health through an additional year represents a market for about one ton of food.

There can be no doubt of the economic and civic importance of research in this field of nutrition when we recognize that eight of the 10 leading causes of death in the United States today are associated with the metabolic and chronic diseases that are not primarily a result of infections. This situation brings the fruits of research progress into practically every home and every group in our society.

REFERENCE
1. Hammond, E. C., and Haun, D., J.A.M.A., *155*, 1316 (1954).

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# The Influence of Dietary Fats on Serum-Lipide Levels in Man[1]

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THE RÔLE of unsaturated fatty acids in human nutrition is a topic of rapidly increasing popularity in both scientific and lay publications. It has been claimed that ingestion of this group of fatty acids decreases the concentration of lipides in the serum and that an inadequate intake of these acids may be the cause of the high serum-lipide levels so prevalent in Western civilization. Since these high levels are allegedly involved in the pathogenesis of arteriosclerosis and coronary disease, it has been tempting to implicate lowered intakes of unsaturated fatty acids in the rising incidence of these diseases. Although existing experimental data do not as yet warrant such conclusions, there is mounting evidence that the precise chemical composition of ingested fatty acids, in particular the degree of saturation, may have considerable significance in both health and disease (14).

E. H. Ahrens Jr.

The most recent stimulus to study in this field comes from clinical investigations which suggest that levels of serum-lipides may be related to the dietary intake of unsaturated fatty acids. Kinsell and Michaels (11) found that the feeding of coconut oil (a highly saturated fat) produced higher levels than the feeding of a highly unsaturated oil, soybean oil. Long-term feeding tests in this laboratory (4) indicated that serum levels of cholesterol and phospholipides varied inversely with the iodine number of six different dietary fats. The findings of these two laboratories were greatly extended in the detailed report by Bronte-Stewart *et al.* (8) and also were confirmed by the experiments of Beveridge *et al.* (5).

None of the experiments known to us has proven conclusively that the effect on serum-lipides of these dietary fats is due to the double-bond structure of their fatty acids. Nevertheless there has been widespread acceptance of this hypothesis by workers in human nutrition, and broad applications of the hypothesis have already been made. Since we think these extensions premature, it seems appropriate for us to set forth in some detail the studies carried out by us over the past three years which bear upon the important questions at issue. The unique design of these studies was based on the belief that nutritional experiments can be carried out as precisely in man as in animals. In view of numerous well-known differences in species response to nutritional variation, such an approach seemed essential. All recognized extra-dietary factors influencing serum-lipide levels were kept to a minimum. Patients were carefully selected and then observed within the closely supervised environment of the metabolic ward for periods long enough to assure establishment of equilibrium states. Dietary intakes were simplified and rigidly standardized; even the variable of cooking was eliminated. Yet all known requirements for essential foodstuffs, minerals, and vitamins were generously supplied. Medical and hospital care was provided to the patients without charge, and constant attention was paid to socio-economic factors which might disrupt their adjustment to the study situation. Under these circumstances the data obtained have quantitative as well as qualitative usefulness.

## Methods of Present Studies

A technique of oral feeding of liquid formulas was developed (3) and has been used almost exclusively in all feeding experiments since 1953. With this method patients were maintained at constant body weight by suitable adjustments of caloric intake; the proportions of foodstuffs remained unchanged. Changes in body weight rarely exceeded 1 kg. The basic formula consisted of protein 15%, fat 40%, and carbohydrate 45% of total calories. There was no source of calories other than the formula. Formula feeding has been used uninterruptedly for 36 months in one patient without recognized ill effect; however in most of the experiments discussed below the feeding tests lasted four to six months. Thirty-eight of 40 patients were observed continuously under strict metabolic-ward conditions; four of the 40 were sufficiently motivated and intelligent to follow the regimen at home. Iodized salt (2 g. sodium chloride with 200 μg. potassium iodide),[4] two multi-vitamin capsules, and ferrous gluconate (0.3 g.) were given

[1] This condensation of a paper appearing in The Lancet, *1*, 943–953 (May 11, 1957), was prepared by F. G. Dollear.
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[4] We are indebted to Clayton Rich, Rockefeller Institute Hospital, for demonstrating a requirement for supplemental iodine in our patients maintained solely by oral formula feeding. Unpublished serial radioactive iodine studies showed significant increases in uptake in some patients in less than one month.

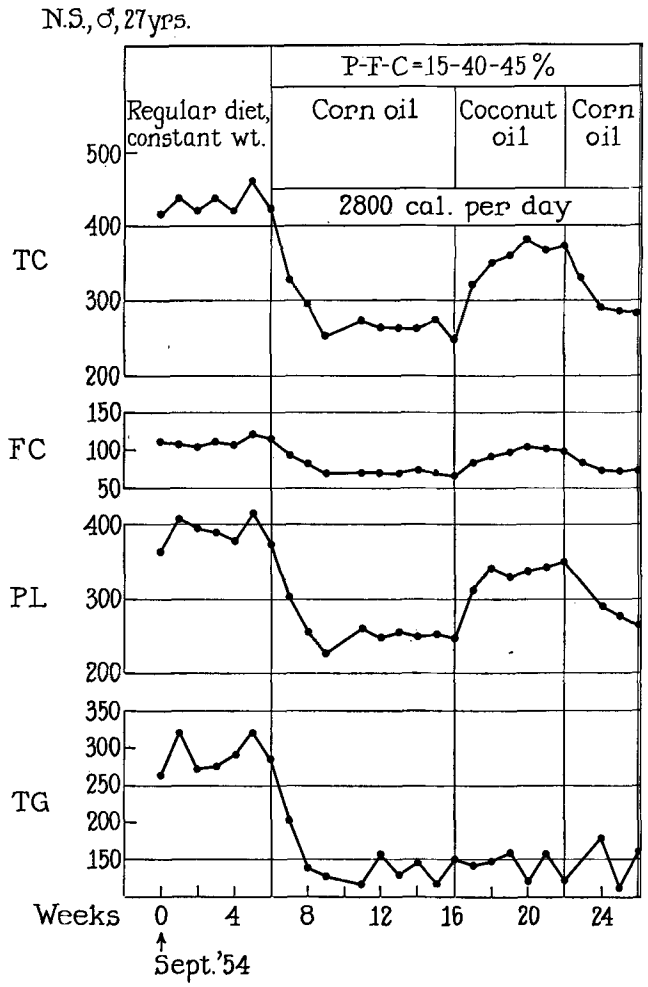


FIG. 1. 26-week study of serum lipides of patient 9, maintained at constant body weight on *ad libitum* feeding, then by oral formula feeding exclusively.
TC = total cholesterol, FC = free cholesterol, PL = phospholipides, TG = triglycerides—all in mg. per 100 ml. serum. P-F-C = dietary proteins, fats, and carbohydrates, as percentages of total calories.

daily as supplements. The entire protein intake was derived from milk proteins; the intake of nitrogen and pattern of amino-acid intake were considered completely adequate for adult men and women (13).

