PAPERS AND ORIGINALS

Mutants, Hyperlipoproteinaemia, and Coronary Artery Disease*

DONALD S. FREDRICKSON

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This week America was recommended a diet that some may find hard to digest. The occasion was a report of the Inter-society Commission for Heart Disease Resources, a group sponsored by the Federal Government's Regional Medical Program (Atherosclerosis Study Group, 1970). The Commission was originally created to survey the nation's resources for treating circulatory diseases. Quite probably appalled by the rising demand for acute coronary care, it has instead dedicated this one of its several reports to suggestions for eliminating some of the need through preventive action. These are impeccable motives, and the goal will have unanimous support. All the interested parties, however, will not agree with all of the Commission's present recommendations.

On the dietary side the Commission urges three precautions on all Americans from infancy to the grave. The first, to maintain optimal weight, will inspire no opposition, but perhaps will always fail to obtain maximal participation. The second, a requiem for the egg, is to eat less than 300 mg of cholesterol each day. The third is to consume what comes to about 30% of total calories from fat, to be equally divided between saturated, monounsaturated, and polyunsaturated sources. The last two strictures are sure to be controversial.

The Commission offers some praiseworthy suggestions for implementing its advice. These include several directed at the cholesterol-raising elements in cows' milk, shortcomings that this animal doubtless would gladly overcome if she could, but which seem to be locked into the changeless biochemical programme of her rumen.

The Commission advocates upsetting the general use of butter fat content to set the wholesale prices of dairy products, ending the prohibition against adding vegetable fats to meat products, a process now declared "adulteration," and reversing the regulations of some states and of certain federal

institutions and food programmes that insist that butter be the only spread allowed on the table. The dairy cow has powerful sympathizers, some in Congressional quarters, but on the above counts the report merits strong consumer support. It would seem time to allow these aspects of the diet to be regulated by the play of the market-place rather than by old-fashioned protectionism.

American housewives seeking to stay in step with advice about "prudent diets" are also in need of more comprehensive labelling of foods. This is especially so in view of the deplorable but steady trend towards reliance on frozen preassembled meals. The Commission urges the Food and Drug Administration to overcome previous reluctance and to compel manufacturers to stamp foods with their contents of calories, cholesterol, saturates (S), monounsaturates (M), and polyunsaturates (P).

Critics of the labelling proposal can no longer argue that most Americans will never learn the meaning of S, M, and P. Dietary trends in the U.S.A. have already been shifting in the direction of greater unsaturation of national adipose tissue reserves. Stimulated by margarine advertisements, and countless popular references to the "diet controversy," public awareness is high. Probably more Americans are concerned about an increase in their cholesterol than about antiballistic missiles, the popularity of certain political figures, or a host of other preoccupations sampled regularly in public opinion polls.

The report is not to be scorned. The majority of expert opinion is undoubtedly in sympathy with the general, and many of the specific, objectives of the Commission.

There are some things about the report to be pitied, however. In the light of what is actually known, the injunctions on consumption of cholesterol and fats seem too radical as they stand. What evidence do we have that an egg yolk a day spells jeopardy for *all* Americans? Do we have enough information about marginal hyperglyceridaemia or incipient diabetes to advise everyone to eat a diet which will tend to provide more than half of the calories as carbohydrates? What of sucklings and older infants? The Commission's report leads to an inference that a third of their calories from fat should also be polyunsaturated. Are we convinced of the safety of a diet containing 10% of polyunsaturates to the extent that we want to insist on this in baby's formula?

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National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland, U.S.A.

DONALD S. FREDRICKSON, M.D., Director of Intramural Research

Finally, are we certain enough of the efficacy of such sweeping changes that we, as physicians, can convincingly follow them ourselves?

Observing that, despite a mass of suggestive and encouraging evidence, there is no conclusive proof of the preventive value of the dietary change it advocates, the Commission urges the Government to get on with large-scale trials of the effect of its diet on the incidence of ischaemic heart disease. It wants, however, its recommendations concerning diet, and others aimed at widespread treatment of mild hypertension and a phasing out of the cigarette industry, to be implemented at the same time that trials to determine their effects are being planned.

