndrome" include linoleic and arachidonic acids, and possibly linolenic acid -- although there is still controversial evidence regarding this last acid. (Burr and Burr 1930: 1932) (Evans and Lepkovsky 1932) (Quakenbusch, Kumerow and Steenbock 1942) (Turpeinen 1938) (Deuel 1947).

It has been stated that essential fatty acids are essential for one species only -- the rat (Hansen 1937), although Burr and Burr (1930) and Anderson and Williams (1937) believe that warm-blooded animals in general cannot synthesize appreciable quantities of linoleic or some of the other highly unsaturated fatty acids. These acids, it is thought, are the normal constituents of essential cellular phospholipids and are required in greater amounts for the more active tissues. Further confirmatory evidence was produced by Hansen, Knott and Wiese (1947), who recently showed that more than three-quarters of a number of eczematous children examined had an iodine value for serum fatty acids below normal. As a result of feeding these patients diets rich in unsaturated fatty acids (lard and some vegetable oils), a favourable response in the eczematous condition was obtained. The specific rôle of the fatty acids, these authors believe, is to restore and maintain skin nutrition.

Rancidity is an important problem which is closely related to the unsaturated nature of fats and oils. With the production of peroxides, natural antioxidants (alpha tocopherols in vegetable oils)

are destroyed, and the oxidative destruction of carotene, vitamin A and the essential unsaturated acids continues unhindered. The practice of hydrogenation has been developed as a fairly successful preventative measure (Miller 1943). It is not harmful to the nutritive value of the oil, unless, by increasing its melting point to above 50°C., the digestibility is affected.

From a summary of this literature, it is evident that fat has an important nutritional rôle in the diet which is based on both its caloric and non-caloric functions. As political events have curtailed the supply of many edible fats to Canada and have consequently forced the investigation of some of the less well-known products, the nutritional import of these products becomes a matter of great consequence.

Deuel (1946a) states that "the first essential for a food to be considered of high nutritive value is that it be relatively completely digested and absorbed". According to Cowgill (1945), edible fats are all digested and absorbed to about the same degree providing their melting points are not too high to prevent liquefaction in the alimentary tract. In an extensive series of experiments carried out by the office of Home Economics of the United States Department of Agriculture on a wide variety of animal and vegetable fats, it was found that most of the seventy fats studied were digested to an extent of 95% or higher by human subjects (Langworthy 1923). Exceptions to this rule include fats which have a melting point above 50°C. such as muttonfat (Langworthy and Holmes 1915), oleostearin (Holmes 1919), deer fat (Deuel and Holmes 1922), and some almost completely hydrogenated fats

melting at 52° to 60°C (Deuel and Holmes 1921). There seems to be an inverse ratio between digestibility and melting point, the latter being dependent upon the stearic acid content of the oil (Mattil and Higgins 1945) (Crockett and Deuel 1947). A high content of this saturated fatty acid increases the melting point of the product and thus hinders its absorption. It is apparent, therefore, that high digestibility is a characteristic common to most edible fats and oils with the exception of the list already mentioned, and also rape-seed oil. Rape-seed oil has a high content of erucic acid (50%) and the poor absorbability of this acid probably accounts for the low digestibility of rape-seed oil (Deuel, Cheng and Morehouse 1948). Although no information concerning the digestibility of other erucic acid oils is available, it seems feasible to believe that they would behave in a similar manner to rape-seed oil.

In many rat growth tests, butter was found to be superior to other vegetable oils when incorporated in a basal diet containing skim-milk powder. This difference was attributed to the presence of certain long-chain fatty acids which had the specific function of stimulating growth (Deuel and Movitt 1945). Boer, Jansen and Kentie (1947) isolated vaccenic acid, an isomer of oleic acid, from Summer butter and claimed that it was the growth promoting factor. The result of more recent research, however, has shown conclusively that these previous claims are unfounded. Deuel et al (1948a) found no growth promoting potency in vaccenic acid, while numerous other workers have shown that all vegetable and animal fats are equally well

utilized for tissue formation and growth in rats, if incorporated in a diet containing mixed carbohydrates. It is only when lactose is the sole source of carbohydrate, as would be the case with suckling animals, that butter fat shows superiority over vegetable oils (Harris and Mosher 1940) (Boutwell et al 1943) (Geyer, Boutwell, Elvehjem and Hart 1943) (Deuel, Movitt and Hallman 1943: 1944) (Deuel, Hallman and Movitt 1945a). Deuel et al (1945a) for example, fed seven types of oleomargarines of animal and vegetable origin to young rats. When the sole source of carbohydrate was lactose, the growth rate of these animals averaged 6.4% lower than animals fed butter, but with a mixed carbohydrate ration all fats gave identical results. A possible explanation was offered by Geyer et al (1943). They believed that lactose had an unknown effect upon intestinal conditions which was counteracted by butter but not by other animal or vegetable oils. All workers are in agreement however, that on an adequate basal diet and vitamin intake, most oils are nutritionally comparable to butter fat, and are used with equal efficiency for transformation into body tissue (Deuel, Movitt and Hallman 1943) (Deuel, Hallman and Movitt 1945a) (Cowgill 1945).

Reports in the literature indicate that with the exception of the erucic acid oils already discussed, Canadian oil bearing crops (linseed, soybean, mustard seed and rape-seed) do not differ nutritionally from other vegetable oils. Schär (1946) reports from Germany that there is no objection to the use of linseed oil as a human foodstuff on either physiological or technical grounds, while

Rose (1943) claims that it is possible to transform the oil into an edible product of good consistency by hydrogenation. The shortening can then be used directly for making biscuits. Nutritionally, linseed oil has been shown to support normal growth in laboratory animals (Molotkow 1932) (Sherman 1941a), and to increase lactation and the fat content of the milk when added at a 3% level to the low fat feed of goats (Maynard 1942). Soybean oil has also been shown to support normal growth in laboratory animals and to be efficiently used for conversion to body tissue (Deuel, Movitt and Hallman 1944: 1943) (Deuel and Movitt 1945).

Besides good nutritional qualities, there are additional criteria by which an oil is judged to be fit for human consumption. According to Lips, Grace and Hamilton (1948) these criteria include attractive colour, agreeable texture to the palate, bland or pleasing flavour, good keeping quality, high smoke point (400°F.) and clarity at refrigeration temperatures (40°F.). The main problem preventing the use of Canadian oil bearing crops is the one of flavour reversion. Flavour reversion in soybean and linseed oils is believed to be due to isolinoleic acid, a fatty acid derivative of linoleic acid which is formed during hydrogenation and which might be responsible for the development of the unpleasant odour and flavour (Armstrong and McFarlane 1944) (Lemon 1947). Rose (1943) states that the use of linseed oil for human consumption is not economical, as large amounts of nickel catalyst and a long operating time are required to free the

oil of phosphatides which are largely responsible for its unpalatability. Similarly, Schär (1946) suggests that for culinary reasons and on the score of taste, it is preferable to use hydrogenated linseed oil mixed with other edible oils such as peanut oil. Hot pressed rape and mustard seed oils have had little culinary application except in Germany and the Orient, due to the development of a strong odour and flavour as a result of this process (Lips, Grace and Hamilton 1948) (Grace 1948). Lips et al (1948) found that although alkali refining and bleaching of rape-seed and mustard seed oils resulted in a light coloured product suitable for salad oils, its use for the preparation of doughnuts was not satisfactory regarding flavour. Extensive work done by Armstrong and McFarlane (1944) to produce a non-reverting linseed oil product by hydrogenation, deodorization, and the use of anti-oxidants to inhibit reversion, was unsuccessful. Lemon, Lips and White (1945) found that the addition of other vegetable oils to the product, and the use of hydro-quinone or a wheat germ oil preparation as anti-oxidants, slightly retarded the onset of reversion but failed to produce a satisfactory, stable product. Preliminary treatment of the oil with heat at 270° to 275°C. for 12 to 15 hours in the presence of carbon dioxide, however, was found by Privett, Pringle and McFarlane (1945) to be the best method for producing a non-reverting shortening. Following the heat treatment, these workers recommended acetone segregation (the acetone soluble fraction being used), and final hydrogenation of the product to n_{60} 1.4615 to 1.4605.

