was markedly retarded in the liverless rat. When hypercholesteremia was provoked in normal rats by constant infusion of phosphatide, the elevated blood cholesterol concentration was not reflected in any rise of cholesterol level in hepatic or intestinal lymph, or in bile. The elevated phospholipid level was not reflected in bile or intestinal lymph, but did cause a moderate rise in the phospholipid level of hepatic lymph.

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## Effects of Dietary Fat upon Plasma Polyunsaturated Acids.* (23583)

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It has been amply demonstrated that plasma cholesterol concentration can be altered by changes in type of dietary fat(1-4). Dietary cholesterol and physiological stresses which induce hypercholesterolemia accelerate development of essential fatty acid (EFA) deficiency(5,6). It is commonly known that cholesteryl esters and phospholipids of plasma contain highly unsaturated acids. It has been postulated that polyunsaturated acids are required for normal transport of cholesterol and other lipids(7).

To test this hypothesis, a study of the effects of alterations in dietary fat upon polyunsaturated acids of plasma was desirable. The fatty acid composition of human plasma lipids was therefore determined in individuals in whom the effect of dietary fat upon plasma cholesterol was being studied. The difficulty of the analysis for polyunsaturated acids limited the number of individuals to be studied. The results presented here are from 4 individuals from Exp. I and II, and 8 individuals from Exp. III, IV, V, and VI of an investigation published earlier(8). In addition, an experiment was performed on one case of familial hypercholesterolemia in whom the effects of dietary ethyl linoleate were observed.

*Exp. A.* Four normal volunteers were fed experimental diets prepared under supervision of dietitian in the University Hospital in Lund. They consumed sufficient amounts of the diets to maintain their body weights constant. Blood was drawn periodically for analysis of total plasma cholesterol(9) and of plasma polyunsaturated acids(10). Two subjects were changed from their unrestricted diet to one in which 40% of calories was provided by corn oil, but in which no other fat was added. This regime was maintained for 4 weeks, then the diet was replaced for another 4 weeks by one in which butterfat provided 40% of the calories. The other 2

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TABLE I. Exp. A. Effects of Corn Oil and Butterfat upon Polyunsaturated Acids of Plasma.

| Subject | Diet | Unsaturated acids as % of total lipid | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Hexaene | Pentaene | Tetraene | Triene | Diene | Total | Tri/Tetra |
| G.N. | Free | .69 | .79 | 1.78 | .60 | 11.0 | 14.9 | .34 |
| | Corn oil (2 wk) | .21 | .21 | 1.01 | .51 | 13.3 | 15.2 | .50 |
| | (4 " ) | .35 | .42 | 2.72 | 1.31 | 24.0 | 28.8 | .48 |
| | Butterfat (2 " ) | .13 | .30 | .97 | 1.07 | 9.2 | 11.7 | .91 |
| | (4 " ) | .22 | .46 | 1.32 | 1.13 | 10.2 | 13.3 | .86 |
| G.A. | Free | .58 | .87 | 2.43 | .79 | 10.2 | 14.9 | .33 |
| | Corn oil (2 wk) | .25 | .29 | 1.81 | .79 | 10.7 | 13.8 | .44 |
| | (4 " ) | .36 | 1.54 | 1.67 | 1.01 | 15.2 | 19.8 | .61 |
| | Butterfat (2 " ) | .31 | .56 | 1.70 | 1.52 | 10.2 | 14.3 | .89 |
| S.L. | Butterfat (2 wk) | .52 | .54 | 1.31 | 1.22 | 8.2 | 11.8 | .91 |
| | (4 " ) | .51 | 1.05 | 2.20 | 1.15 | 9.9 | 14.8 | .52 |
| | Corn oil (2 " ) | .33 | .25 | 1.25 | .64 | 15.3 | 17.8 | .51 |
| S.H. | Free | .57 | .82 | 2.60 | .87 | | | .33 |
| | Butterfat (4 wk) | .25 | .64 | 1.83 | 1.41 | 9.5 | 13.6 | .77 |
| | Corn oil (2 " ) | .34 | .39 | 1.80 | .98 | 13.2 | 16.7 | .54 |
| | Corn oil + chol. (2 wk) | .25 | .37 | 1.57 | 1.00 | 10.4 | 13.6 | .64 |

subjects received the 2 experimental diets in reverse order.