Here the Commission seems to find itself somewhat at cross-purposes. If its recommendations were wholeheartedly adopted tomorrow would a field trial then be possible in the general population? Surely no one could accept seriously the results of a study in which the control group would necessarily consist solely of non-adherers.

It seems to me that the recommenders have thus rejected the best defence for so dramatic a demand on the national palate. They could have pleaded an end of patience and reached a regretful conclusion that the costly and arduous definitive experiment will never come about. For in the event of no further scientific tests of the "dietary hypothesis," the weight of evidence would force many neutral observers to place themselves somewhere behind the Commission report and push it with varying enthusiasm, depending on their individual prejudices.

The hard truth is that, without its conversion to public policy—with the official acceptance of the legitimacy of the diet prescription, the mass education, and the Federal supports to the endangered sectors of the food industry this implies—the momentum of a drive to lower the mean cholesterol enough to lower convincingly the collective incidence of ischaemic heart disease is likely to fail. And one looks vainly in the Commission Report for something new and strong enough to change the present governmental non-position concerning dietary fat consumption.

A Matter of Priorities

The anxieties generated by comparing plasma lipid levels with prevalence or incidence of premature atherosclerosis are genuine and widely felt. It is noteworthy, however, that events in this arena have not always followed in orderly progression. One wonders whether there has not been scattered to the general public advice of a stringency that too far out-distances its validation, and whether more attention should not first be aimed at the most susceptible fractions of the population. One of these is represented by patients with the extremes of hyperlipidaemia, particularly those whose plasma concentrations of cholesterol lie in the upper quartile of the population.

The risk factor data make it clear that the vascular hazard if one has a little hypercholesterolaemia is worse when one has a lot. The translation of hypercholesterolaemia to hyperlipoproteinaemia also leaves no doubt that all hypercholesterolaemia is not the same and will not respond adequately to the same course of treatment.

I admit here to the bias of one long preoccupied with genetically determined hyperlipidaemia, which for practical reasons is today easy to recognize only when cholesterol or triglyceride concentrations exceed roughly the upper 5% limits. To be sure, such subjects probably represent a small proportion of the total affected with premature ischaemic heart disease. In the Framingham Study (1968) data the new "coronary events" observed in the younger population (30-45 years), where hyperlipidaemia has its highest association with premature ischaemic heart disease, were insufficient to support firm predictions. But it appears that men with cholesterol

above the 5% limit (about 290 mg/100 ml) may account for about a quarter of the myocardial infarcts. Even among this fraction there is no present evidence to tell how many of these highly susceptible individuals are likely to have familial hyperlipidaemia.

It is none the less ironic that the dilemma represented in the report of the Commission just referred to has come lately and even more dramatically to the province of severe hyperlipoproteinaemia. The problem in the majority of the "most susceptible" is not to be solved by general dietary advice. Their therapy usually combines diet and drug in a manner tailored to the disorder by further diagnostic exercise. It is now possible to lower lipoprotein levels systematically in many of these subjects. But to do so apparently will require either a religious adherence to diet and the regular ingestion of drugs for the lifetime of the patient or something more radical, like ileal bypass (Buchwald and Varco, 1967).

The confrontation between this new power and the expectations is a distillate of the problem facing the general population. How is it possible to sustain the enthusiasm of patient and physician for a lifetime of diet and medication without more proof of ultimate benefits to be realized many years later? The question has implications beyond the limits of this single medical problem. It may be that patients with severe hyperlipoproteinaemia, in providing the answer needed for themselves, might also offer the proof needed for extension to their less susceptible but still vulnerable fellows in the rest of the population.

Let us examine this possibility more closely, taking as examples two forms of severe hyperlipoproteinaemia apparently due to mutant genes. These are familial type II (hyperbetalipoproteinaemia) and type III ("broad beta disease") (Fredrickson, Levy, and Lees, 1967).

Familial Type II

Familial type II hyperlipoproteinaemia, or the most common kind of familial hyperbetalipoproteinaemia, is an old disease. It has been successively refined and renamed to the present impersonality of a Roman numeral (Fredrickson *et al.*, 1967). Earlier it had been variously known as xanthoma tuberosum multiplex, xanthelasma multiplex, essential hypercholesterolaemic xanthomatosis, xanthoma tendinosum, and essential familial hypercholesterolaemia. Familial hypercholesterolaemia is an acceptable but less precise synonym than familial hyperbetalipoproteinaemia.