It is well-known that changes occur in the physical structure of many oils when they are subjected to high temperatures. Depending

upon the unsaturated nature of an oil, heat at certain temperatures will cause polymerization to take place (Terrill 1946). This is a process for which the unsaturated centres of the fatty acids are largely responsible (Kass 1947). According to Kass (1947), linseed oil requires a minimum temperature of 240° to 285°C. for polymerization to take place. Grace* reports that the heating of rape-seed oil at 275°C. for 24 hours caused polymerization to occur and produced changes in the erucic acid fraction of the oil which rendered it unrecognizable by its physical constants. Kass goes on to explain that although the term polymerization is widely used, very little advanced polymerization occurs in oils irrespective of the type of fatty acid unsaturation involved. The predominant products are more usually dimers and trimers, with only a small portion, if any, of higher polymers being formed. In addition, the dimers from various sources are not identical as their physical and chemical constants point to structural differences ultimately determined by the original degree of unsaturation of the parent fatty acid. Also, dimers and trimers from any one unsaturated acid are not homogeneous, but rather a mixture of isomeric compounds. Even in the absence of oxygen, polymerization is accompanied by decomposition, isomerization and disproportionation (Kass 1947) (Terrill 1946).

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* Information to authors from N.H.Grace,1947.

The extent of these side reactions is proportional to the severity of the thermal treatment and becomes quite prominent at or above 300°C. Above 330°C., Kass believes polymerization is inhibited by the formation of non-polymerizable by-products. Heat treated oils, as long as they are not jelled, retain their solubility in hydrocarbon solvents to a large degree, but they become progressively insoluble in oxygen bearing solvents such as acetone (Kass 1947). Segregation of polymerized rape-seed oil, for example, was found by Grace* to give a 50:50 split in acetone. Terrill (1946) states that acetone segregation of a heated oil is capable of removing the unpolymerized portion containing the more saturated components.

There is little evidence in the literature regarding the nutritive value of heated oils, nor is any mention made of the ability of the digestive system to deal with the large polymer molecule. Work done in the Nutrition Department, Macdonald College, established the fact that the acetone soluble fraction of linseed oil heated for 12 hours at 275°C. was toxic to rats when fed at a level of as low as 8% by weight of the diet. The animals failed to grow and died before the end of a 28 day test period (Millar 1947). Similar information was reported by Lane and Ivy (1948) who found that lard heated for 30 minutes

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* Information to authors from N.H.Grace, 1947.

at 350°C. failed to support the normal growth, or maintain the feed consumption of rats. Lard subjected to less severe treatment (350°F. for 120 minutes) was also found to reduce the growth rate of rats when fed at a 50% level of the diet (Morris and Larsen 1943). This heated material caused the animals to lose weight over a period of eighteen months, and in some cases, to develop a progressive paralysis resembling vitamin E deficiency.

Other workers have claimed that the ingestion of heated fats leads to the occurrence of cancer (Steiner, Steele and Koch 1943). Roffo (1938: 1942) for example, heated lard, suet, mutton tallow, olive oil and cholesterol to 350°F. and fed them to rats. He reported the development of some sarcomas in these animals, and adenocarcinomas of the glandular portion of the stomach. Roffo believed that heated fats were carcinogenic due to the conversion of the sterols to carcinogens, but when Kirby (1944) fed heated cholesterol to rats, he failed to observe any gastric lesions. The theories advanced by these workers seem inconclusive, and in addition, it has been claimed by Miller et al (1944) that any diets high in fat (using the unheated material) will contribute towards the incidence of cancer by increasing the number of calories consumed. There is no real evidence that heating fats results in the formation of hydrocarbons of the benzyprene type which are believed to be capable of stimulating cancerous growths (Anon.1945).

OBJECT OF RESEARCH

In view of the reports in the literature (Lane and Ivy 1948) (Morris and Larsen 1943) and as a result of experiments carried out in this laboratory (Millar 1947), it has been suggested that heat may reduce the nutritive value of oils, or may even cause toxic products to develop. It is thus of great importance that further investigations be conducted, especially since the refining processes necessary to develop acceptable shortenings out of the crude, Canadian vegetable oils, generally require relatively high temperatures.

EXPERIMENTAL PROCEDURE

I GENERAL.

The general plan of this study involved feeding to male, white rats basal diets in which various vegetable shortenings were incorporated at levels of either 10, 20 or 36% by weight. These shortenings included linseed, soybean, corn, peanut and rape-seed oils heated for varying lengths of time at 275°C., and in some cases subjected to acetone segregation.

II ANIMALS.

The feeding trials were each based on a simple randomized block design, and were conducted for 7 to 42 days depending upon the nature and the object of the test. Three hundred and seventy-six male, white rats were involved, the animals being approximately 28 days old at the start of the test unless otherwise indicated. They were allotted at random to individual wire bottom cages, and according to the test were grouped into lots of 3 to 12 animals each. Feed and water were given ad libitum and weekly records of feed consumption and liveweight changes were kept. In certain cases, feces collections were made during the second or third week of the test periods to allow the calculation of digestibility coefficients.

III DIETS.

Three basal diets were used in these tests, which differed primarily in fat content. Increases in the fat level from 10% to 36% (by weight) were made at the expense of the wheat flour,

thus allowing the protein content of the diets to be maintained at an approximate level of 23% by weight. The following table shows the percentage composition of the three different basal diets used.

Table 1. The Percentage Composition of Basal Diets

Ingredients	Percentage (by weight) in the diet		
Wheat flour	57.0	47.0	26.5
Casein	11.5	11.5	15.0
Milk powder	19.0	19.0	20.0
Fat	10.0	20.0	36.0
Bone meal	2.0	2.0	2.0
Salt	0.5	0.5	0.5
% protein (by weight)	21.27	24.05	24.76

As this study involved shortenings which might ultimately be used for human consumption, the ingredients of the basal diet were chosen partly by the requirement that they could be made into a table biscuit and the whole finally baked in the oven. All the ingredients were mixed together thoroughly without the addition of water, and were then baked in the oven at 375°F. for approximately 18 minutes. Subsequently the biscuits were granulated, air-dried and stored in the refrigerator until required.

Vitamin supplements were administered orally to all test animals twice weekly, to provide the following daily intake of vitamins to each rat:

Thiamine	0.1 mg.
Niacin	2.3 mg.
Riboflavin	0.05 mg.
Vitamin A	25 i.u.
Vitamin D	5 i.u.

A description of the diets fed in this study, together with information regarding the number of animals fed each diet, the level of fat in the diet, the length of the test period and the various supplements administered, appear in table 2.

Table 2. Description of the Diets

Diet No.	No. Rats	Oil	% Oil	Heating period at 275°C. (hours)	Acetone fraction used	Supplement	Level of administering supplement	Test period (days)
1	6	Linseed R.	36	12	Soluble			28
9	6	Linseed C.	36	12	Soluble			28
18	6	Linseed R.	36					28
27	4	Linseed C.	20	12	Soluble	Cortin	15 units daily (curatively)	28
28	4	Linseed C.	20	12	Soluble	NaCl+Cortin	15 units daily + 1% saline drinking water (curatively)	28
29	4	Linseed C.	20	12	Soluble	NaCl	1% saline drinking water (prophylactically)	28
30	4	Linseed C.	20	12	Soluble	NaCl	1% saline drinking water (curatively)	28
31	12	Corn	10		Soluble			42
32	12	Corn	10	15	Soluble			42
33	12	Corn	10	30	Soluble			42
34	12	Corn	10	30	Insoluble			42

Table 2 (continued) Description of the Diets

Diet No.	No. Rats	Oil	% Oil	Heating period at 275°C. (hours)	Acetone fraction used	Supplement	Level of administering supplement	Test period (days)
36	4	Corn	10	30	Soluble	Rat feces	20% by weight	17
37	12	Peanut	10		Soluble			42
38	12	Peanut	10	15	Soluble			42
39	12	Peanut	10	30	Soluble			42
40	12	Peanut	10	30	Insoluble			42
42	4	Peanut	10	30	Soluble	Rat feces	20% by weight	7
49	5	Linseed R.	10					27
50	5	Linseed R.	10	12				27
51	5	Linseed R.	10	12	Soluble			27
52	5	Linseed R.	10	12	Insoluble			27
53	4*	Linseed C.	20	12	Soluble			33
54	4*	Linseed C.	20	12	Soluble	Vitamin C	0.5 mg. daily	33

Table 2 (continued) Description of the Diets

Diet No.	No. Rats	Oil	% Oil	Heating period at 275°C. (hours)	Acetone fraction used	Supplement	Level of administering supplement	Test period (days)
56	4*	Linseed C.	20	12	Soluble	Animal lard	50% of fat level	33
57	4*	Linseed C.	20	12	Soluble	Fish meal	Replacing 11.5% dietary protein	33
63	10*	Linseed C.	10	12	Soluble			21
64	10*	Linseed C.	10	12	Soluble	Rat feces	20% by weight	21
65	10*	Linseed C.	10	12	Soluble	Water extr. feces	20% by weight	21
66	10*	Linseed C.	10	12	Soluble	Ether extr. feces	20% by weight	21
67	10*	Linseed C.	10	12	Soluble	Niacin	0.1 mg. daily	12
68	10*	Linseed C.	10	12	Soluble	Vitamin K	0.02 mg. every third day	12
69	10*	Linseed C.	10	12	Soluble	Liver extract	0.5 cc daily	21
70	10*	Linseed C.	10					21
72	10	Soybean	10		Soluble			35
73	10	Soybean	10	15	Soluble			35