The analyses for polyunsaturated acids are summarized in Table I. Inasmuch as plasma is a transport vehicle in which total lipid varies, data are presented as concentrations of total lipid. As one might expect, more dienoic acid was found in plasma lipids when corn oil was fed than when butterfat was the dietary fat, for corn oil is about 50% linoleic acid. The trienoic acid concentration in plasma lipids was generally greater at times when butterfat was fed than when corn oil was fed. The tetraenoic acid contents showed no consistent pattern nor did the pentaenoic and hexaenoic acid contents. The principal and most consistent shift in the polyunsaturated acid picture aside from the reflection of dietary linoleate, was that the ratio of trienoic to tetraenoic acids varies with the kind of dietary fat. When corn oil was fed, tetraenoic acid (arachidonic acid) was the dominant non-diene polyunsaturated acid, but when butterfat was fed, the ratio of trienoic acids to tetraenoic acids increased. This is reminiscent of the increased trienoic acid contents of tissues from rats which are EFA-deficient(11).

*Exp. B.* In a more extensive study, effects of hydrogenated coconut oil (HCO) and corn oil upon plasma cholesterol and polyunsaturated acids were observed. In this experiment, the diets contained 40% of calories as either corn oil, hydrogenated coconut oil or a mixture of hydrogenated coconut oil and corn oil, 2:1. In another diet, 50 g corn oil daily supplemented an unrestricted diet. One subject was given 100 g lean meat daily plus 40% of calories as corn oil.

Correlation between dietary change and individual polyunsaturated acid types in plasma could not be easily seen. However, plasma contents of trienoic acid were higher when saturated fat was fed and the reverse was true for tetraenoic acids. A shift in the ratio of trienoic acids to tetraenoic acids parallels the shift in dietary fat (Table II). In this experiment the content of total polyunsaturated acids and cholesterol in plasma lipids also remained remarkably constant despite radical changes in polyunsaturated acid content of dietary fat. The cholesterol to polyunsaturated acid ratios varied from 1:1 to 3:1 with most samples near 2:1. (The ratio in cholesteryl linoleate in 1:38.) These observations may indicate that when saturated fat is a large proportion of the dietary fat transported to and from fat depots, polyunsaturated acids and cholesterol travel with it in approximately constant proportions. If transport of saturated fat is of large magnitude or of long duration, polyunsaturated acids may be released from tissue stores and cholesterol may be synthesized to travel with

TABLE II. Exp. B. Effects of Corn Oil and Hydrogenated Coconut Oil upon Plasma Polyunsaturated Acids.

| Subject | Diet | Chol. Lipid | PUFA Lipid | Triene PUFA | Tetraene PUFA | Tri/Tetra |
|---|---|---|---|---|---|---|
| | | % | | | | |
| G.N. | Free | 24.3 | 8.4 | 6.0 | 9.9 | .60 |
| | HCO (2 wk) | 24.7 | 21.0 | 4.3 | 4.1 | 1.05 |
| | HCO + CO (1 " ) | 24.8 | 15.7 | 5.4 | 8.0 | .67 |
| | " (2 " ) | 25.8 | 18.8 | 4.8 | 7.1 | .34 |
| | " (3 " ) | 25.0 | 17.4 | 6.8 | 7.8 | .87 |
| | Free + CO | 24.3 | 13.7 | 8.1 | 9.3 | .87 |
| E.Lif. | Free | 28.5 | 12.4 | 8.0 | 17.1 | .47 |
| | HCO (2 wk) | 27.8 | 18.8 | 14.7 | 11.3 | 1.30 |
| | CO (1 " ) | 25.0 | 14.9 | 3.8 | 8.6 | .44 |
| | " (2 " ) | 25.6 | 16.8 | 4.6 | 7.8 | .59 |
| | " + meat (1 " ) | 25.0 | 17.4 | 6.9 | 9.1 | .76 |
| | " + free | 29.2 | 14.7 | 6.9 | 7.8 | .88 |
| J.R. | Free | 24.2 | 11.1 | 9.2 | 12.3 | .75 |
| | HCO (2 wk) | 24.0 | 12.9 | 7.0 | 9.3 | .75 |
| | CO (1 " ) | 22.0 | 10.1 | 4.1 | 8.0 | .51 |
| | " (2 " ) | 24.2 | 14.3 | 5.2 | 8.3 | .63 |
| | " + meat (1 " ) | 24.7 | 16.2 | 9.4 | 10.4 | .90 |
| | " + free | 21.8 | 10.3 | 8.5 | 10.5 | .81 |
| A.L. | Free | 28.1 | 11.7 | 9.6 | 13.2 | .72 |
| | HCO (2 wk) | 27.4 | 14.0 | 19.1 | 11.4 | 1.67 |
| | HCO + CO (1 " ) | 21.9 | 14.2 | 6.9 | 9.6 | .72 |
| | " (3 " ) | 22.0 | 13.0 | 7.0 | 8.3 | .84 |
| A.H. | Free | 25.4 | 13.6 | 10.5 | 10.6 | 1.00 |
| | HCO (2 wk) | 26.6 | 15.2 | 13.0 | 9.9 | 1.31 |
| | HCO + CO (1 " ) | 21.9 | 15.0 | 7.3 | 7.2 | 1.00 |
| | " (2 " ) | 26.3 | 20.1 | 7.4 | 7.6 | .97 |
| | " (3 " ) | 23.6 | 15.7 | 7.7 | 7.9 | .97 |
| | Free (2 " ) | 25.2 | 9.4 | 8.9 | 9.4 | .95 |
| R.G. | Free | 22.0 | 7.1 | 8.6 | 12.0 | .72 |
| | HCO (2 wk) | 23.8 | 9.4 | 7.2 | 9.4 | .77 |
| | " (3 " ) | 20.5 | 9.6 | 10.2 | 11.2 | .91 |
| | " (4 " ) | 23.2 | 11.3 | 9.6 | 10.0 | .96 |
| | CO (1 " ) | 22.9 | 20.5 | 10.3 | 12.6 | .82 |
| | Free + CO (1 " ) | 24.1 | 8.7 | 10.9 | 10.1 | 1.08 |
| E.L. | Free | 22.2 | 10.4 | 8.8 | 10.0 | .88 |
| | HCO (2 wk) | 23.9 | 8.1 | 8.1 | 9.5 | .85 |
| | " (3 " ) | 21.6 | 8.8 | 7.8 | 7.7 | 1.01 |
| | " (4 " ) | 24.3 | 11.7 | 6.9 | 10.1 | .69 |
| | CO (1 " ) | 22.1 | 17.1 | 5.8 | 8.1 | .72 |
| | Free + CO (1 " ) | 24.9 | 11.7 | 5.5 | 7.7 | .71 |