The subjects of these studies were hypercholesteræmic or hyperlipæmic patients (some with clinical evidence of arteriosclerosis and xanthomatosis) and normocholesteræmic patients with arteriosclerotic heart disease. All patients were free from conditions which might complicate the interpretation of long-term metabolic studies, such as renal, hepatic, endocrine, or infectious disease, or nutritional deficiencies. All were ambulatory.

In addition to routine clinical tests, weekly measurements were made of total and free serum-cholesterol (15), total serum-lipide phosphorus (17), and total serum-lipides (2). Cholesterol esters and serum triglycerides were calculated from these basic data; generally accepted mean molecular weights for the cholesterol esters, 645, and phospholipides, 775, were used in these calculations. Rapid determinations of total cholesterol (1) made each week checked (within 5%) with the Sperry-Webb values.

### Base-line Levels on Corn-oil Formula

Figure 1 is a graphic demonstration of the data of a typical feeding experiment. For the first eight weeks the patient ate *ad libitum*. After relatively constant levels of serum-lipides had been achieved, formula feeding was begun. In the three succeeding periods all dietary fat was derived from corn oil, coconut oil, and corn oil, respectively. There were striking decreases in all serum-lipide components during the two corn-oil periods and rises in all except triglycerides when coconut oil was fed. After each change of formula, lipide levels shifted upward or downward in an orderly manner through a three-week transitional phase. This was followed by a new steady state, a plateau of levels marked by only minor fluctuations. Body weight remained unchanged in all periods.

Not all patients responded to the corn-oil formula in the same measure, and only occasional patients with high serum-cholesterol achieved normal levels. To compare responses to various dietary fats in different patients, it was necessary to establish in each individual a base-line of serum-lipides attained on corn-oil feeding. Then, when other fats were substituted isocalorically for corn oil in the basic formula, the serum-lipide data obtained during successive steady states could be related to the corn-oil base-line. Thus in Figure 1 the level of total cholesterol on the coconut-oil diet was 40% higher than that on corn oil.

Measurements during transitions were, of course, omitted in these calculations. The length of each transition phase was determined on an individual basis by inspection of the assembled data. Transitions varying from three days to eight weeks have been encountered, but usually transitions were completed in two to three weeks. Consequently with each change in formula at least the first two weeks' determinations were discarded when selecting data for comparative calculations; when transitions were slower, more data were discarded.

It is relevant to define the extent of variation of week-to-week values during the terminal portion of each feeding period designated as the steady state. The duration of the steady state was at least three weeks and usually four or more. During the steady state the standard deviation of the mean level of total cholesterol, as well as of phospholipides, was less than $\pm 20$ mg. per 100 ml. serum in all cases except patient 3, and the pooled variation of the 120 steady states reported in this paper was $\pm 9.9$ mg. for total cholesterol, $\pm 4.2$ mg. for free cholesterol, and $\pm 9.9$ mg. for phospholipides. The deviations of each of these components were unrelated to their total mean concentration. Both technical and true biological variations (18) of triglycerides were greater than in the case of cholesterol and phospholipides. In 95 out of the 100 steady states, standard deviations of mean triglyceride levels were less than $\pm 60$ mg. per 100 ml. serum; the pooled variations in these 100 periods was $\pm 31$ mg. The deviations of triglycerides were larger at higher concentrations of this lipide component, as would be expected from the method of measurement. Independent tests showed that about one-third of the total variation of each lipide level could be ascribed to methodological error. Differences in mean cholesterol or phospholipide levels of 25 mg. per 100 ml. of serum are statistically significant at the 1% level when four data are collected in each of two steady states under comparison and standard deviations of mean levels are $\pm 10$ mg.

It is central to the issue under discussion that the

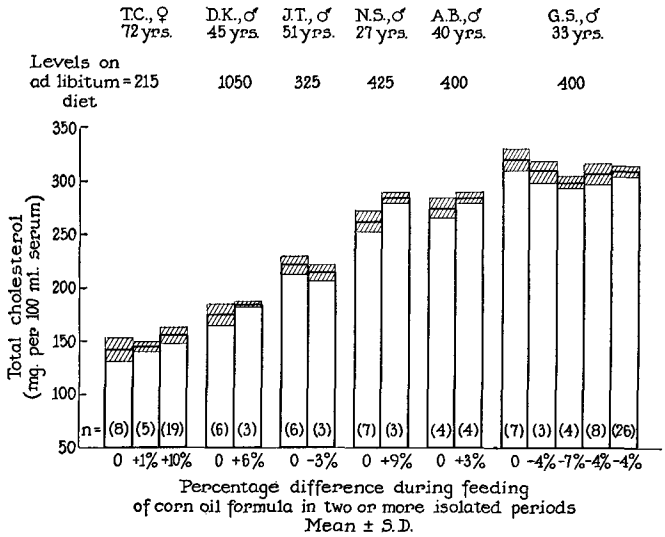


FIG. 2. Reproducibility of serum-cholesterol levels in six patients (from left to right, patients 34, 1, 27, 9, 12, and 13) retested on basic corn-oil formula (P-F-C = 15-40-45%), with other regimens in intervening periods.