Table 2 (continued) Description of the Diets

Diet No.	No. Rats	Oil	% Oil	Heating period at 275°C. (hours)	Acetone fraction used	Supplement	Level of administering supplement	Test period (days)
75	10	Soybean	10	30	Soluble			35
76	10	Soybean	10	30	Insoluble			35
77	3	Soybean	10	30	Soluble	Rat feces	20% by weight	7
79	10	Rape-seed	10		Soluble			35
80	10	Rape-seed	10	15	Soluble			35
82	10	Rape-seed	10	30	Soluble			35
83	10	Rape-seed	10	30	Insoluble			35
84	3	Rape-seed	10	30	Soluble	Rat feces	20% by weight	7
86	6	Corn	10		Soluble			21
87	6	Corn	10					21
89	6	Soybean	10		Soluble			21
90	6	Soybean	10					21

Table 2 (continued) Description of the Diets

Diet No.	No. Rats	Oil	% Oil	Heating period at 275°C. (hours)	Acetone fraction used	Supplement	Level of administering supplement	Test period (days)
91	6	Peanut	10		Soluble			21
92	6	Peanut	10					21

* Denotes mature animals

R.Denotes refined oil

C.Denotes crude oil.

IV CHEMICAL PREPARATION OF OILS

Preparation of the oils was carried out by the Chemistry Department, Macdonald College. The following general methods were employed.

1. Heating of Oils

Crude or refined oils were heated in a salt bath for the required length of time with the temperature accurately maintained at 275°C. Heating of the oils was invariably carried out at this temperature. From the start of the procedure until the product was finally cooled, carbon dioxide was bubbled through the oil to reduce oxidative decomposition to a minimum.

2. Acetone Segregation

A batch extraction method was employed in which seven volumes of acetone were shaken up with the oil. The mixture was allowed to stand overnight in a water bath held at a temperature just below the boiling point of acetone, during which time separation of the soluble from the insoluble fraction took place. The acetone from both fractions was subsequently removed under vacuum.

V SUPPLEMENTS TO HEATED OIL DIETS

Various supplements were fed in conjunction with heated oil diets in either a curative or a prophylactic capacity. The level and method of their administration to rats are shown in Table 3. Appendix II contains a description of these supplements and where applicable, the method in which they were prepared.

Table 3. Level and Method of Administering Supplements to Rats

Supplement	Method of Administration	Dose Level
Liver extract	orally	0.5 cc per rat daily
Rat feces	Ground and added to basal diet	20% by weight of diet
Ether extracted feces	Ground and added to basal diet	20% by weight of diet
Water extracted feces	Ground and added to basal diet	20% by weight of diet
Cortin	Intra-peritoneal injections	15 units per rat daily
Vitamin K	Orally, mixed in corn oil and bilesalts	0.02 mg. per rat every third day
Vitamin C	Orally, dissolved in distilled water	0.5 cc per rat daily
Fish meal	Added to basal diet	Replacing 11.5% protein of diet
Animal lard	Baked into basal diet	Constituting 50% dietary fat

VI CRITERIA

The criteria used to evaluate the effect of heat on the nutritive value of the oils included the growth rate of the rats, their feed consumption, general condition of health and thrift and their survival time. Post-mortem examinations were performed on all rats failing to survive the allotted test period, and also on a representative number of the apparently normal animals. The term "toxicosis" will be used to describe the syndrome characterized by loss of body weight, severe diarrhea and death of the animals within ten to fourteen days. Where the growth rate of the rats remains positive but is less than the control animals, the oils they consume will be termed "reduced in nutritive value" or "mildly toxic."

OBSERVATIONS DURING TESTS

Following the ingestion of certain heated oil diets, young rats developed typical toxicosis which has been defined above. Various non-specific symptoms were also exhibited by these animals, such as a progressive muscular weakness, lack of thrift and appetite, a nervous disposition and a rough, discoloured coat. In a significant number of animals "bloody nose" and blood in the urine were noted, although post-mortem examinations failed to reveal any visible lesions or hemorrhagic areas in the viscera. The adrenal glands of many of the sick animals, however, were found to be enlarged and darkened.

Plate 1. shows two young rats with typical linseed oil toxicosis in comparison with a normal animal.

Plate 1. A Comparison of a Young Rat with Toxicosis (right and below) and a Normal Animal (left)*

* The diets consumed by these rats contained unheated linseed oil or the acetone soluble fraction of the heated oil. (12 hours at 275°C.).

Oils described as being mildly toxic or having low nutritive values, failed to support normal growth in rats but did not result in actual weight losses. The animals did not die or develop diarrhea but showed mild, non-specific symptoms which appeared to be similar in nature to the toxicity symptoms. These included muscular weakness, lack of thrift, a poor coat condition and in a few cases, enlarged, darkened adrenal glands.

Plate 2. compares normal rats with others receiving an oil low in nutritive value.

Plate 2. A Comparison of Normal Rats (left) with Animals Receiving Oils Low in Nutritive Value (right)*

* The diets consumed by these rats contained unheated peanut oil or the acetone soluble fraction of the heated oil (30 hours at 275°C.).

In view of the fact that the toxicity syndrome involved the death of the animals before the end of the allotted test periods, it was impractical to analyse the data by statistical methods (except where indicated later). In order to make the growth rates and feed consumption independent of the survival time of the rats, these criteria were therefore compared on an average daily basis.

Interpretation of the results of one feeding trial was further complicated by a respiratory infection which was contracted by many of the animals. This test involved the feeding of corn, peanut, soybean and rape-seed oils to young rats. Upon post-mortem examination, these animals were found to have severe adhesions around the lungs and heart which were not associated with the heat treatment of the oils. Accordingly, only the data collected from these rats up to one week before death was used. It is felt, however, that in spite of making this allowance, no accurate conclusions may be drawn from this data until further work has been shown to support it.

RESULTS

I THE EFFECT OF HEAT

It was shown in these feeding trials, that under certain conditions heat had a detrimental effect upon the nutritive value of the oils tested.*

1. Heating for 12 Hours

Heat treatment for 12 hours at 275°C. followed by acetone segregation according to the method of Privett, Pringle and McFarlane (1945) rendered linseed oil toxic to young rats when fed at either a 36% or 10% level by weight. The average daily gains and feed consumption of animals fed the heated oil diets are shown in comparison with a control group in Table 4. The percentage of rats surviving the trial is also given.

Table 4. The Effect of 12 Hours Heating on the Nutritive Value of Linseed Oil
(The acetone soluble fraction of the heated oil was used)

Diet No.	Oil	% Oil	Average daily gain (gms.)	Average daily feed (gms.)	% Survival
18	Unheated linseed oil	36	3.5	7.0	100
1	Heated linseed oil	36	-2.1**	4.5	0
51	Heated linseed oil	10	-0.9**	5.8	60

** Denotes severe diarrhea

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* For summary of data see Appendix I

Lowering the level of heated oil in the diet from 36% (diet 1) to 10% (diet 51), appeared to lessen the severity of the toxicosis as judged by the weight losses of the rats and the percentage which survived the entire 28 day test period. It is doubtful, however, whether variations in weight losses can be accurately correlated with degrees of toxicity, therefore no attempt will be made to compare toxic diets one with another. When mature rats were fed the heated linseed oil diet, milder symptoms and longer survival period were noted. This seemed to indicate that the older animals were probably able to resist the toxic factor more effectively than weanling rats. Table 5 shows a comparison between mature and young rats fed a diet containing 10% of heated linseed oil.

Table 5. The Effect of a Heated Linseed Oil Diet on the Growth Rate, Feed Consumption and Survival Time of Mature and Young Rats
(The acetone soluble fraction of linseed oil heated for 12 hours at 275°C. was used)

Diet No.	Age of rat at start of test (days)	Average daily gain (gms.)	Average daily feed (gms.)	% survival
51	21	-0.9**	5.8	60
53	60	-0.01	10.8	100

** Denotes severe diarrhea

2. Heating for 15 Hours

Corn, peanut, soybean and rape-seed oils were heated for 15 hours and after acetone segregation, were included in the diets of young rats at a 10% level by weight. The results are presented in Table 6.

