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Factors Influencing the Requirement for Polyunsaturated Fatty Acids

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THE purpose of this presentation is to discuss several factors or conditions which have an influence upon the requirement of animals for polyunsaturated acids, and which may influence the metabolism of these substances. No attempt is made to be comprehensive, and reference is made only to representative examples of the phenomena mentioned. For a more detailed discussion of essential fatty acid deficiency, and for more complete documentation of the general statements made in this presentation, the reader is referred to recent reviews on the subject.[1,2]

EFFECTS OF THE STRUCTURE OF POLYUNSATURATED ACIDS

The polyunsaturated fatty acids exist in several families, the members of which differ in total unsaturation and chain length but are alike in the position of the double bond nearest the terminal methyl group. Thus the linoleic acid family, known as essential fatty acids, has two or more *cis* double bonds, the first of which occurs at the sixth carbon atom from the methyl group. The oleic acid family has its first double bond at the ninth carbon atom.

Linoleic and related acids allow growth and maintenance of normal skin in rats and other species. The linolenate family allows growth but cannot cure the dermal symptoms of essential fatty acid deficiency. Oleate is synthesized by tissue and is not required in the diet. 5,8,11-Eicosatrienoic acid, of the oleate family, occurs in abnormally large amounts in essential fatty acid-deficient animals. It is thus clear that polyunsaturated acids are not all equal in biological activity. The metabolic function connected with maintenance of normal skin cannot be met by linolenate or oleate-type acids. Linoleate and arachidonate are the most abundant fully effective essential fatty acids. The former is found in plants and animals, the latter only in animal lipids. Arachidonate is more potent as an essential

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fatty acid than is linoleate from which it is derived metabolically. Only the all-*cis* forms of the unsaturated acids are effective as essential fatty acids. Recently many reports have indicated that polyunsaturated fatty acids have a hypocholesterolemic effect. At first this was assumed to be solely an essential fatty acid activity, but it is now known that oils rich in linolenate-type acids and poor in linoleate-type acids have an even stronger hypocholesterolemic effect than does linoleate.[3] Thus it appears that the transport function of polyunsaturated fatty acids can be met by both linoleate- and linolenate-type acids. The efficacy of the individual types of polyunsaturated fatty acids in meeting the requirement for reproduction or other functions has not been tested.

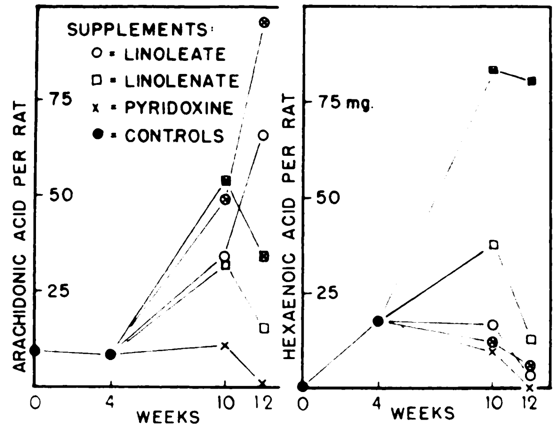

FIG. 1. Effect of pyridoxine upon synthesis of arachidonic and hexaenoic acids in the intact rat. From: WITTEN, P. W. and HOLMAN, R. T. *Arch. Biochem.*, 41: 266, 1952.[8]

THE EFFECT OF SEX

The essential fatty acids are known to be required for normal reproduction in animals. Severely deficient females are unable to conceive when mated with normal males, and deficient males are unable to sire litters with normal females. The reproductive organs of animals contain lipids in which the concentration of polyunsaturated fatty acids is extremely high. A fat-free diet fed to female rats during the gestation period causes the formation of defective young that survive only a few days. The requirement of essential fatty acids has been found to be higher for males than for females.[4] The fundamental cause of this difference is not yet determined.