Bars show mean levels during steady states, hatched area one S.D. n = number of weekly data during each steady state. Percentage differences along base-line calculated with reference to mean levels of first feeding periods.

concentration of a serum-lipide during ingestion of corn oil be a reproducible constant for a given subject. The similarity of these levels in re-test periods is shown in Figure 2, in which data from six re-tested patients are graphed. It is seen that mean concentrations of serum-cholesterol varied by no more than 10% from one corn-oil period to another. In the same patients phospholipide levels varied by 7% or less from period to period, but the range of triglyceride fluctuations was as great as 50%. In patients 34 and 13 there was no drift of levels during administration of the corn-oil formula for 19 and 26 weeks, respectively, as shown by the small standard deviations of values from the mean of these periods. Thus, for the period set as a minimum in all our studies (four to five months), the corn-oil base-line remained fixed and showed no "escape."

A comparison was made of the total serum-cholesterol levels in patients eating diets of their own choice with their base-line levels obtained with corn-oil formula feeding. Decreases of 20% or more were produced in 36 of 39 patients during corn-oil formula feeding. In patient 1 a decrease of 84% was achieved while a 12% decrease was seen in patient 27, and in patient 15 the corn-oil formula produced no change. Excepting these three cases, the percentage decreases ranged from 20–67% (mean 37%, standard deviation ±11%). These data show clearly the unpredictability of response of individual patients to a given dietary fat intake.

### The Responsible Factors in Corn Oil

Experiments such as that shown in Figure 1 suggested that certain factors in the diet raised the levels of serum-lipides, and others depressed them. When corn oil was fed as the sole source of dietary fat, the lowered levels could have been caused by an absence of elevating factors, or to the presence or dominance of depressing factors. Since the protein and carbohydrate portions of these formulas were identical and the total quantity of fat remained constant, the factors in question resided in the qualitative differences between dietary fats.

An obvious difference between fats of animal and vegetable origin is the presence of cholesterol in the former and of poorly absorbed plant sterols in the latter. However experiments of the type shown in Figure 3 convinced us that the depressions of serum-lipide levels during feeding of the corn-oil formula were not due to the absence of cholesterol from that formula. Similar experiments have been carried out with a lard formula; the ingestion of cholesterol (0.6 g. per day) with lard produced no higher levels of serum-lipides than lard alone.

### Variations in Fatty-acid Composition

A number of informative tests has been carried out by substituting various natural fats and oils for corn oil in the basic formula. These fats were chosen to obtain wide variations in fatty-acid composition. The chemical characteristics of the fats used are shown in Table I.[5] Except in the cases of butter and coconut oil, all fats fed were composed of fatty acids of similar chain length (predominantly $C_{16}$ to $C_{18}$). To simplify the interpretation of such experiments, oils rich in

[5] We are greatly indebted to Donald H. Wheeler, General Mills Inc., to Roy W. Riemenschneider and Waldo C. Ault, Eastern Utilization Research Branch, U. S. Department of Agriculture, and to Fred Mattson, Procter and Gamble Company, for numerous analyses and for their helpful advice on matters of fat technology.

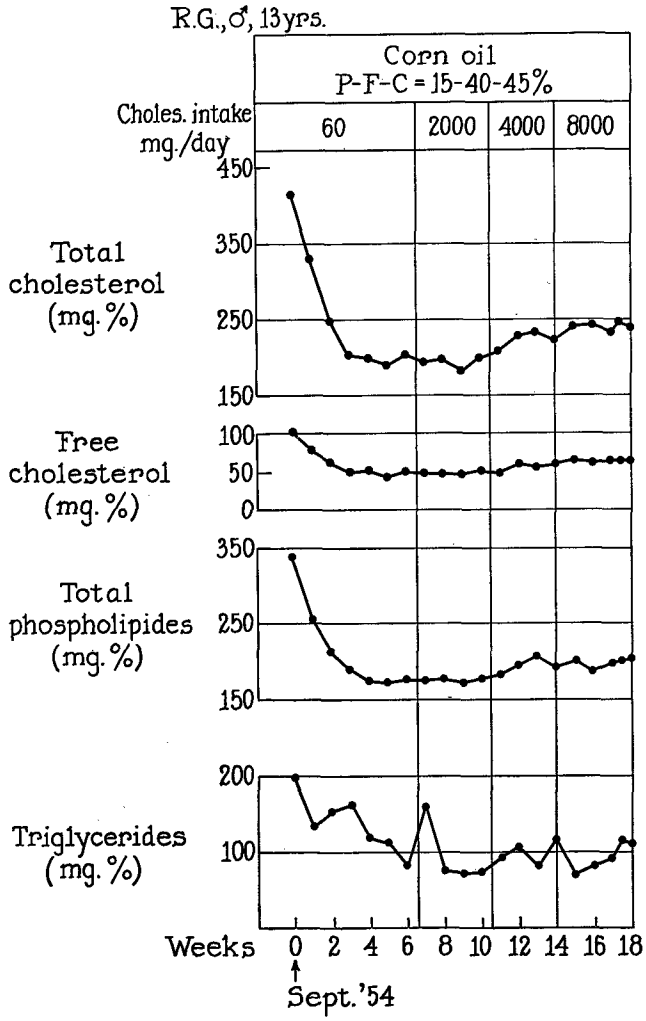


FIG. 3. Effect on serum-lipides of additions of cholesterol to the basic corn-oil formula. Crystalline cholesterol was dissolved in warm corn oil and homogenized in the formula. Increases in cholesterol and phospholipide levels on intakes of 4 and 8 g. per day were significantly higher ($P<0.01$) than on 0.06 and 2 g. per day, but differences were small.

trienes were purposely avoided (soybean, linseed oils).

The levels of serum-lipides produced by the feeding of each of these fats were determined. Calculations were made of the absolute and percentage differences of these levels from the base-line levels reached during the feeding of corn oil. The results were related to a number of characteristics of natural fats—iodine number, linoleic-acid content, and biological activity in rats deficient in essential fatty acids.[6] Absolute differences in lipide levels failed to correlate with any of these parameters. However a roughly linear relationship was obtained by relating percentage differences in cholesterol or phospholipide levels to the iodine values of the fed fats. This analysis is shown in Figure 4. The use of fats with iodine values below 90 produced levels of cholesterol significantly higher than the corn-oil base-line ($P<0.01$) in 23 of 26 tests. However fats with iodine values over 100 all produced equally low levels of cholesterol, suggesting a limit to the effect produced by the ingestion of unsaturated fats. With the feeding of fats with iodine values below 90, the cholesterol content of the serum increased as the saturation of the dietary fat increased. The plot of the phospholipide data showed the same type of correlation with a somewhat smaller dispersion of data. In the case of the triglycerides there was no correlation with iodine value; all percentage differences fell within the ±25% limits of reproducibility noted above in the analysis of the corn-oil refeeding tests.