Table 6. The Effect of 15 Hours Heating on the Nutritive Value of Corn, Peanut, Soybean and Rape-seed Oils (The acetone soluble fraction of the heated oils were used)

Diet No.	Treatment to oil	Average daily gain (gms.)	Average daily feed (gms.)	Gain to feed ratio (gms.)
31	Corn oil, unheated	3.4	10.1	0.34
32	Corn oil, heated to 15 hours	2.6	8.2	0.32
37	Peanut oil, unheated	3.6	8.3	0.43
38	Peanut oil, heated 15 hours	3.6	9.5	0.38
72	Soybean oil, unheated	3.4	9.6	0.35
73	Soybean oil, heated 15 hours	3.7	10.5	0.35
79	Rape-seed oil, unheated	3.4	10.0	0.34
80	Rape-seed oil, heated 15 hours	2.9	9.5	0.31

In view of the fact that many of these animals developed the respiratory infection already described, no significance can be attached to the slight reduction in growth rate shown by the rats fed heated corn and rape-seed oils. In addition, as these animals consumed less food than the control groups, it was concluded that the lowered growth values were not indicative of a reduction in the nutritive value

of these two oils, but rather a consequence of lowered feed intake. The clinical appearance of the animals supported this view, because no visible symptoms of ill-health developed and post-mortem examinations of the viscera showed no abnormalities which could be associated with the test diets.

3. Heating for 30 Hours

The nutritive value of corn, peanut, soybean and rape-seed oils was reduced by heat treatment at 275°C. for 30 hours. The extent of the damage was not comparable with the effect of heat on linseed oil as these rats showed no diarrhea or weight losses. Their rate of growth, however, was found to be significantly reduced as compared with their respective controls.

Table 7 compares the data collected from animals fed the heated oil diets with their respective controls.

Table 7. The Effect of 30 Hours Heating on the Nutritive Value of Corn, Peanut, Soybean and Rape-seed Oils
(The acetone soluble fraction of the heated oils was used)

Diet No.	Treatment to oil	Average daily gain (gms.)	Average daily feed (gms.)	Gain to feed ratio (gms.)
31	Corn oil, unheated	3.4	10.1	0.34
33	Corn oil, heated 30 hours	2.0	7.5	0.27
37	Peanut oil, unheated	3.6	8.3	0.43
39	Peanut oil, heated 30 hours	1.0	5.6	0.18
72	Soybean oil, unheated	3.4	9.6	0.35
75	Soybean oil, heated 30 hours	2.2	8.1	0.27
79	Rape-seed oil, unheated	3.4	10.0	0.34
82	Rape-seed oil, heated 30 hours	2.3	7.8	0.29

Although these heated oil diets cannot be described as toxic, the animals receiving them developed mild symptoms which appeared to be similar in nature to those described for the linseed oil syndrome. They included muscular weakness, lack of thrift, a poor coat and in a few cases enlarged, darkened adrenal glands which were observed during post-mortem examinations.

II INVESTIGATIONS INTO THE CAUSE OF TOXICITY

It was not possible to conduct digestibility trials with the animals fed heated linseed oil because severe diarrhea formed a part of the clinical picture of toxicosis in young rats. Data collected from animals fed heated rape-seed and soybean oils, however, showed that weight losses could not be correlated with poor digestibility. On the average, the dry matter of these diets was found to be 94% digested, while the coefficients of digestibility of the ether extract was 96%.

Various attempts were therefore made to isolate or concentrate the harmful factor found in heated oils, with the object of removing it and possibly identifying it.

1. Refining

The supposition that impurities in crude linseed oil might be changed by heat and rendered toxic was shown to be incorrect. Preliminary refining of the oil did not prevent a toxic product from developing. (Compare diets 1 and 9 in Appendix I).

All the heated oil diets thus far discussed were subjected to acetone segregation and the soluble fraction fed to the rats. This

step was included in the preparation of corn, peanut, soybean and rape-seed oils for two reasons: (a) Because it was similar to the method of preparing a non-reverting product out of heated linseed oil. It was felt that if the treatment of the different oils could be made more uniform, the interpretation of the results would be simplified. (b) Because acetone segregation of the heated oils might concentrate the harmful factor in a single fraction which could then be removed. It was also hoped that this might aid in its eventual identification.

2. Acetone Poisoning

The possibility that incomplete removal of acetone from the oil was responsible for the toxic condition was not considered likely when the results of feeding corn, peanut, soybean and rape-seed oils, heated for 15 hours, were studied. However, the strong odour of acetone in the diet during baking demanded that this possibility should not be disregarded. A test was therefore designed, in which unheated oils were treated with acetone and fed to young rats at a 10% level by weight. In comparison, control groups of rats received unheated, untreated oil diets. The results of this test appear in Table 8.

Table 8. The Effect of Acetone Treatment on the Nutritive Value of Unheated Corn, Peanut and Soybean Oils

Diet No.	Treatment of unheated oils	Average daily gain (gms.)	Average daily feed (gms.)
86	Corn oil, acetone treated	3.3	8.3
87	Corn oil	3.6	10.5
91	Peanut oil, acetone treated	3.4	8.6
92	Peanut oil	4.1	10.4
89	Soybean oil, acetone treated	3.5	9.3
90	Soybean oil	3.9	10.3

Table 8 shows that a consistent decrease in feed consumption occurred with acetone treatment of the oils. Statistically, these differences were shown to be real. The decrease in feed consumption, however, was reflected in the average daily gains produced by these diets, giving a regressional value of 0.28 grams. It may be concluded, therefore, that the effect of acetone has been to decrease the palatability of the diets, thus indirectly affecting weight gains. This is in keeping with the clinical appearance of the animals which exhibited no visible toxicity symptoms or differences between groups.

3. Acetone Segregation

In view of the extent of heat treatment required to effect corn, peanut, soybean and rape-seed oils, and because subsequent

acetone treatment resulted in the appearance of an insoluble fraction, it was suggested that the formation of decomposition products or polymers might be responsible for the nutritional depreciation of the oils. Linseed oil, it was supposed, polymerized more rapidly and completely than the other oils, and thus contained a greater concentration of the toxic products. In addition, it was thought that heat treatment of linseed oil might give rise to a particularly potent product which was not formed to the same extent in the other oils. Acetone segregation of the oils was therefore employed, in an attempt to remove the toxic fraction. Table 9 shows the response of rats fed heated linseed oil with and without acetone segregation, in comparison with a control group.

Table 9. The Effectiveness of Acetone Segregation in Concentrating the Toxic Products Present in Heated Linseed Oil

Diet No.	Oil	Acetone fraction used	% survival	Average daily gain (gms.)	Average daily feed (gms.)
49	Linseed oil, unheated	untreated	80	3.8	11.2
50	Linseed oil, heated 12 hours	untreated	60	0.2**	7.9
52	Linseed oil, heated 12 hours	insoluble	100	1.8	9.5
51	Linseed oil, heated	soluble	60	-0.9**	5.8

** Denotes severe diarrhea

It will be noticed from Table 9 that 20% of the control animals receiving unheated linseed oil failed to survive the test period. As this percentage constituted only one rat out of the group of five, and as this animal developed no symptoms which suggested toxicosis, it was concluded that death in this case was not the result of the test diet.

In accordance with previous results, the acetone soluble fraction of heated linseed oil was found to be toxic to young rats. It caused the animals to lose body weight and develop diarrhea. As the toxic products are presumably more diluted in the whole, heated oil, it is not surprising to find this diet less toxic than the acetone soluble fraction. These animals developed typical toxicity symptoms but did not show actual weight losses. The low nutritive value of the acetone insoluble fraction of heated linseed oil was not due to absorption failure. The animals receiving this fraction gained weight, did not develop diarrhea and remained fairly normal in appearance. It was therefore presumed that although acetone segregation was not completely efficient in isolating the toxic products, these products were sufficiently diluted in the insoluble fraction to allow slow growth to be maintained.

Similar results were obtained when corn, peanut, soybean and rape-seed oils, heated for 30 hours, were subjected to acetone treatment. Although the difference between the two fractions is not marked as in the case of linseed oil, the same trend is apparent.

Table 10 shows the average daily gain and the average daily feed intake of rats fed these fractions of the four oils.

Table 10. The Effectiveness of Acetone Segregation in Concentrating the Harmful Products Present in 30 Hour Heated Corn, Peanut, Soybean and Rape-seed Oils

Diet No.	Oil	Length of heat treatment (hours)	Acetone fraction used	Average daily gain (gms.)	Average daily feed (gms.)
31	Corn	0	Soluble	3.4	10.1
34	Corn	30	Insoluble	2.1**	11.2
33	Corn	30	Soluble	2.0	7.5
37	Peanut	0	Soluble	3.6	8.3
40	Peanut	30	Insoluble	2.1**	9.0
39	Peanut	30	Soluble	1.0	5.6
72	Soybean	0	Soluble	3.4	9.6
76	Soybean	30	Insoluble	3.0	11.2
75	Soybean	30	Soluble	2.2	8.1
79	Rape-seed	0	Soluble	3.4	10.0
83	Rape-seed	30	Insoluble	2.5	9.1
82	Rape-seed	30	Soluble	2.3	7.8

** Denotes severe diarrhea

It will be noticed that severe diarrhea developed among the animals fed the insoluble fraction of corn and peanut oils heated for 30 hours. It is believed that this condition was not the result of toxicity, as the animals continued to gain weight and otherwise showed no signs of ill-health. Instead, it is thought that unabsorbable polymers

may have been formed in these particular oils, thus giving rise to the diarrhea. No accurate digestibility data could be collected from the rats to substantiate this explanation, but the extremely oily condition of the animals' coats suggested that these oil fractions probably behaved like a mineral oil.