THE EFFECT OF AGE

The older the animal when first fed a fat-free diet, the longer is the time required to develop essential fatty acid deficiency. In weanling (twenty-one day old) male rats fed a fat-free diet a severe deficiency develops in nine to twelve weeks. Adult rats are resistant to the development of deficiency. In one study it was necessary to reduce the animals to half weight and then feed the fat-free diet *ad libitum* to induce transitory symptoms of deficiency during the period of regrowth.[5] In another study, in adult rats fed an essential fatty acid-free diet *ad libitum* mild and transitory dermal symptoms developed at about thirty-five weeks[6] and stress of dietary cholesterol had little effect on adult rats. However, a stress of dietary cholesterol has an accelerating effect upon essential fatty acid deficiency which is greater in fifteen day old rats than in twenty-one day old rats.[7] The feeding of an essential fatty acid-free diet to pregnant female rats causes the young to be born with definite abnormalities. These several observations indicate that the younger an animal is when given a fat-free diet, the more readily is it depleted of essential fatty acid reserves.

THE EFFECT OF WATER BALANCE

Essential fatty acid deficiency increases the permeability of the skin, decreases capillary resistance, impairs renal function and increases water intake. The excessive evaporation of water from the skin probably contributes to the dry character of the dermatosis. The dermal symptoms of essential fatty acid deficiency can be accentuated by a dry atmosphere and minimized by a humid one. The onset of dermal symptoms can be hastened by restricting the water intake of rats fed a fat-free diet. Whether this really accentuates the deficiency or only the dermal difficulties is not known.

EFFECT OF PYRIDOXINE

Both pyridoxine and essential fatty acid deficiencies induce dermatites which are superficially somewhat similar, and the double

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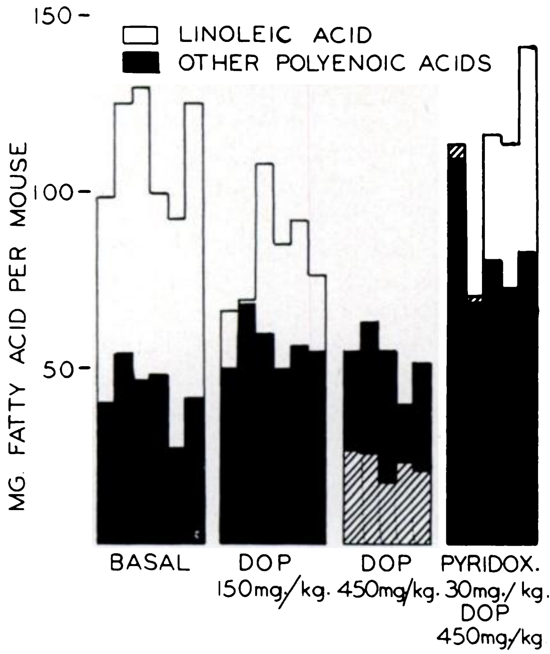

FIG. 2. Effect of desoxypyridoxine (DOP) and pyridoxine supplements upon polyunsaturated acids in intact mice. From: HOLMAN, R. T. Proceedings of the Second International Conference of Biochemical Problems of Lipids.[9]

deficiency of these nutrients induces severe deficiency more rapidly than either singly. Rats deficient in both pyridoxine and essential fatty acid were given supplements of pyridoxine, linoleate, linolenate, pyridoxine plus linoleate and pyridoxine plus linolenate.[8] The total tetraenoic, pentaenoic and hexaenoic acids in the carcass of the several groups of rats were determined by alkaline isomerization. The synthesis of tetraenoic acid from linoleate was found to be enhanced when pyridoxine was fed with linoleate and the synthesis of hexaenoic acid from linolenate was enhanced by pyridoxine (Fig. 1). Thus it appears that this vitamin is involved in the conversion of dietary linoleate and linolenate to the more highly unsaturated longer-chain acids in tissue.

Administration of the antimetabolite, desoxypyridoxine, to mice depressed the deposition of linoleate in their carcasses.[9] Supplementation of mice with pyridoxine as well as desoxypyridoxine restored the total body linoleate and increased the synthesis of polyenoic acids other than linoleate (Fig. 2). Recent preliminary results from our laboratory suggest that even the synthesis of eicosatrienoic acid is impaired when desoxypyridoxine is fed.

EFFECT OF DIABETES

In an attempt to accelerate essential fatty acid deficiency, a condition was sought in which fat catabolism is increased. Alloxan diabetes was induced in rats fed a fat-free diet, and the onset of dermal symptoms took place rapidly,[10] reaching, at one month, a stage of severity found in simple essential fatty acid deficiency only after three to four months. Stress of dietary cholesterol had no significant additional effect upon the deficient rats with diabetes. The diabetic subject is known to have hyperlipemia and hypercholesterolemia and excessive lipid transport may be the basis for the accelerated essential fatty acid deficiency.