PUFA = polyunsaturated fatty acids; HCO = hydrogenated coconut oil; CO = corn oil.

saturated fat and to maintain homeostasis. This concept agrees with the observations that when cholesterol is fed or when animals are rendered hypercholesterolemic by hypothyroidism or diabetes, symptoms of EFA deficiency are induced more rapidly(4). Thus, essential fatty acids (or polyunsaturated fatty acids) may be regarded as necessary for normal transport of saturated fatty acids and/or cholesterol.

*Exp. C.* A female patient aged 44, diagnosed as familial hypercholesterolemia with xanthomatosis and angina pectoris, was studied for the effect of linoleate upon plasma cholesterol level. For some time previous to the experiment she had been on a low-fat diet, and immediately prior to the experiment she was allowed an unrestricted diet for a few days. During the first experimental period she was given a formula diet, each liter of which contained 50 g hydrogenated coconut oil and 100 g skim milk powder containing 0.8% fat. She consumed 1.5 liters of this formula daily, and her diet was supplemented with 100 g glucose, boiled rice (100 g dry weight), one portion fruit juice jelly, coffee

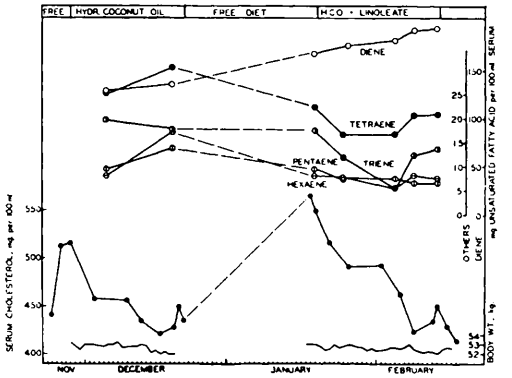


FIG. 1. Response of plasma cholesterol and polyunsaturated acids to dietary hydrogenated coconut oil and ethyl linoleate in a case of familial hypercholesterolemia.

with sugar, and one apple. Between Dec. 20, 1955 and Jan. 16, 1956, she was released and was not subject to dietary restrictions. Upon returning she was again given the formula diet, except that half of the hydrogenated coconut oil was replaced by ethyl linoleate (Jan. 19). On Jan. 30 the formula was altered to contain 25 g hydrogenated coconut oil and 20 g ethyl linoleate/liter. Vit A, $B_1$, C and D and iron were administered in adequate doses throughout the experiment.