Statistical analysis showed a significant correlation between P (the percentage decrease of total cholesterol or phospholipide levels with a given dietary fat, relative to the corn-oil base-line) and the total iodine values of the fats fed.

It is a matter of great practical and theoretical interest to learn whether the observed effects of dietary fats on serum-lipides are caused by mean unsaturation or by the content of a specific unsaturated acid, such as linoleic acid, but it is clear that this issue cannot be resolved by tests made with commonly occurring natural fats. The fatty-acid compositions of these fats are so constituted that there is great interdependence between $I_T$, the total iodine value of the dietary fat, and linoleic acids, L. In fact, the coefficient (r) between $I_T$ and L of all fats used in this study, excluding butter and coconut oil, is $0.88 \pm 0.09$ ($P<0.01$). When butter and coconut oil are included, $r = 0.79 \pm 0.12$ $P<0.01$), also a high degree of correlation. If $I_T$ and L are to be separately evaluated, it is obvious that tests must be made with fats synthesized from pure acids. The hypotheses suggested by our calculations can be critically evaluated only when $I_T$ is held constant and L varies maximally, and *vice versa*. It is idle at this time to carry the interpretations of the present data further than to state that the effect on serum-cholesterol and phospholipide levels is strongly correlated with the iodine value of dietary fat.

## Hydrogenated Fats

If unsaturation of fatty acids is responsible for the effects under study, the obliteration of double bonds by hydrogenation would be expected to mitigate the depressing action of these acids. Complete hydrogenation of such long-chain fatty-acid oils as corn or cottonseed produces triglycerides composed of palmitic and stearic acids—fats with high melting-points which are poorly absorbed by the small intestine (9). To insure complete absorption of the fed fat the investigator is forced to use partially hydrogenated oils with melting-points below 50°C. Partial hydrogenation is however not a simple process, and isomers are formed as well as simple hydrogen addition products (16). The nutritional effects of partially hydrogenated oils have received extensive study (9), and in terms of growth, maturation, and reproduction such fats are equivalent to their natural precursors. However in terms of the parameters under study here the effects of these complicated mixtures are unknown. Since *trans* acids do not replace *cis* acids in remedying essential-fatty-acid deficiency (10, 12), it seems probable that the isomers found in partially hydrogenated fats are metabolized through different pathways.

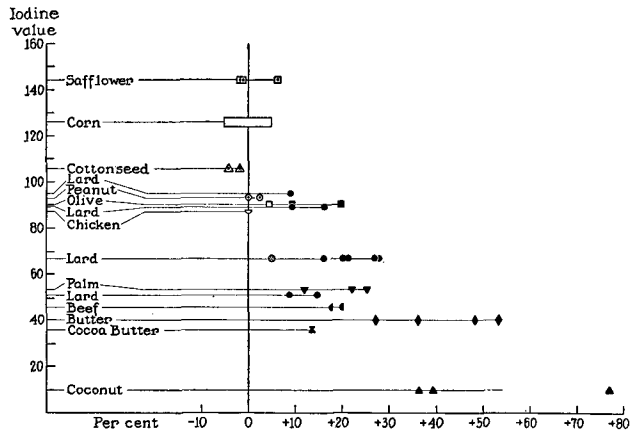


FIG. 4. Relationship between iodine values of dietary fats and serum cholesterol levels, expressed as percentage differences from base-line established during ingestion of corn oil.

Iodine values of fats listed along vertical axis. Open bar at I.V. 126 = estimated reproducibility of corn-oil base-line (fig. 2). Open symbols represent results not significantly different from base-line, solid symbols different at level of $P<0.01$, hatched symbols different at level of $P<0.05$.

Three feeding tests were carried out with partially hydrogenated oils, fed in the basic formula as 40% of calories. The most clear-cut response was shown by patient 20 (Figure 5) in whom the feeding of corn oils, hydrogenated to iodine values of 80 and 58 in a moderately selective process, produced progressively higher levels of cholesterol and phospholipides in the serum. In patient 30 however the ingestion of corn-oil, hydrogenated to an iodine value of 80, produced no higher levels of serum-lipides than the unhydrogenated oil. In patient 18, serum-lipides were equally depressed on unhydrogenated corn and cottonseed oils, but the subsequent feeding of cottonseed oil, hydrogenated to an iodine value of 68 in a selective manner, produced a small but significant rise ($P<0.01$) in cholesterol and phospholipide levels. The compositions of these products are listed in Table I.

It is worth emphasizing that in these three patients the entire fat intake consisted of fats hydrogenated more heavily than in the case of the margarines and shortenings commonly marketed in the United States (16), yet in two of three cases serum-lipide levels were not so high as those found during *ad libitum* feeding.

## Variations in Length of Fatty-acid Chains

The highest lipide levels encountered in our tests were produced by the feeding of butter and coconut oil (Figure 4). While these oils were the most satu-

[6] A number of bio-assays on fats used in these tests were generously performed by H. J. Thomasson, Unilever Research Laboratory, Holland, by the methods described in *Internat. Rev. Vitamin Research*, 1953, *25*, 62.