THE EFFECT OF HYPOTHYROIDISM

Hypothyroidism is also accompanied by hypercholesterolemia. Therefore this abnormal metabolic condition was induced in rats fed a fat-free diet to determine if it also accelerates essential fatty acid deficiency. Thiouracil fed in a fat-free diet also accelerated essential fatty acid deficiency in weanling male rats.[11] Hypothyroidism increased the requirement for essential fatty acid so much that 1 per cent linoleate no longer fully protected the rats from essential fatty acid deficiency.

EFFECT OF HYPERCHOLESTEROLEMIC AGENTS

To further test the hypothesis that hypercholesterolemia and the attendant fat transport are the cause of accelerated essential fatty acid deficiency in diabetes and hypothyroidism, three hypercholesterolemic substances were tested. Dietary tetramethyl benzidine (0.2 per cent) induced accelerated symptoms of essential fatty acid deficiency in rats fed a fat-free diet without an appreciable hypercholesterolemia. Intraperitoneal injections of a non-ionic detergent, triton, and intraperitoneal injections of an amino nucleoside derived from aureomycin also accelerated the essential fatty acid deficiency. Corn oil offered some protection only against the effects of the latter, which induced a profound hypercholesterol-

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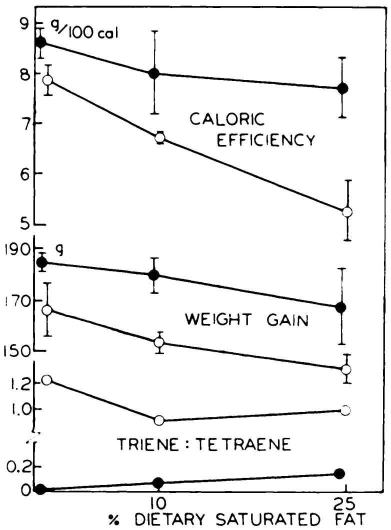

FIG. 3. Effects of dietary saturated fat upon essential fatty acid-deficient rats. From: PEIFER, J. J. and HOLMAN, R. T. *J. Nutrition*, 68: 155, 1959.[12]

emia. Dietary cholic acid, which is known to induce hypercholesterolemia, has been found to accelerate essential fatty acid deficiency as well.

EFFECT OF DIETARY CHOLESTEROL

Dietary cholesterol is known to induce hypercholesterolemia in animals, and the effect of this stress upon essential fatty acid deficiency has been studied in some detail.[7] Cholesterol (1 per cent) fed in a diet free of essential fatty acids hastens the appearance of dermal symptoms of essential fatty acid deficiency and the effect is more regular and severe with fifteen day old male rats than with twenty-one day old weanlings. Linoleate fed with the cholesterol either prevents or cures the deficiency, indicating that dietary cholesterol is no stress if intake of essential fatty acids is adequate. Dermal symptoms of deficiency continue to become more severe even after cholesterol is withdrawn from the diet, indicating that once the accelerating effect of cholesterol had begun depletion of the animal, continued stress of cholesterol is not needed to maintain the deficiency. That feeding cholesterol accentuates essential fatty acid deficiency is evidenced by a lower food efficiency, a lower weight gain, retardation of testicular development and an accentuated pattern of polyunsaturated fatty acids in heart lipids characteristic of essential fatty acid deficiency. The ratio of trienoic to tetraenoic acids in heart lipids, which is normally of the order of <0.4, was 1.8 for rats fed an essential fatty acid-free diet. Dietary cholesterol in a diet free of essential fatty acids raised this ratio to 2.4, indicating a more severe essential fatty acid deficiency. When cholesterol was fed with linoleate, no increase was observed. Thus objective chemical evidence indicated an intensified essential fatty acid deficiency. This indicates that dietary cholesterol increases the requirement for essential fatty acids, but no quantitative evidence on this point is at hand.