Possibly the disease was first described in England (Fox, 1879). I had thought so, at least, some years ago when I made a pilgrimage to Guy's Hospital to view the models of xanthomas fashioned by Towne (Addison and Gull, 1851; Fagge, 1876). Only then did I fully appreciate that all of the lesions so artfully preserved in wax had nothing to do with familial hyperbetalipoproteinaemia. They were related instead either to the remarkable precipitability of British bile in the Victorian period or at least to a frequency and severity of biliary cirrhosis different from that we see today.

The first familial examples of tendon or subcutaneous xanthomas, sometimes associated with sudden death in young people, appeared around 1900 (Arning, 1910). Hypercholesterolaemia did not completely displace xanthomas 'as the more common marker for the disease until the 1940s (Wilkinson, Hand, and Fliegelman, 1948), and lipoproteins did not begin to displace cholesterol as the determinant until mid-1950 (McGinley, Jones, and Gofman, 1952).

LOW-DENSITY LIPOPROTEINS

In our own still-evolving genetic studies we had found it desirable to define familial type II strictly on the basis of an abnormal increase in the plasma concentration of low density

FIG. 1—Type II.

or β -lipoproteins (LDL), with the added provision that the LDL have the normal density for this lipoprotein (Fig. 1). This distinction is critical to the separation of type II from type III. The type II pattern is not specific for a single disorder, however, and we use other major criteria to assign one of the two phenotypes conventionally recognized (Fredrickson and Levy, 1971).

The heterozygote is defined as having (1) the type II pattern and either (2) a first-degree relative also with type II or (3) tendon xanthomas. The homozygote is defined as one who has (1) the type II pattern, and (2) type II in both parents or, as sometimes necessitated by excessive mortality of such relatives, at least evidence of type II in both parental lines, and (3) LDL concentrations that are roughly twice as great as those in heterozygotes in the same kindred.

The LDL concentrations used as the discriminant have been arbitrarily defined as the upper 5% limits established in apparently "normal" subjects (Fredrickson *et al.*, 1967). Cholesterol in LDL is the measurement used, and upper cut-off limits are shown in Table I. The requirement of a quan-

TABLE 1—Low-density Lipoprotein Cholesterol (mg/100 ml) in Normal Subjects and Type II Heterozygotes

Age in Years	LDL Cholesterol		
	Normal		II Heterozygotes
	Mean	Upper 5%	
—19	100	(170)	250 $\pm$ 60
20—29	110	(170)	300 $\pm$ 90
30—39	125	(190)	300 $\pm$ 75
40—49	135	(190)	315 $\pm$ 85
50—59	160	(210)	350 $\pm$ 85

tity not directly measurable in most laboratories has prompted us to test an indirect estimation of LDL concentrations (CLDL) from more readily available values (Friedewald, Levy, and Fredrickson, 1971): the plasma cholesterol (C_{plasma}) and triglyceride (TG) concentrations, and the cholesterol attributed to high-density lipoproteins (CHDL), which can be obtained after a simple precipitation reaction (Burstein and Samaille, 1960). The equation $CLDL = C + \mu - (CHDL + TG/5)$ is valid except under two conditions: (1) in type III hyperlipoproteinaemia in which the contribution of very low density lipoproteins (VLDL) to total plasma cholesterol cannot be estimated from the usual C: TG ratio of 1:5 in normal VLDL; and (2) when the plasma TG is above 400, at which time the precipitation reaction becomes unreliable.

FAMILIAL INVOLVEMENT

Admittedly, by insisting on familial involvement as a requisite to phenotype, it is possible that the affected population we have assembled, now nearly 600 patients in over 200 kindreds, gives an image of enhanced severity to type II. Mean LDL concentrations from a sample of heterozygotes

(Levy and Fredrickson, 1971) may be compared with the normal in Table I.

There is obvious bimodality in the distribution of LDL in a comparison of affected and non-affected relatives from many kindreds (Fredrickson and Levy, 1971). The same is true, with less separation, for plasma cholesterol concentrations. It is evident, however, that no precise cut-off point can be set to divide familial type II from the normal by using either quantity alone. In fact, we have estimated from the data that if one uses cholesterol concentration alone it will be necessary to consider patients with cholesterols above the following concentrations as possibly having familial type II: 220 mg/100 ml at ages less than 30 years, 240 mg between 30 and 40 years, and 250 mg between 40 and 50 years (Fredrickson and Levy, 1971). These are values far below the upper 5% limit for cholesterol, a downward extension mainly necessitated by varying contributions of HDL to total plasma cholesterol.