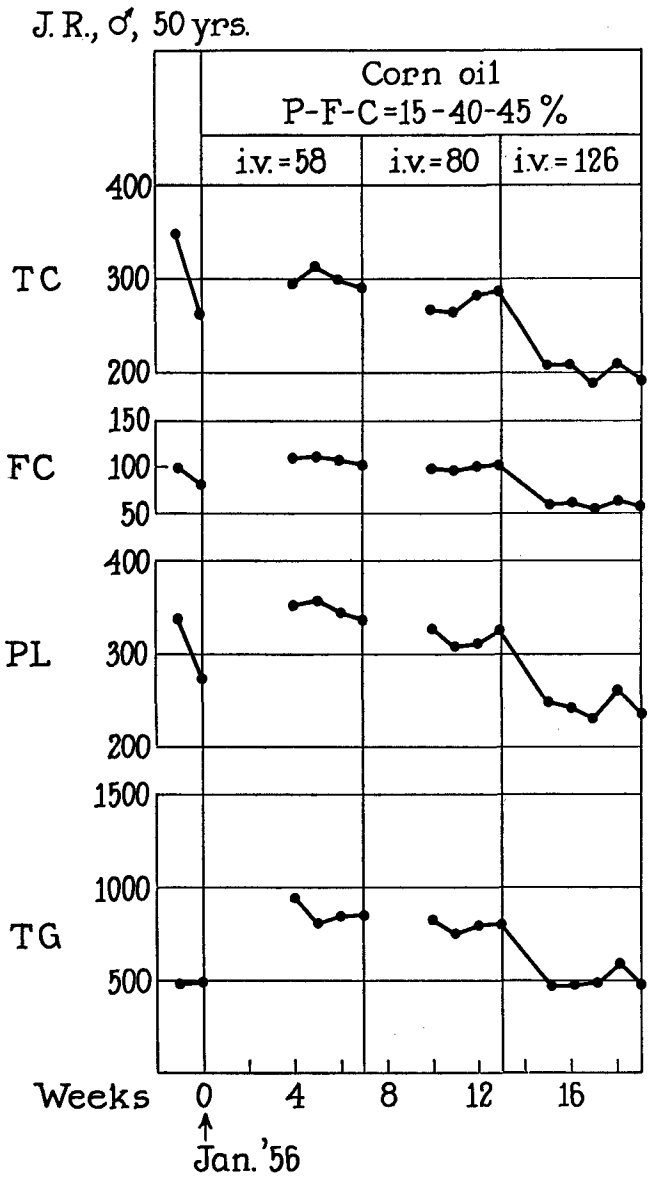


FIG. 5. Effect in patient 20 of ingestion of corn oil hydrogenated to iodine values of 58 and 80, compared to unhydrogenated oil. Levels of lipides significantly different ($P<0.01$) in three test periods.

rated of the dietary fats tested, it must be stressed that they were also the only fats which contained a high content of fatty acids with chain lengths less than $C_{16}$. Twenty-five per cent of butter fatty acids have chain lengths of $C_4$ to $C_{14}$ while 60% of coconut-oil fatty acids have chain lengths of $C_8$ to $C_{14}$. Thus it is possible that the effectiveness of these fats in raising serum-lipide levels is due in part to the low content of unsaturated acids but also to the presence of short-chain acids.

Experiments have been completed in patients 26 and 37 which were designed to test the importance of chain length as a variable. In these tests butter was compared with cocoa-butter. These fats are almost identical in iodine value and in percentage composition of saturated, oleic, and linoleic acids (Table I). However the saturated acids of cocoa-butter are predominantly $C_{16}$ and $C_{18}$ acids whereas 40% of the saturated acids of butter are $C_4$ to $C_{14}$ acids. Serum-cholesterol and phospholipide levels were significantly higher ($P<0.01$) during butter-feeding periods in both patients (Figure 6). The fats were apparently equally well absorbed, as evidenced by the constancy of body weights over the many weeks of these tests when the formulas were isocalorically exchanged.

These results are interpreted as presumptive evidence that fatty acids of short and intermediate chain length cause higher lipide levels in the serum than do the long-chain saturated acids. The short-term feeding tests carried out by Beveridge *et al.* (7) with fractions of butter prepared by molecular distillation seem to confirm this postulate. Their highest cholesterol levels were produced by the fractions of butter containing the shortest and most saturated fatty acids. However in both laboratories' experiments the non-glyceride portions of the test fats also differed. Therefore final proof of this point awaits use of fats synthesized from pure fatty acids. It seems clear that butter and coconut oil are not ideal prototypes of highly saturated fats for our experiments because of inhomogeneity in chain length of their fatty acids.

### Variations in Caloric Mixture

The response of patients to any given dietary fat is influenced not only by the chemical structure of the fat but also by the proportional contribution of fat to the total caloric intake. These relationships are complex (Figure 7). When only 10% of total calories was fed as corn oil, serum-lipide levels were significantly higher in all categories than when 40% or 70% of calories were derived from corn oil. In fact, the lowest serum levels were attained on the highest fat intake. Similar findings were made in three other patients. This may be related to the presence in corn

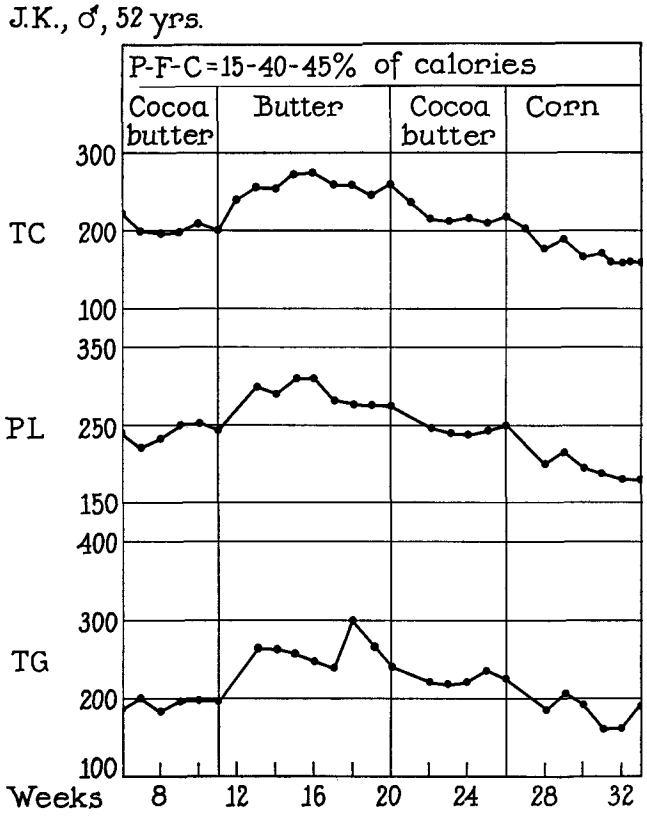


FIG. 6. Feeding tests in patient 37 comparing butter and cocoa butter. These two fats have similar contents of saturated, oleic, and linoleic acids (Table I), but butter is rich in $C_4$-$C_{14}$ fatty acids.