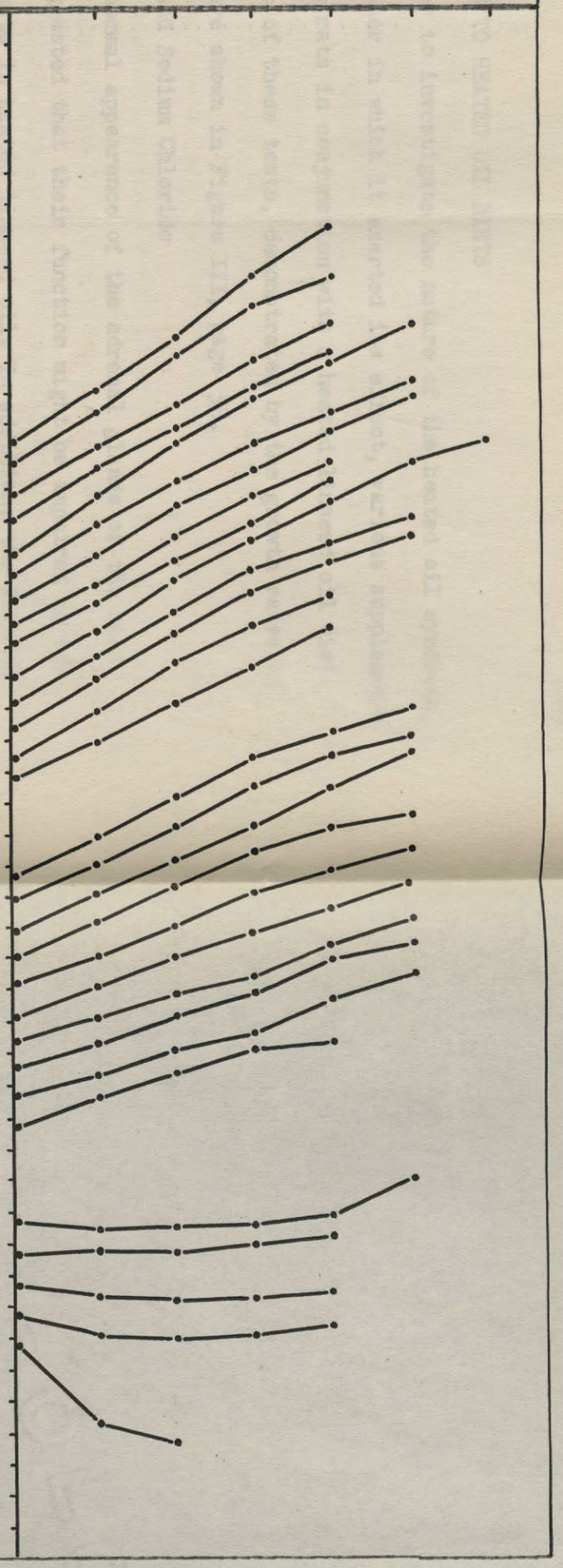
A summary of the data contained in the first section of this paper was compiled by plotting the cumulative, average daily gains (per week) of the rats fed the various oils. It was found that three groups were formed. The first group contained the unheated oils and soybean and peanut oils heated for 15 hours. The second group contained heated corn, soybean and rape-seed oils and the acetone insoluble fraction of heated linseed oil and of peanut oil heated for 30 hours. Into the third group fell the toxic linseed oil diets and the acetone soluble fraction of peanut oil heated for 30 hours. This last oil was not considered as severely toxic as the heated linseed oils diets included in the same group, because it did not cause diarrhea. However, the growth rates of the animals fed this oil were sufficiently low to warrant its inclusion in group III.

This graphic summary of the effect of heat on the nutritive value of the oils is shown in Figure I.

Figure I. Effect of Heat in the Nutritive Value of Vegetable Oils, as Measured by the Growth Response of Rats.

Oil	Heating period at 275°C. (hours)	Acetone fraction used	Diet No.
Group I.			
Peanut			92
Corn			87
Soybean			90
Linseed			49
Peanut		Soluble	37
Soybean	15	Soluble	73
Peanut	15	Soluble	38
Linseed			18
Soybean		Soluble	89
Corn		Soluble	31
Soybean		Soluble	72
Rape-seed		Soluble	79
Peanut		Soluble	91
Corn		Soluble	86
Group II.			
Soybean	30	Insoluble	76
Rape-seed	15	Soluble	80
Corn	15	Soluble	32
Rape-seed	30	Insoluble	83
Rape-seed	30	Soluble	82
Soybean	30	Soluble	75
Peanut	30	Insoluble	40
Corn	30	Insoluble	34
Corn	30	Soluble	33
Linseed	12	Insoluble	52
Group III.			
Peanut	30	Soluble	39
Linseed	12		50
Linseed	12	Soluble	53
Linseed	12	Soluble	51
Linseed	12	Soluble	1.

Cumulative average daily gain of rats per week (2 gram unit changes in weight)



III SUPPLEMENTS TO HEATED OIL DIETS

In order to investigate the nature of the heated oil syndrome and the manner in which it exerted its effect, various supplements were fed to rats in conjunction with a heated linseed oil diet. The results of these tests, demonstrated by the growth rates of the rats, are shown in Figure III, page 55.

1. Cortin and Sodium Chloride

The abnormal appearance of the adrenal glands of the sick animals suggested that their function might be impaired by the toxic products in heated linseed oil. In addition, the linseed toxicity symptoms such as severe diarrhea, loss of appetite and weight and ultimate death were found to be very similar to those described for Addison's Disease and adrenalectomized animals by Soskin and Levine (1946). These authors state that the absence of adrenal cortical extract disturbs the normal environment of all cells and thus leads to abnormalities in metabolism. Dehydration, hemoconcentration and the toxic action of high serum potassium levels leads to a shock-like condition and causes the rapid decline and death of the animals. According to Hartman and Lewis (1940), there are two factors in the adrenal cortical extract which are essential for preserving the life and normal function of the adrenalectomized animals. One maintains serum sodium levels and thus prevents the accumulation of toxic levels of potassium, while the other (cortin) is potent in preserving life, appetite, body weight and normal behaviour, even though serum sodium levels may remain low. It was found that the administration of NaCl

to adrenalectomized animals did not restore them to normal, but enabled them to survive indefinitely provided they were not exposed to any stresses or strains (Soskin and Levine 1946) (Anderson and Joseph 1941). Cortin, however, was found by Hoskins (1946) to maintain the animals in a healthy state. It is believed to be a general cell stimulant and consequently influences all vital activities.

In the studies conducted in this laboratory, the prophylactic or curative administrations of either cortin, salt or both, to young rats receiving a heated linseed oil diet was found to be ineffective in relieving the toxicity symptoms. It was concluded, therefore, that the function of the adrenal glands was not impaired by the toxic factors in the heated linseed oil. The enlarged, darkened appearance of the adrenals of sick rats was probably a non-specific manifestation of the general ill-health of the animals.

2. Vitamins C and K

The "bloody nose" and appearance of blood in the urine associated with the toxic linseed oil diet, suggested that normal synthesis of vitamins C and/or K might be inhibited, thus leading to an upset in cell permeability and structure and the occurrence of hemorrhage. Bay et al (1943) states that vitamin K is essential for the proper function of the liver cells in the formation of prothrombin. Vitamin K appears to be effective against hypothrombinemia from any causes (Kornberg 1944), but a number of compounds are able to counteract its effect, e.g. sulfonamides, which cause a deficiency as a result of

inhibition of bacterial synthesis. According to Sullivan (1943), the action of 4-hydroxycoumarin in producing hypothrombinemia is accentuated by a vitamin C deficiency. Hypothrombinemia has been found clinically in various conditions of impaired digestive function, and treated effectively in most instances as a K-avitaminosis (Sherman 1941a).

In these feeding trials vitamin C and K were not active in preventing linseed oil toxicosis in mature animals. It may therefore be concluded that some other factor or deficiency must be responsible for the hemorrhagic symptoms observed.

3. Animal Lard and Fish Meal

Animal lard was added to the toxic diet fed to rats, on the presumption that heat might modify the essential fatty acids present in linseed oil and thus destroy their biological activity. Fish meal was also chosen as a supplement because it was believed that an amino acid unbalance (caused by the toxic factor) might develop when casein and milk powder were the sole source of protein in the diet. Fish meal would supply the rats with additional amino acids, notably tyrosine and tryptophane.