Perhaps the most troublesome problem in phenotyping type II is the concomitant presence of increased triglyceride or VLDL concentrations. The mean triglyceride concentration in type II is greater than normal at all ages and rises more sharply with age than it does in normals. It is very rarely over 500 mg/100 ml and averages nearly 150 mg. We have informally called the lipoprotein pattern in which LDL and VLDL were increased type II_b (as opposed to II_a in which only LDL was increased), designations accorded "official" status in a forthcoming W.H.O. report on classifying hyperlipidaemia (Beaumont, Carlson, Cooper, and Fredrickson, 1971).

The preservation of both kinds of patterns under the rubric of type II is quite important in present-day genetic concepts of the disorder. Either pattern may appear in different affected members of the same kindred. The VLDL concentration does not yet form a valid marker for different subspecies of familial type II. It remains a potential one, however, and the mechanism of VLDL elevation in so many of these patients is an interesting problem to be solved.

We have found the distribution of lipoprotein patterns in parents, sibs, or children of probands with type II to be disconcertingly close to Mendelian predictions for an autosomal dominant (Fredrickson and Levy, 1971). For example, of 733 such close relatives, 362 had type II, 355 were normal, and 16 had a type IV pattern; the latter is somewhat less than might be expected from the upper limits of triglyceride or VLDL concentrations used to define type IV (Fredrickson *et al.*, 1967). No type III patterns were found in these kindreds. A great deal of previous work by many workers has established that familial hypercholesterolaemia seems to behave as a highly penetrant dominant trait. It seems improbable, though, that only one mutation at a single locus could account for all of these abnormal subjects, considering the quantitative definition used and the certainty of polygenic influences on LDL and cholesterol concentrations.

If we assume that familial type II might be caused by a single mutant allele or similar alleles, it is of great interest to determine the gene frequency. No large, unbiased sample of any population around the world has been studied adequately for this purpose. The most recent information is preliminary and comes from the study of cord bloods.

Kwiterovich, Levy, and Fredrickson (1970), following up other studies indicating that type II may be detectable at birth (Lewis, Brown, and Green, 1967), found that 7 out of 15 offspring having one parent heterozygous for type II had cord blood LDL concentrations more than 2 standard deviations higher than the mean of controls. One apparent false negative and possibly one false positive were observed in later follow-up. As it does later in life, LDL proved a sharper discriminator of type II in cord blood than cholesterol.

Glueck, Heckman, Schonfeld, Steiner, and Pearce (1970) are carrying out a much larger study of cord bloods in a mixed

population of blacks and whites. The parents of children with cord blood >100 mg/100 ml are being sought and tested. The preliminary results, though not yet corrected for all false positives or false negatives that may show up in subsequent months and lacking complete data on all parents, suggest that the frequency of familial type II could be as high as 1:200.

Atherosclerosis

Most patients with type II are at present detected because they or one of their parents have premature ischaemic heart disease. In our experience less than half as many patients, at any age, are detected because they have xanthomas or raised plasma cholesterol (Fredrickson and Levy, 1971).

The most comprehensive data on the prevalence of ischaemic heart disease in familial type II have been collected by Slack (1969) in Britain and by Jensen, Blankenhorn, and Kornerup (1967) in patients in Denmark. The former selected 104 heterozygotes on the basis of hypercholesterolaemia without hyperglyceridaemia. In men the chance of ischaemic heart disease appearing by age 30 was 5%, 51% by age 50, and 85% by age 60. For women the risks were less, but much greater than normal (Slack, 1969). Jensen *et al.* (1967) found that 32% of 181 patients with type II had ischaemic heart disease compared with a prevalence of only 1.3% in their normal relatives. An adequate sample of unaffected blood relatives, matched for age, has not yet been studied to prove that the prevalence of ischaemic heart disease is similar to that in controls in unaffected families, but this is probably the case.