TABLE I

Composition Data on Fats and Oils Used in Formula-Feeding Experiments

Fatty-acid compositions listed as percentages of total fatty acids. Data derived from spectrophotometric analysis after alkali isomerization and from iodine values. *Trans* acids obtained by infrared analysis. Analyses performed as follows: GM = General Mills Inc. (D. H. Wheeler); EURB = Eastern Utilization Research Branch, U. S. Department of Agriculture (R. W. Riemenschneider); DPI = Distillation Products Industries (P. L. Harris); P & G = Procter and Gamble (F. H. Mattson)

| No. | Oil or fat | Description and origin | Analysis by | Iodine value | % Saturated acids | % Oleic | % Linoleic | % n.s. | % *trans* monoethenoid | % *trans* di-ethenoid | % Conjugated diene | Melting-point |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Beef tallow | Oleo oil, Cudahy | GM | 46.8 | 47.6 | 50.1 | 2.3 | .... | .... | .... | .... | .... |
| | | | GM | 47.6 | 45.2 | 49.2 | 2.3 | .... | .... | .... | .... | .... |
| 2 | Butter oil | University of Wisconsin Agricultural College (H. C. Jackson) | GM | 39.5 | 57.8 | 38.3 | 3.9 | .... | .... | .... | .... | .... |
| 3 | Chicken fat | Ocoma Foods Co. | GM | 86.5 | 23.4 | 52.9 | 23.7 | .... | .... | .... | .... | .... |
| 4 | Cocoa-butter | Tower Brand | GM | 36.6 | 60.1 | 37.0 | 2.9 | .... | .... | .... | .... | .... |
| 5 | Coconut oil | E. F. Drew & Co. | GM | 9.7 | 90.7 | 7.3 | 2.0 | .... | .... | .... | .... | .... |
| 6 | Corn oil | C.P., winterized | GM | 126.8 | 8.8 | 35.5 | 55.7 | .... | .... | .... | .... | .... |
| 7 | Corn oil | C.P., hydrogenated | EURB | 79.8 | 18.0 | 48.9 | 6.2 | 1.22 | 21.1 | 4.7 | .... | 34–35°C. |
| 8 | Corn oil | C.P., hydrogenated | EURB | 57.7 | 34.4 | 33.1 | 0.0 | 1.16 | 31.4 | 0.8 | .... | 44–47°C. |
| 9 | Corn oil | C.P., m.d.-distillate | DPI | 123.2 | .... | .... | .... | 1.91 | .... | .... | .... | .... |
| 10 | Corn oil | C.P., m.d.-residue | DPI | 129.0 | .... | .... | .... | 0.32 | .... | .... | .... | .... |
| 11 | Corn oil | E. F. Drew & Co. | GM | 125.9 | 9.0 | 36.1 | 54.9 | 1.2 | 4 | | .... | .... |
| 12 | Corn oil | E. F. Drew & Co., random rearranged | GM | 124.1 | 10.6 | 35.3 | 54.1 | .... | 4 | | .... | .... |
| | | | GM | 119.6 | 12.6 | 36.1 | 51.3 | 0.2 | | | | |
| 13 | Corn oil | E. F. Drew & Co., rearranged, low n.s. | | | 26.7 | 25.4 | 47.5 | 0.42 | 7 | | 10 | .... |
| 14 | Cottonseed oil | Procter & Gamble | P & G | 105.8 | | | | | .... | .... | .... | .... |
| 15 | Cottonseed oil | Procter & Gamble, hydrogenated | P & G | 68.1 | 25.5 | 70.2 | 4.6 | 0.59 | 32 | | .... | 29–36°C. |
| | | | EURB | 67.4 | 26.4 | 69.1 | 4.3 | .... | .... | .... | .... | .... |
| 16 | Lard | Cudahy, prime steam | GM | 70.0 | 33.1 | 52.9 | 13.5 | .... | .... | .... | .... | .... |
| 17 | Lard | Cudahy, m.d.-distillate | P & G | 67.4 | 36.1 | 51.3 | 12.2 | 0.20 | .... | .... | 0.2 | .... |
| 18 | Lard | Cudahy, m.d.-residue | P & G | 68.2 | 33.2 | 55.1 | 8.9 | 0.07 | .... | .... | 2.3 | .... |
| 19 | Lard | R.I. | EURB | 89.4 | 30.3 | 37.8 | 30.5 | 0.12 | .... | .... | .... | .... |
| 20 | Lard | R.I. | EURB | 51.5 | 42.8 | 55.0 | 1.8 | 0.20 | .... | .... | .... | .... |
| 21 | Lard | Procter & Gamble | P & G | 66.5 | 37.7 | 49.4 | 12.3 | 0.28 | 0.7 | | 0.2 | .... |
| 22 | Lard | P & G, random rearranged | P & G | 66.7 | 37.3 | 50.5 | 11.3 | 0.78 | 0.6 | | 0.2 | .... |
| 23 | Lard | P & G, directed interesterification | P & G | 94.5 | 9.5 | 71.3 | 18.0 | 1.8 | 0.9 | | 0.6 | .... |
| 24 | Olive oil | Ehmann Olive Co. | GM | 89.7 | 2.9 | 89.5 | 7.6 | .... | .... | .... | .... | .... |
| 25 | Palm oil | Durkee Famous Foods | EURB | 53.1 | 50.1 | 39.1 | 9.9 | .... | .... | .... | .... | .... |
| | | Borden Co. | GM | 53.6 | 47.3 | 42.9 | 9.8 | .... | .... | .... | .... | .... |
| 26 | Peanut oil | Planters (A. C. Eaton) | GM | 93.0 | 17.7 | 56.5 | 25.8 | .... | .... | .... | .... | .... |
| 27 | Safflower-seed oil | E. F. Drew & Co. | GM | 144.3 | 5.7 | 21.7 | 72.6 | .... | .... | .... | .... | .... |
| 28 | Sesame oil | Welch, Holmes, & Clark | GM | 113.0 | 11.8 | 45.4 | 42.8 | .... | .... | .... | .... | .... |

C.P. = Corn Products. R.I. = Rockefeller Institute. n.s. = non-saponifiable fraction. m.d. = molecular distillation.

oil of factors which depress serum-lipide levels, but clearly the proportions of non-fatty elements in the diet also may play an important modifying rôle. It is important to note that the substitution of carbohydrate for fat calories produced major changes in the serum-triglyceride levels; other lipides increased less strikingly. The regularity and dominance of this triglyceride response in all four patients contrasts with the lack of orderly change in this fraction in experiments where metabolic mixtures were held fixed while fats of varying fatty-acid composition were exchanged.