As a result of these feeding tests, it was found that neither animal lard nor fish meal were effective in preserving the health of mature animals. It may be concluded, therefore, that heated oils do not cause a deficiency or an unbalance of essential fatty acids or amino acids.

4. Liver Extract, Rat Feces and Niacin

It is believed by many workers that the intestinal flora of certain animals is capable of synthesizing members of the B vitamins which are then utilized by the host either directly by absorption, or indirectly by coprophagy (Geyer 1947). The importance of these intestinal bacteria in the general nutrition of animals has been widely acknowledged, and it has been shown by numerous workers that diet may influence the type of intestinal flora and thus control the amount of biosynthesis which can take place (Gall et al 1948) (Tessier 1908) (Belonovsky 1907) (Crecelius et al 1943) (Porter et al 1940) (Sanborn 1931). Working with rats, Steenbock (1923) and Roscoe (1931) have independently shown that the feeding of feces from normally fed animals will correct dietary deficiencies of vitamin B. Assuming that heated linseed oil might destroy intestinal bacteria or in some way alter the type of flora, rat feces or liver extract, a rich source of the B vitamins, were included as supplements to the toxic diets fed to mature rats. Extra niacin was also fed as a specific supplement because diarrhea is known to occur in its absence (Smith 1942). Although niacin was among the supplements fed to all test animals, it was believed that the nature of the toxic diet might increase its requirement. Table 11 shows the growth response of mature rats to supplements of feces, feces fractions, liver extract or niacin added to the heated linseed oil diet.

Table 11. The Effectiveness of Feces, Liver Extract and Niacin as Supplements to a Toxic Linseed Oil Diet
(The toxic diet contained the acetone soluble fraction of linseed oil heated for 12 hours at 275°C.)

Diet No.	Supplement	Method and level of administering supplement	Average daily gain (gms.)	Average daily feed (gms.)	Gain to feed ratio (gms.)
70	Positive control diet containing unheated linseed oil		3.5	10.3	0.34
65	Water extracted feces	20% mixed in diet	3.3	12.4(9.9)*	0.34
64	Rat feces	20% mixed in diet	3.0	11.9(9.5)*	0.31
66	Ether extracted feces	20% mixed in diet	2.6	11.0(8.8)*	0.30
69	Liver extract	0.5 cc.daily by mouth	2.5	8.8	0.28
67	Niacin	0.1 mg.daily by mouth	1.5	8.8	0.17
63	Negative control diet containing heated linseed oil		1.4	8.3	0.17

* Denotes the amount of basal ration consumed minus the feces.

It is apparent from the figures presented in Table 11 that all fractions of feces were effective in preventing the development of toxicosis. The ether extracted material (diet 66) did not appear to be as active as the whole or water extracted feces (diet 65 and 64), but this might possibly be due to the removal of the fat, which, although small in amount, would nevertheless reduce the caloric concentration of the feed. It is believed that dilution of the toxic oil with feces was not responsible for the results, as it was found that the feed consumption of these animals increased by approximately 20% after the addition of the feces.

Liver extract was not as potent as feces in supplementing the toxic diet, although it effectively maintained the growth rate of these animals at a higher level than the negative controls (diet 63). Niacin was considered to be ineffective.

Work done with rat feces in a curative capacity showed that this material was also able to cure the symptoms developed by rats fed heated oil diets and to stimulate their growth. The beneficial effect of this supplement was extremely marked and occurred within three to four days. Figure II shows the curative potency of feces when they are added at a 20% level to the heated oil diets of rats. These diets contained the acetone soluble fraction of corn, peanut, soybean and rape-seed oils heated for 30 hours at 275°C.

A summary of the results of supplementing heated linseed oil diets with various materials is shown in Figure III. It is apparent that only feces and liver extract are effective in preventing toxicosis and stimulating the growth of rats.

Figure II. The Curative Effect of Feces as Measured by the Growth Response of Rats. (The acetone soluble fraction of the oils heated for 30 hours were used.)

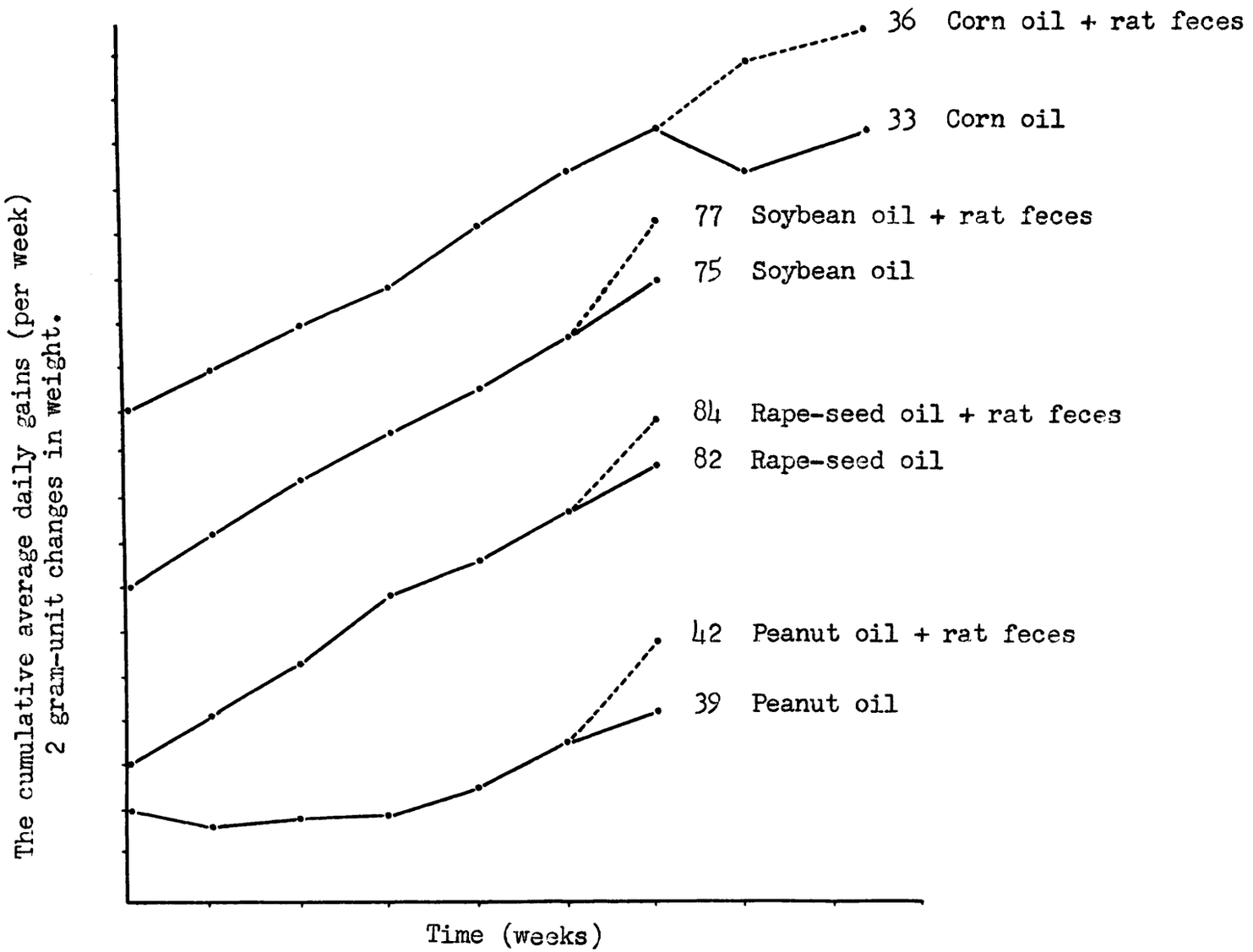
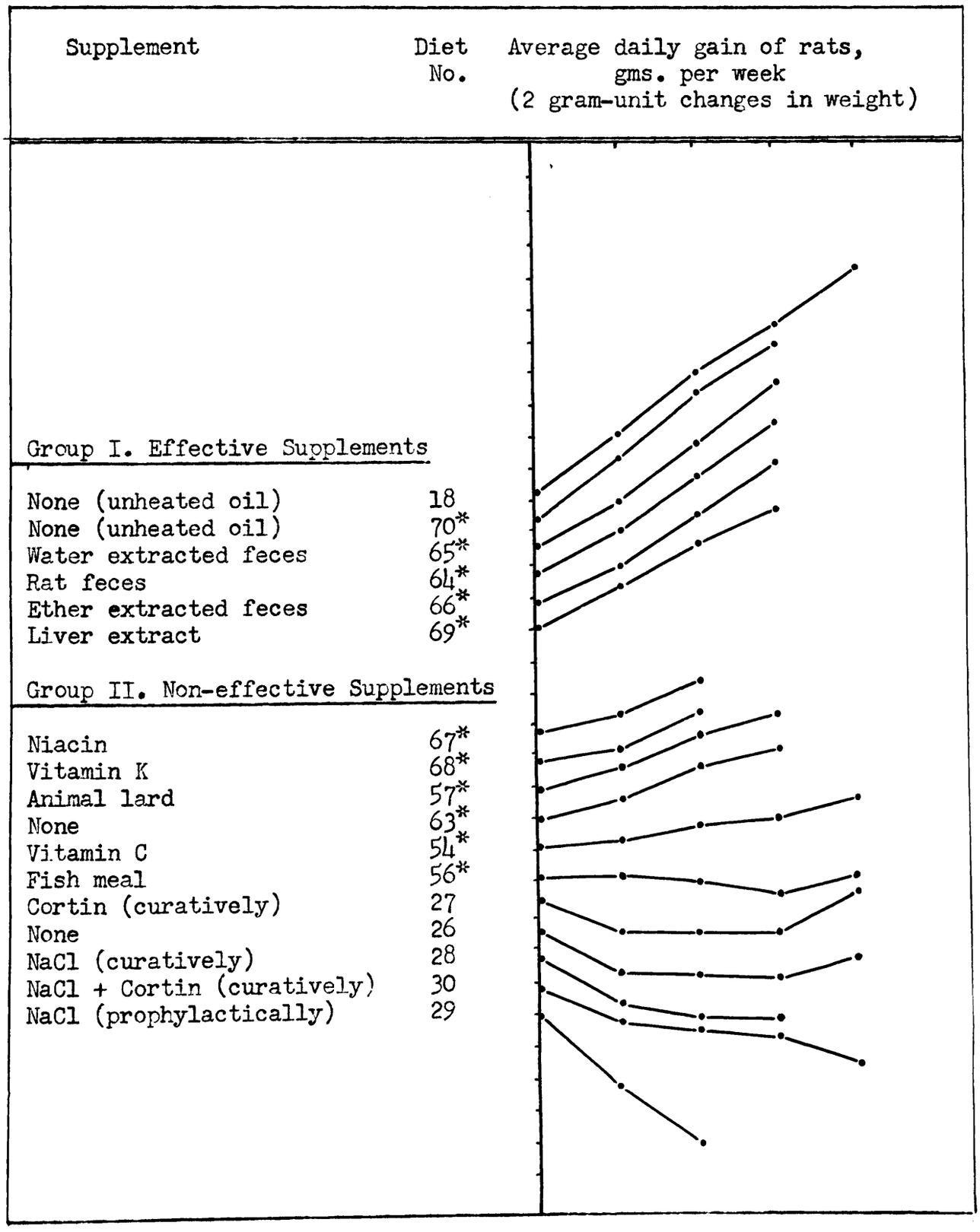