EFFECT OF DIETARY SATURATED FAT

The influence of saturated fat upon essential fatty acid metabolism has been recently studied from several aspects in our laboratory.[12] Six groups of weanling male rats were fed two sets of diets. In these diets, essential fatty acid was lacking, and the content of hydrogenated coconut oil (fully saturated fat) was 1, 10 or 25 per cent. In the other three diets, 0.5 per cent ethyl linoleate was incorporated and the content of hydrogenated coconut oil was 0.5, 9.5 or 24.5 per cent. Weight gain and caloric efficiency were determined upon all groups. After seven weeks, the animals were sacrificed, and the polyunsaturated fatty acid content of the heart tissue was determined. The data are summarized in Figure 3.

Caloric efficiency decreased as the content of saturated fat increased in the presence or absence of essential fatty acids in the diet. The caloric efficiency decreased more drastically in the absence of essential fatty acids. Weight gain decreased as the content of saturated fat in the diet increased, whether or not essential fatty acids were fed. The chemical index of essential fatty acid deficiency, trienoic acid :tetraenoic acid ratio, likewise indicated that high levels of saturated fat imposed a metabolic strain. The ratio was high and rather constant in all animals that were not fed essential fatty acids. However, the normally low ratio induced when essential fatty acids were fed rose significantly when higher levels of saturated fat were fed. These three criteria indicate that the require-

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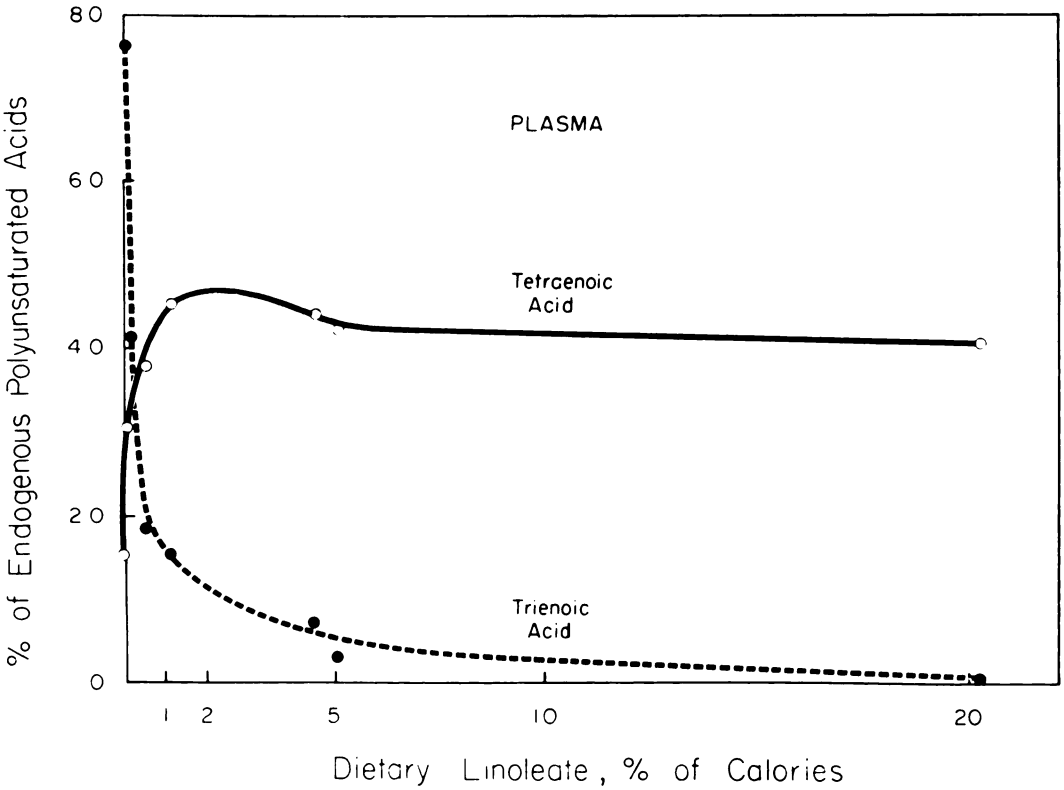

FIG. 4. Relationships between dietary linoleate and the trienoic and tetraenoic acids of plasma of rats.

ment for essential fatty acids is increased when saturated fat is fed at higher levels. That is, the requirement of essential fatty acids is not a fixed amount per day but is proportional to the quantity of non-essential fat in the diet.