## Discussion

The results discussed above favor the hypothesis that serum lipides can be lowered by ingestion of highly unsaturated oils. The salient points supporting this contention are as follows:

Feeding tests with dietary fats rectified in various ways suggest that the major influence on serum-lipide levels is exerted by the glycerides, not the non-saponifiable fraction.

The lowest serum-lipide levels were seen when corn oil, safflower-seed oil, or cottonseed oil constituted the sole dietary fats. Higher levels were found with all other fats tested. The differences in these levels are directly related to the degree of saturation of the glyceride fatty acids as measured by the iodine value of the fat.

Use of hydrogenated corn and cottonseed oils as sole dietary fats resulted in higher serum-lipide levels than when the corresponding unhydrogenated oils were fed.

The experiments of Kinsell and Michaels (11), Bronte-Stewart *et al.* (8), and Beveridge *et al.* (5) produced qualitative data which accord with this hypothesis. It is noteworthy that, despite major differences in experimental design, the investigations of four groups led to the same tentative conclusions. Thus the same type of response in serum-cholesterol levels was obtained when unsaturated fats were substituted for saturated fats in the diets of young normocholesteræmic medical students, vitamin- and protein-deficient normocholesteræmic Bantus, and Americans with and without high serum-cholesterol levels and arteriosclerotic heart-disease. Three groups kept caloric intakes and proportions of foodstuffs in the diet unchanged in comparative feeding periods; the South African experiments yielded qualitatively similar results even though these controls were lacking.

In our investigations the number of patients studied was limited by the long duration of the feeding tests, but our design allowed a quantitative comparison of the effects of various dietary fats. Minimum feeding-periods of five weeks for each diet made possible the accumulation of serum-lipide data after steady states were reached, and minimum study-periods of four months provided data from several steady states in each patient. During these periods all the major lipide classes in serum were measured at weekly intervals, affording for the first time a total analysis of the serum-lipide picture. The base-line data obtained during the feeding of the corn-oil formula served as a common denominator when it was desired to compare quantitatively the varying responses of patients to other dietary fats. The use of this common denominator was validated by the finding that patients' lipide levels returned to the same point whenever they ingested this oil. It is probable that any dietary fat may serve the same purpose for in a limited number of refeeding trials other fats and oils have evoked

serum-lipide responses which were reproducibly characteristic of the fat fed.

This quantitative technique has provided evidence which strongly suggests that the effect of dietary fats on serum-cholesterol and phospholipide levels is a function of their iodine value. It cannot be stated at present if this effect is more dependent on mean unsaturation of the fed fat than on its content of a specific unsaturated acid, such as linoleic; this issue remains to be clarified in future experiments. Whether linseed oil (rich in triethenoid $C_{18}$ acids) or fish oils (rich in tetraene, pentaene, and hexaene acids of $C_{20}$ to $C_{22}$ chain length) will produce effects as great as, or greater than, the oils so far tested has not been investigated under our experimental conditions.

Our present line of investigation has aimed at defining the specific dietary factors which cause the rises and falls in serum-lipide levels demonstrated above. An understanding of the mechanisms involved in these changes clearly demands a thorough preliminary exploration of food factors. If it is finally established that the phenomena under study are in fact due to the nature of the fed fatty acids and not to trace components in the oils so far tested, it may be profitable at this point to enumerate some of the questions which will require answers. If unsaturated acids cause lower lipide levels, is this due simply to the number of double bonds per unit weight of methylene groups, as our data suggest at present, or is the effect caused by certain unsaturated fatty acids and not by others? Do the monoenes have half the potency of the dienes? Are trienes more effective than dienes? Are conjugated double bonds as effective as non-conjugated? Do saturated acids, *per se,* cause higher serum-lipide levels, and if so, do all chain lengths have the same effect? What is the effect of ingestion of the fatty acid isomers produced by hydrogenation processes?

Assuming that the net effect of ingestion of a mixture of various fatty acids can be defined at one caloric level, what changes will be produced when the proportions of fat, carbohydrate, and protein are varied? It was stressed that this manipulation of dietary intakes had its major effect on the serum triglycerides, other serum-lipides varied in parallel but to a smaller degree. There have been many proponents of the low-fat diet, but few have measured the triglyceride response to this metabolic mixture. We recognize that high levels of cholesterol and phospholipides on *ad libitum* diets may be reduced on a low-fat regimen, at least temporarily, but in our experience a high intake of unsaturated fat leads to even more striking decreases in these serum components and in addition avoids increases in triglycerides.

Our readiness to accept the unsaturated hypothesis as firmly proved has been tempered by the realization that none of the experiments performed here or elsewhere has ruled out the possibility that the factors sought may lie in the non-glyceride portion of the fed fats. In fact, in a recent abstract Beveridge *et al.* (6) concluded that the "plasma cholesterol depressant effect of corn oil depends to a large extent upon its sterol content." Our tests with molecularly distilled corn oils and with reconstituted corn-oil glycerides containing small amounts of non-saponifiable material suggest that, if a trace factor is involved, it must be active in extremely low concentration. By chemical processes on a pilot-plant scale it was possible to reduce sixfold the content of non-saponifiable material in corn oil. The same order of magnitude of change in intake of non-saponifiable material was also achieved by reducing the intake of whole corn oil from 70% to 10% of calories. In the latter experiment (Figure 7) the depressing effect of corn oil on serum-lipide was lost, yet after a sixfold decrease in non-saponifiable material by chemical treatment, the depressing effect was not lost. Clearly the resolution of this issue awaits completion of feeding tests with glycerides synthesized from pure fatty acids. The great cost of producing sufficient amounts of pure acids has delayed the accomplishment of these critical experiments.