Figure III. Effect of Various Supplements in Relieving Linseed Oil Toxicity, as Measured by the Growth of Rats over a Four-week Test Period.
(Unless otherwise indicated, the diets contained the acetone soluble fraction of linseed oil heated for 12 hours at 275°C.)



* Denotes mature animals

DISCUSSION

I. EFFECT OF HEAT

The immediate query which arises from a review of the results of these feeding trials, is whether the effect of heat on all the oils is similar or whether linseed oil toxicosis is a manifestation of a different type of disorder. The nature of the symptoms developed by rats fed heated corn, peanut, soybean and rape-seed oils was similar (although milder) to the linseed oil toxicity symptoms, especially those developed by mature rats. In addition, it was shown that the effect of all the heated oils could be counteracted by supplementation with feces. Thus it was concluded that heat rendered all oils toxic to a varying degree.

It was clear that toxicity did not result from acetone poisoning or from the action of heat on impurities present in the crude oil, therefore it was presumed that heat degradation products or polymers, which are known to form at 275°C., must be responsible for the disorder. The term "polymer" is used here in a wide sense of the word. It is doubtful whether true polymers developed in these oils, but the use of the term includes any addition products such as monomers, dimers or trimers.

According to an earlier premise (Privett, Pringle and McFarlane 1945), it was believed that all polymers were insoluble in acetone. As the acetone soluble fraction of heated linseed oil was observed to be more toxic than the insoluble fraction, it was therefore

believed that polymers could not be the harmful agents. By this reasoning, the mildly toxic effect of the acetone insoluble fraction could only be explained on the basis that acetone segregation was not 100% efficient. It was evident that digestibility was not involved unless the polymers formed soaps in the feces and thus escaped detection.

As a result of more recent work (Kass 1947) it is now known that some polymers may be soluble or partially soluble in acetone. The identity of the toxic factor however, still remains a matter of conjecture, as the presence or the development of other substances in the oil apparently influence the nature of the changes which occur within the fatty acid structure, and thus might cause variations in the type of end-product formed. Assuming polymers are the harmful agents, the severely toxic nature of heated linseed oil might, therefore, be due to the formation of a particular type of compound which is not readily formed in the other oils. A second possibility is that a less toxic polymer may develop in corn, peanut, soybean and rape-seed oils owing to inherent differences in fatty acid composition.

II. THE NATURE OF TOXICITY

The clinical picture of the heated oil disorder does not indicate the manner in which the toxic factor acts. As a result of post-mortem examinations it is evident that no gross lesions occurred in the viscera which might account for the symptoms, while tests showed that the functions of the adrenal glands were apparently unimpaired. Although no strong evidence exists, it was concluded that

the heated oils caused an acute deficiency of some essential nutrient. Thus, mature rats raised on an adequate diet would probably possess sufficient body stores of this nutrient to allow them to survive the toxic diet longer than weanling rats.

Following this hypothesis, it is evident that the essential nutrient is some substance common to both feces and liver extract. The assay of the liver supplement showed it to be a rich source of vitamins, but it is possible that other substances might also have been present. As animal lard and fish meal were ineffective in preventing toxicosis, it seems improbable that either fatty acids or amino acids were involved. It is concluded, therefore, that the essential nutrient must be either a vitamin or a trace element.

Assuming the deficiency to be due to a vitamin, the results of the tests indicate that niacin, vitamin C and vitamin K are probably not involved, while thiamine, riboflavin, vitamins A and D were administered to all test animals without success in preventing toxicosis. Red and white blood cell counts were made on blood samples taken from sick animals, and as no abnormalities were noticed, it was presumed that folic acid was not associated in the deficiency either. Evidence therefore suggests that if the essential substance is a vitamin, it must be one of the less well-known members. According to this theory rat feces should show a reduction in potency following extraction with either water or ether. As this did not occur it must be concluded that the extraction methods were inefficient in removing the vitamins. No assays were performed to test this possibility, but the consistency of the wet material seemed to support the explanation.

It is not clear how the toxic factor in heated oils causes a vitamin or trace element deficiency to develop, but considering the potency of the feces supplement, it is suspected that bacterial synthesis might be involved. Feces presumably contain material originating from bacterial synthesis as well as from ingested food. A bacteriological investigation* showed that the toxic factor did not destroy or alter the type of intestinal flora, therefore two alternative hypotheses have been proposed to explain the action of the heated oil diets.

(a) It is possible that the toxic factor might tie up the end-product of bacterial synthesis, i.e., the essential nutrient, and thus render it unavailable to the host. This interference could be through direct destruction or through the formation of an addition compound which destroys the biological activity of the nutrient. By supplying the animal with an excess of the essential nutrient (present in liver and feces) the effect of the toxic factor could be overcome. By this theory, it is possible to explain the greater potency of feces, simply that it supplies the animal with more adequate amounts of the essential nutrient than liver extract.

(b) Alternatively the toxic factor might destroy or completely tie up the enzyme systems involved in the synthesis of the essential nutrient. Supplementation of the toxic diet with feces or liver extract would therefore supply the host with the preformed nutrient, which in itself would not be affected by the toxic factor.

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* We are indebted to Mr.R.Lachance for his assistance in the bacteriological work.

CONCLUSIONS

1. Heat treatment for 12 hours at 275°C., rendered linseed oil toxic to young rats when incorporated in their basal diet at levels of 10, 20 or 36% by weight.
2. The nutritive value of corn, peanut, soybean and rape-seed oils was not affected by heat applied for 15 hours but was reduced by treatment for 30 hours.
3. Acetone segregation was effective in partially concentrating the toxic factor in the soluble fraction of the oils.
4. Since neither acetone poisoning nor impurities in the crude oil were responsible for the toxic condition, heat degradation products or polymers soluble in acetone are probably the toxic agents.
5. The toxic factor did not impair adrenal function or cause a deficiency of essential fatty acids or amino acids.
6. Heated oils appear to exert their effect by producing a deficiency of some essential nutrient ----- possibly a vitamin or a trace element.
7. Since niacin, riboflavin, thaimine, folic acid, vitamins C,K, A or D were probably not involved in the syndrome, the deficiency, if due to a vitamin, must be caused by the lack of one of the less well-known members.