The ethyl linoleate was prepared from safflower oil by repeated urea fractionation of the ethyl esters and fractional distillation to separate esters of $C_{18}$ acids. Infrared analysis showed only a trace of *trans* unsaturation and paper chromatography revealed ethyl oleate to be the only significant impurity, approximately 5%.

The changes in plasma cholesterol and polyunsaturated acids are shown in Fig. 1. In the first experimental period, the feeding of hydrogenated coconut oil was accompanied by a decreased plasma cholesterol and by small changes in polyunsaturated acids. In the period in which ethyl linoleate was fed, plasma cholesterol concentration was reduced and dienoic acid was increased. During this time, trienoic acid and tetraenoic acids both exhibited a transitory decrease and pentaenoic and hexaenoic acids remained essentially constant. The transitory change in a polyunsaturated acid following a dietary change has been observed in other experiments, and may represent a biological readjustment. Thus, in the present experiment, the decrease in trienoic and tetraenoic acids may be a substitution of dietary linoleate for circulating polyunsaturates, followed by increased synthesis from accumulated linoleate.

*Discussion.* From these studies it is concluded that measurement of polyunsaturated acids in single samples of plasma does not allow one to assess the nutritive status of an individual with respect to polyunsaturated acids. This is apparent from the considerable variation between individuals and from the different degrees to which each responds to dietary change. Assessment of nutritive status is precluded because one can not determine *a priori* whether polyunsaturated acids are travelling to or from tissue stores.

This study indicates that a diet containing a high proportion of saturated fat causes a shift in polyunsaturated acids of plasma which is somewhat akin to the effect seen in tissues of animals deficient in EFA. It appears that polyunsaturated acids other than diene tend to substitute for linoleic acid when dietary fat contains little of the latter. These may be provided by mobilization of tissue polyunsaturated acids or by an increased synthesis of trienoic acid from non-essential fatty acid precursors. 5,8,11-Eicosatrienoic acid is known to increase in animals deficient in EFA(12). It is structurally related to oleic acid(13) and may therefore be a substituted metabolite produced whenever normal supplies of dietary essential polyunsaturated acids are inadequate.

*Conclusion.* If the increase in trienoic acids may be taken as chemical evidence of EFA deficiency, the results of this preliminary investigation suggest that prolonged ingestion of high levels of saturated fat may lead to a relative deficiency of essential fatty acids.

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## Effect of Urea and Methylamine on Plasmin.* (23584)

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Plasmin, partially purified from human plasma, is unstable in neutral solutions, as demonstrated by rapid loss of proteolytic activity at 37°C. The experiments presented here show the lability to be due to self-digestion of plasmin and describe inhibition of this process by urea and methylamine. This study was undertaken after the report of the preserving effect of urea on trypsin(1), which also undergoes auto-destruction at neutral reaction. The proteolytic activity of trypsin is completely inhibited in 8 M urea, thus accounting for the ability of that concentration of urea to prevent auto-digestion of trypsin(2). The preserving and inhibiting effect is entirely reversible on sufficient dilution, indicating that trypsin does not undergo permanent change upon exposure to urea(1). With plasmin a similar situation can be shown to exist not only with urea but also with methylamine, another highly soluble simple amine.

*Methods and materials.* The caseinolytic assay previously described was used for determination of plasmin and plasminogen(3). The method has been modified from that originally described in that 15% rather than 10% trichloroacetic acid was added for precipitation of undigested casein. Slightly more consistent results are obtained by the use of the higher concentration of acid. *Plasmin.* The inactive precursor, plasminogen, was prepared from human plasma fraction III† by the method of Kline(4), and dried with alcohol and ether as described by Cliffton and Cannamela(5). Several lots of 400 mg were converted to plasmin as follows: The dry powder was dissolved in 30 ml distilled water with the aid of a few drops of N HCl. The volume was brought to 200 ml with 0.05 M borate-saline buffer, pH 7.4, with the formation of a finely divided precipitate. 3.0 mg commercial streptokinase‡ was added and the mixture was incubated at 25°C for 30 minutes with occasional stirring. 200 ml 2 M NaCl was added and the reaction adjusted to pH 2.0 with N HCl. As reported by Troll and Sherry, active plasmin was precipitated by this technic and streptokinase lost(6). The precipitate was separated by centrifugation for 10 minutes at 1,500 rpm and washed twice with 1 M NaCl adjusted to pH 2.0 with N HCl. The precipitate was dried by washing twice with 95% alcohol and 3 times with anhydrous ether. About

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