It is often asked whether patients are clinically improved when their serum-lipides are held continuously at a lower level by the regimens discussed in this report. On the whole, our patients have shown considerable subjective improvement while under our care. But we believe that this is primarily due to daily medical attention, to prolonged restriction of

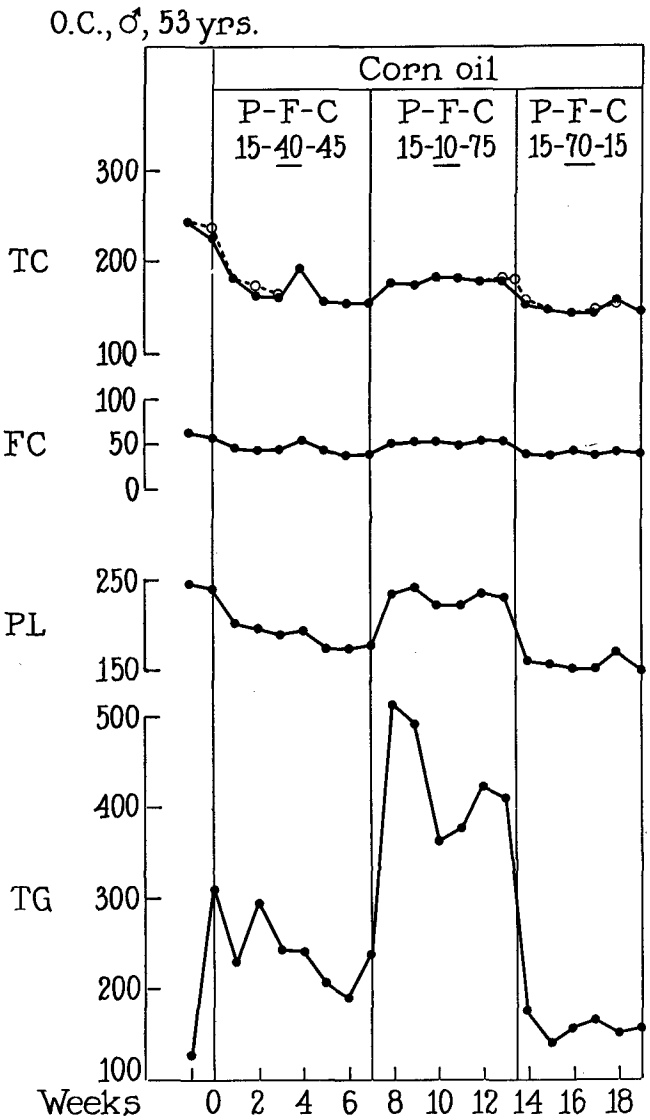


FIG. 7. Effect on serum-lipides (patient 31) of varying the proportions of fat and carbohydrate calories reciprocally, keeping total calories, protein, and body weight constant. Major changes in triglycerides, smaller differences in phospholipides, and least striking effects on cholesterol. Lowest serum-lipides on highest intake of corn oil, highest levels on lowest intake.

physical activity under pleasant hospital circumstances, and to our continual efforts to educate them about their disease. Patients who have gained the most reassurance have unquestionably benefitted the most. Because so many non-specific factors have profoundly affected our patients' clinical status, it is impossible at this time to attribute to our dietary management any specific therapeutic merit. In fact, we consider hazardous any short-term evaluation of a therapeutic regimen in patients with coronary-artery disease. Until it becomes possible to assess the progress of the atheromatous process during life, it will be exceedingly difficult to determine whether a therapeutic measure is effective. It has been gratifying to observe the disappearance of skin xanthomata in patients with hyperlipæmia or hypercholesteræmia, but we have seen no changes in tendon xanthomata despite marked decreases in serum-cholesterol levels as long as two years. It is an open question whether atheromata respond to this regimen as do skin xanthomata, or fail to be appreciably influenced like tendon xanthomata.

It is our present conclusion that recommendations for radical changes in food habits, even by those populations most seriously threatened by atherosclerosis, should await a clearer definition of the specific food factors which control serum-lipide levels. It is entirely possible that an understanding of the mechanisms evoked by these factors will lead to practical measures for control of serum-lipide concentrations. Only then can large-scale epidemiological experiments be planned to determine whether the incidence of atherosclerosis and its complications in the human species will be affected by decreasing the levels of lipides in the serum.

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# Progress in the Metabolism of Lipides[1]

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NOT TOO MANY years ago a review of recent advances in fat metabolism would have presented no particular problem. Today the task is a difficult one. Between 75 and 100 papers on fat metabolism were presented at the Federation of American Societies for Experimental Biology held in Chicago during the week of April 15, 1957. The spectrum of the subject matter of these papers was very wide, yet almost all carried very important implications.

Raymond Reiser

Being faced with this dilemma, I have decided to limit myself to about three areas in which I have been personally interested and which will not duplicate what the others on this program will discuss.

## Digestion and Absorption

Until very recently it was assumed that a glyceride was either completely hydrolyzed during digestion or it was not hydrolyzed at all. However there have been some revolutionary observations in the study of fat digestion in recent years. These new concepts were anticipated as early as 1935 by Artom and Reale (1), who found that *in vitro* pancreatic fat digestion produced no free glycerol but only fatty acids and mono- and diglycerides. This was confirmed independently 10 years later by Frazer and Sammons (2). In the years between 1945 and 1950 Desnuelle and others (3, 4, 5), in a series of studies, reported that the action of pancreatic lipase on fat produced diglycerides readily and monoglycerides only in the presence of calcium ions, also that the production of free glycerol required calcium ions, bile salts, and a large excess of water.

These three groups of authors, in about four or five papers, demonstrated that concepts of all-or-none hypotheses of fat digestion are untenable and that the end product of fat digestion is a partial glyceride; the truth, as usual, lies between the two extremes.

These observations brought up the questions as to which of the three positions of the glyceride molecule are the most resistant and the most susceptible to lipase action and as to whether there is any difference in specificity toward saturated or unsaturated or long- or short-chain acids.

[1] Presented at the Symposium on Fats in Nutrition and Health, Animal meeting, American Oil Chemists' Society, New Orleans, La., April 30, 1957.