8. The deficiency of the essential nutrient is probably due to the inhibition of intestinal synthesis.

9. Since heated oils neither destroy nor alter the type of intestinal flora, the toxic factor may interfere with the enzyme systems or in some way involve the end-products of biosynthesis.

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APPENDIX I

APPENDIX II

Appendix Table 1. Summary Table of Data

Diet No.	Oil	% Oil	Heating period at 275°C. (hours)	Acetone fraction used	Supplement	No. in group	Test period (days)	% survival	Av.daily gain (gms.)	Av.daily feed (gms.)
1	Linseed R	36	12	Soluble		6	28	0	-2.1**	4.5
9	Linseed C	36	12	Soluble		6	28	0	-0.7**	3.5
18	Linseed R	36				6	28	100	3.5	7.0
27	Linseed C	20	12	Soluble	Cortin (curatively)	4	28	75	-0.4**	7.0
28	Linseed C	20	12	Soluble	NaCl+Cortin(curatively)	4	28	25	-1.9**	7.0
29	Linseed C	20	12	Soluble	NaCl (prophylactically)	4	28	0	-4.2**	3.0
30	Linseed C	20	12	Soluble	NaCl (curatively)	4	28	0	-1.9**	5.0
31	Corn	10		Soluble		12	42	83	3.4	10.1
32	Corn	10	15	Soluble		12	42	83	2.6	8.2
33	Corn	10	30	Soluble		12	42	75	2.0	7.5
34	Corn	10	30	Insoluble		12	42	83	2.1**	11.2

Appendix Table 1.(continued) Summary Table of Data

Diet No.	Oil	% Oil	Heating period at 275°C. (hours)	Acetone fraction used	Supplement	No. in group	Test period (days)	% survival	Av.daily gain (gms.)	Av.daily feed (gms.)
36	Corn	10	30	Soluble	Rat feces	4	17	100	2.3	10.0 (8.0)
37	Peanut	10		Soluble		12	42	58,	3.6	8.3
38	Peanut	10	15	Soluble		12	42	75	3.6	9.5
39	Peanut	10	30	Soluble		12	42	75	1.0	5.6
40	Peanut	10	30	Insoluble		12	42	65	2.1**	9.0
42	Peanut	10	30	Soluble	Rat feces	4	7	100	4.6	11.2 (9.0)
49	Linseed R	10				5	27	80	3.8	11.2
50	Linseed R	10	12			5	27	60	0.2**	7.9
51	Linseed R	10	12	Soluble		5	27	60	-0.9**	5.8
52	Linseed R	10	12	Insoluble		5	27	100	1.8	9.5
53	Linseed C	20	12	Soluble		4*	33	100	-0.01	10.8

Appendix Table 1. (continued) Summary Table of Data

Diet No.	Oil	% Oil	Heating period at 275°C. (hours)	Acetone fraction used	Supplement	No. in group	Test period (days)	% survival	Av.daily gain (gms.)	Av.daily feed (gms.)
54	Linseed C	20	12	Soluble	vitamin C	4*	33	100	0.8	9.8
56	Linseed C	20	12	Soluble	Animal lard	4*	33	100	1.4	11.4
57	Linseed C	20	12	Soluble	Fish meal	10*	33	100	-0.4	13.2
63	Linseed C	10	12	Soluble		10*	21	70	1.4	8.3
64	Linseed C	10	12	Soluble	Rat feces	10*	21	80	3.0	11.9 (9.5)
65	Linseed C	10	12	Soluble	Water extr. feces	10*	21	100	3.3	12.4 (9.9)
66	Linseed C	10	12	Soluble	Ether extr. feces	10*	21	90	2.6	11.0 (8.8)
67	Linseed C	10	12	Soluble	Niacin	10*	12	100	1.5	8.8
68	Linseed C	10	12	Soluble	Vitamin K	10*	12	90	1.5	8.8
69	Linseed C	10	12	Soluble	Liver extract	10*	21	90	1.4	8.3
70	Linseed C	10				10*	21	90	3.5	10.3

Appendix Table 1. (continued) Summary Table of Data

Diet No.	Oil	% Oil	Heating period at 275oC. (hours)	Acetone fraction used	Supplement	No. in group	Test period (days)	% survival	Av.daily gain (gms.)	Av.daily feed (gms.)
72	Soybean	10		Soluble		10	35	50	3.4	9.6
73	Soybean	10	15	Soluble		10	35	80	3.7	10.5
75	Soybean	10	30	Soluble		10	35	70	2.2	8.1
76	Soybean	10	30	Insoluble		10	35	100	3.0	11.2
77	Soybean	10	30	Soluble	Rat feces	3	7	75	4.8	16.2(13.0)
79	Rape-seed	10		Soluble		10	35	60	3.4	10.0
80	Rape-seed	10	15	Soluble		10	35	80	2.9	9.5
82	Rape-seed	10	30	Soluble		10	35	60	2.3	7.8
83	Rape-seed	10	30	Insoluble		10	35	80	2.5	9.1
84	Rape-seed	10	30	Soluble	Rat feces	3	7	100	3.5	14.3(11.4)
86	Corn	10		Soluble		6	21	100	3.3	8.3

Appendix Table 1. (continued) Summary Table of Data

Diet No.	Oil	% Oil	Heating period at 275oC. (hours)	Acetone fraction used	Supplement	No. in group	Test period (days)	% survival	Av.daily gain (gms.)	Av.daily feed (gms.)
87	Corn	10				6	21	100	3.6	10.5
89	Soybean	10		Soluble		6	21	100	3.5	9.3
90	Soybean	10				6	21	100	3.9	10.3
91	Peanut	10		Soluble		6	21	100	3.4	8.6
92	Peanut	10				6	21	100	4.1	10.4

*Denotes mature rats

**Denotes severe diarrhea

The average daily feed values appearing in brackets are the amounts of basal ration consumed without the addition of feces.

DESCRIPTION OF SUPPLEMENTS TO HEATED OIL DIETS

1. Liver extract

This was a watery extract of liver produced in the Connaught Laboratories as a by-product in the preparation of the liver extract to be taken orally for pernicious anemia. According to Dr.E.W.McHenry of the University of Toronto from whom this material was obtained, it is considered to be a faily good source of all B vitamins except for thiamine.

Per one cc. this material contained:

Thiamine	36 mcg.
Nicotinic acid	3200 mcg.
Riboflavin	207 mcg.
Pantothenic acid	1000 to 1400 mcg.
Pyridoxine	40 mcg.
Biotin	1 mcg.
Choline	24 mg.(total Choline)
Folic acid	present(not assayed)

2. Rat Feces

Feces were collected from healthy adult rats receiving a normal diet. They were air-dried and ground.

3. Ether Extracted Feces

Five hundred grams of whole, air-dried rat feces, collected from healthy, adult rats receiving a nommal diet, were placed in a cheese-cloth bag and washed in petrol ether for 30 minutes. The ether was decanted off, and replaced with fresh ether in which the rat feces were allowed to stand for six hours. The ether was again decanted. The feces were finally air-dried and ground. It was presumed that this supplement was free of fat soluble vitamins.

4. Water Extracted Feces

Five hundred grams of whole, air-dried rat feces collected from healthy adult rats receiving a normal diet, were placed in cheese-cloth stretched over a tripod stand. A layer of cheese-cloth covered the feces and using a sprinkler system, cold water was allowed to filter through the feces for 6 hours. The feces were finally oven-dried at 75°C. and ground. It was presumed that this supplement was free of water soluble vitamins.

The feces used were obtained from Ayerst, McKenna and Harrison Laboratories, Montreal.

5. Cortin

This material was an adrenal cortical extract obtained from the Connaught Medical Research Laboratories, Toronto, with a potency of 30 units per cc.

6. Vitamin K

A solution was prepared by mixing Menadione (2-methyl-naphthoquinone), a synthetic product with vitamin K activity, obtained from Merck and Company Limited, Montreal, with corn oil and bile salts.

7. Vitamin C

Pure crystalline ascorbic acid was dissolved in glass distilled water.

8. Fish Meal

This material was a commercial product consisting of dried whole fish and fish cuttings. It was obtained from the Montreal City Renderers Limited and was guaranteed to contain:

Crude protein	(min.)	55%
Crude fat	(max.)	14%
Crude fibre	(max.)	3%
Salt (NaCl)	(max.)	4%

The fat was extracted from the material with ether before the meal was fed to rats.

9. Animal Lard

The commercial product, Maple Leaf Brand, was used.