In the previous paragraphs, several influences have been shown to increase the requirement for essential fatty acids. These influences have in common their hyperlipemic effect. In some cases the hyperlipemic effect may be only of secondary significance because the major components of plasma lipids usually rise and fall together. The current emphasis on cholesterol content may be a misplaced emphasis dictated by the relative ease with which cholesterol can be measured, and the difficulty of measuring other lipid components. The hyperlipemia induced by the increased transport of saturated fat or cholesterol also involves an increased transport of polyunsaturated fatty acids as components of cholesterol esters, phospholipids and triglycerides. If the polyunsaturated fatty acid is not provided from dietary sources, it must be mobilized from stores and from tissues, for these substances are not synthesized except from required dietary precursors. If mobilization of polyunsaturated fatty acids from tissues is sustained, the tissues will be depleted and deficiency will result. Thus prolonged intake of high levels of fat containing insufficient essential fatty acids, or sustained hyperlipemia from other causes, may produce chronic or marginal essential fatty acid deficiency.

EFFECT OF PROPORTION OF ESSENTIAL FATTY ACID CALORIES

The observation that increasing the dietary saturated fat increases the requirement for essential fatty acids indicates that this requirement is a relative matter. This prompted a practical test designed to evaluate two common dietary fats for their essential fatty acid efficacy at levels of 10 and 40 per cent of calories, corresponding to the approximate proportions in which butterfat and total fat occur in the American diet. Seven groups

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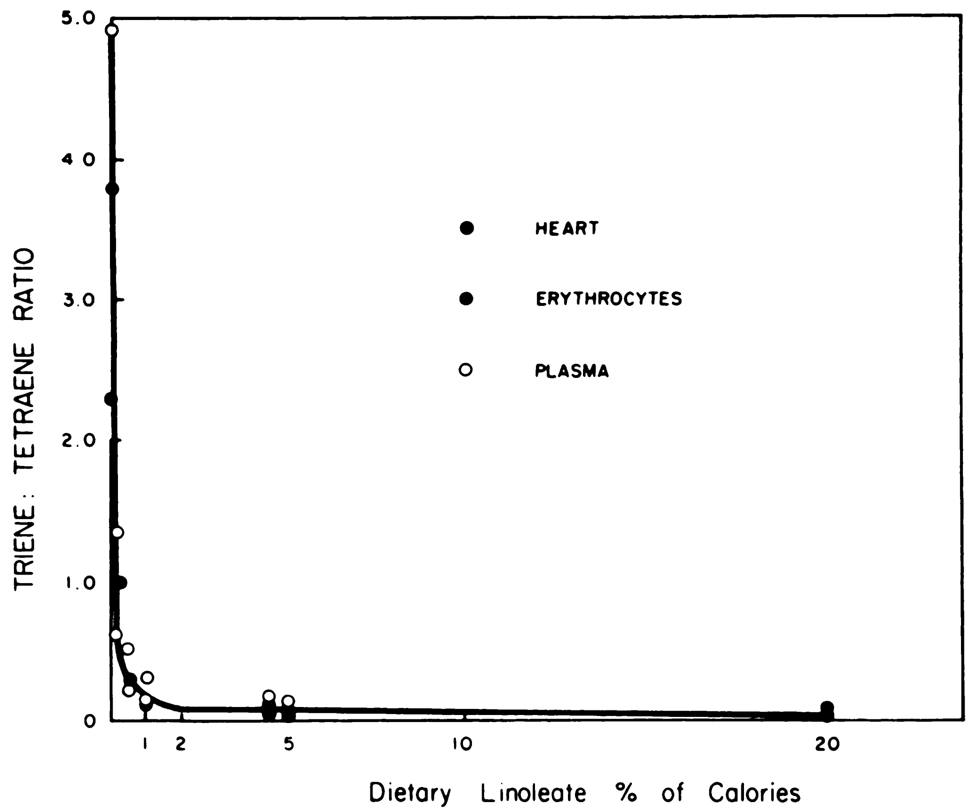

FIG. 5. Relationship between trienoic acid :tetraenoic acid ratios of rat heart, erythrocytes and plasma in dietary linoleate. From: HOLMAN, R. T. *J. Nutrition*, 70:405, 1960.[13]

of male weanling rats were fed synthetic diets containing the following proportions of fat: (1) none, (2) 10 per cent of calories as butterfat, (3) 10 per cent of calories as butterfat:cottonseed oil (4:1), (4) 10 per cent of calories as cottonseed oil, (5) 40 per cent of calories as butterfat, (6) 40 per cent of calories as butterfat:cottonseed oil (4:1), and (7) 40 per cent of calories as cottonseed oil. After eighty-nine days the rats were sacrificed and the polyunsaturated fatty acids were determined in the lipids of plasma, erythrocytes and heart tissue.[13] When the tetraenoic acid content of the endogenous polyunsaturated fatty acid of plasma was plotted against the dietary linoleate expressed as per cent of calories, a curve was obtained having a sharp break near 1 per cent of calories (Fig. 4). As dietary linoleate was increased, the proportion of trienoic acid in the endogenous polyunsaturated fatty acids decreased precipitously and remained relatively constant above 1 per cent of calories. Similar plots made of data from erythrocytes and heart tissue gave essentially the same curves. When the ratio of trienoic acid to tetraenoic acid is plotted against dietary linoleate, a hyperbolic curve is obtained in which an abrupt change in slope occurs near 1 per cent of calories as linoleate (Fig. 5). The curves for data from plasma erythrocytes and heart tissue are superimposable, indicating that the metabolic lesion in essential fatty acid deficiency is reflected in these three tissues and perhaps in all tissues.

In Figure 5, the horizontal leg of the hyperbola represents a normal pattern of polyunsaturated fatty acids and the vertical leg represents an abnormal condition. The point of maximum change in slope represents the minimum requirement for essential fatty acids, 1 per cent of calories as linoleate. This value, being determined by an objective chemical evaluation of changes in composition of tissue, is unaffected by subjective evaluations such as grading dermal symptoms. The evaluation

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TABLE I

Correlation Coefficients Between Dietary Linoleate and Individual Heart Tissue Polyenoic Acids

Dienoic acid	0.88
Trienoic acid	−0.44
Tetraenoic acid	0.70
Pentaenoic acid	0.93
Hexaenoic acid	−0.37

NOTE: At 1 per cent level R = 0.37.

of essential fatty acid requirements by this means is more specific than is measurement of body weight which is a reflection of many influences. The data used for determination of essential fatty acid requirements were derived from fifty-one rats fed seven different diets, involving three levels of dietary fat and three kinds of dietary fat. Even so, the relationship between triene:tetraene ratio and dietary linoleate is unmistakable.

The data obtained from the heart lipids have been treated statistically using a Univac 1103 digital computer, to obtain correlation coefficients between dietary linoleate and the individual heart polyenoic acid types, plus a regression equation relating dietary linoleate to heart polyenes. The correlation between these variables is given in Table I.

These data indicate that of the individual tissue polyenes, pentaenoic acid bears the most direct relationship to dietary linoleate. A plot of pentaenoic acid versus dietary linoleate, given in Figure 6, indicates pentaene to be a reasonable measure of dietary linoleate, but that at low levels of linoleate the function is curvilinear.

The derived regression equation for heart samples relating dietary linoleate to the several variables, and in which the function is more linear, is the following:

$$\begin{aligned}\text{Calc. per cent dietary linoleate} = & -5.086 \\ & + 0.04264\ (\text{dienoic acid}) \\ & + 0.01647\ (\text{trienoic acid}) \\ & - 0.02190\ (\text{tetraenoic acid}) \\ & + 0.07444\ (\text{pentaenoic acid}) \\ & + 0.00602\ (\text{hexaenoic acid})\end{aligned}$$

where values for heart fatty acids are expressed as mg./100 gm.

Values for dietary linoleate calculated from this equation are plotted against the values

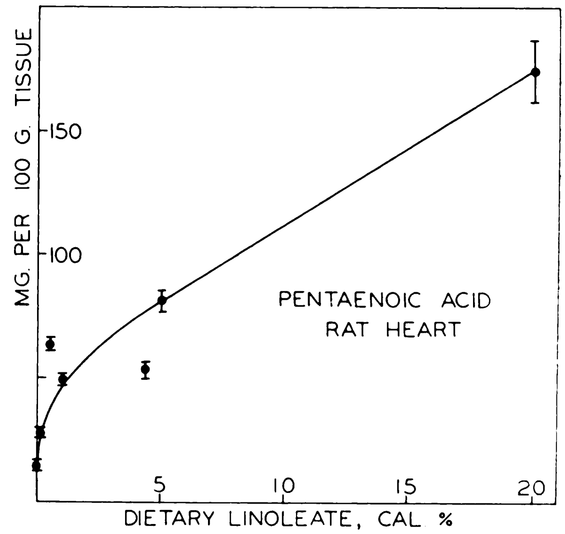

FIG. 6. Relationship between heart pentaenoic acid and dietary linoleate in a population of fifty-one rats fed seven different diets.

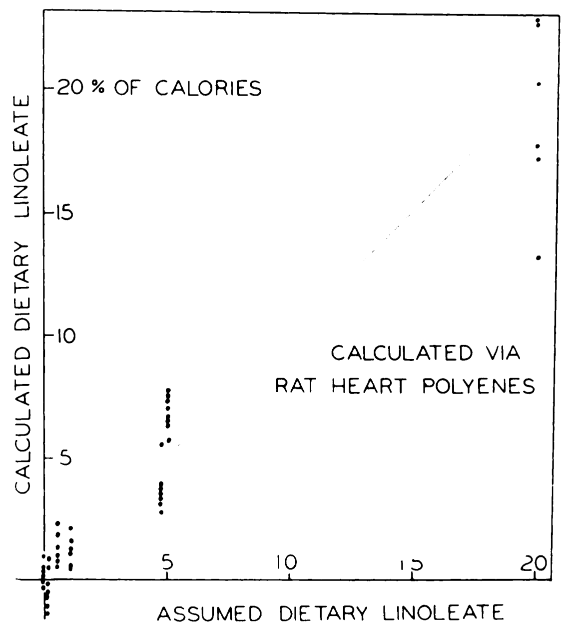

FIG. 7. Comparison of estimated dietary linoleate and values calculated via the regression equation.

predicted from diet composition in Figure 7. The standard deviation between measured and calculated values is 1.89 per cent of calories. This magnitude of error is thus low enough so that the calculation of dietary linoleate from tissue polyunsaturated fatty acids may be used for assessment of the dietary status of essential

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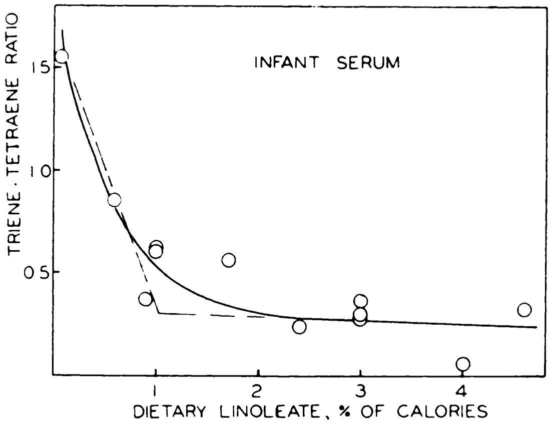

FIG. 8. Relationship between dietary linoleate and serum trienoic acid:tetraenoic acid ratio in infants. Calculated from data of WIESE, H. F., HANSEN, A. E. and ADAMS, D. J. D. *J. Nutrition*, 66:345, 1958.[14]

fatty acids. It should be emphasized that the dietary linoleate was calculated from chemical analyses alone. Other factors which may be included in the calculation to increase the accuracy of prediction are being studied.

This type of determination of essential fatty acid requirement is applicable to human beings. An example of its potential is illustrated in Figure 8 where limited data from the literature have allowed the relating of trienoic acid:tetraenoic acid ratio in infant serum to dietary linoleate.[14] The ratio has been calculated for a series of samples including all the infants who were given diets in which the fat existed as triglyceride. It will be seen that a curve is obtained resembling that obtained from analyses of rats. From the curve the minimum requirement of infants for linoleate is estimated to be 1 per cent, agreeing with the original authors who arrived at this value by a more difficult evaluation of the data. Lack of data for pentaenoic and hexaenoic acids precluded calculation of a predictive equation for dietary essential fatty acids in this case.

A method is now available for an objective measure of the requirement of man or animals for essential fatty acids at any age and in any physiologic condition. From similar data on human subjects, it may also be possible to assess the status of their essential fatty acids and to correct their diets if necessary.

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