In our large collection of patients and relatives a screening for atherosclerosis is under way but is incomplete. The preliminary results in patients appear to be similar to those mentioned above.

A negative opinion obtained from one large kindred study notwithstanding (Harlan, Graham, and Estes, 1966), the evidence available suggests that the heterozygous phenotype for familial type II is associated with many times the normal hazard of premature ischaemic heart disease. Incomplete analyses to date likewise suggest that the familial factor responsible for this hazard is hyperbetalipoproteinaemia. A review of the pathology of ischaemic heart disease in a few patients with type II also does not show that the plentiful atheromas in the coronary arteries and aorta differ from the common or garden variety (Roberts, Levy, and Fredrickson, 1970).

The more dramatic vascular involvement in type II homozygotes has sometimes led to a conclusion that there must be a tissue component to the disease apart from the hyperbetalipoproteinaemia (Watanabe, Tanaka, and Yanai, 1968). These children usually have both xanthomas and ischaemic heart disease before the age of 10. Probably even in utero they have LDL concentrations more than six times normal. Lacking any good evidence to the contrary, we believe that these patients represent nothing more nor less than the extreme exaggeration of the mild hyperbetalipoproteinaemia in industrialized populations that, on the basis of risk factor analyses, is so closely associated with early atherosclerosis.

Mechanism

The abnormal biochemical mechanism(s) accounting for type II are not established. Studies of the turnover of LDL suggest a reduced rate of removal of the lipoprotein from plasma (Langer, Strober, and Levy, 1969). This presumably catabolic defect could involve primarily LDL itself, its apoprotein, cholesterol, or other components. The apoprotein of LDL ("apoLP-serine") has been the most resistant of all the apolipoproteins to characterization. In our laboratory we have repeatedly failed to find differences in either the lipoprotein

or its apoprotein from type II and normal patients in terms of the lipid and amino-acid composition on the immunochemical or optical properties. There is as yet no evidence therefore of an inherited defect in the chemical nature of the lipoprotein itself.

Success of Treatment

The most significant advance in knowledge of familial type II in the past five years is the demonstration that treatment can lower LDL concentrations dramatically and for at least several years. The first report of success with cholestyramine, the anion exchange resin which binds bile acids in the gut and increases cholesterol degradation, was that of Horan, DiLuzio, and Etteldorf (1964). In heterozygotes we have found this drug to be far more effective than clofibrate (Levy and Fredrickson, 1970). Its effect is additive to that obtained with a diet similar to the one discussed earlier as recommended to the American public. We use, however, a diet containing 15% rather than 10% of calories coming from polyunsaturates.

In a double-blind study of 25 adult heterozygotes, reported earlier (Levy and Fredrickson, 1970), the mean fall in LDL with diet alone was 15%. A further 25 to 35% fall was achieved by adding 24 g of the resin daily. An overall decrease in LDL to about 50 to 60% of pretreatment levels has been sustained in a number of conscientious patients (heterozygotes). Children respond best and their LDL levels can sometimes be brought well below the upper limits of normal.

Proof of Efficacy

The demand is now hard on us to prove the efficacy of what is recommended to such patients. There are also other forms of therapy—different drugs or ileal bypass—that seem to be successful when the yardstick is LDL or cholesterol determinations or the slow disappearance of xanthomas. But what is the effect on the vascular tree? No one knows. Are vascular lesions undergoing the hopeful kind of regression seen in monkeys when a diet converting them to type II has been withdrawn (Armstrong, Warner, and Connor, 1969)? Or could lipoproteins be deserting the blood for the vessel wall? Will the halting of atheromatous growth be enough to decrease mortality from ischaemic heart disease? Perhaps we should reserve vigorous treatment for only the young. If so when do we begin and when do we stop?

It is apparent that one or more careful and well-controlled intervention trials in familial type II are inescapable. Whatever the ultimate figure established for the gene frequency, experience to date indicates that there are tens of thousands of such patients in the U.S.A. alone. Most of them are still undetected and only a relative handful under optimal treatment. If treatment will add years to the life expectancy and long retard the disability of vascular complications, all should have the benefit now accorded a few. If the treatment cannot be shown to achieve these goals its trouble and expense can ill be borne by hope alone.

Of the several ingredients necessary for an intervention trial in these susceptibles, most are available. The decision to begin, however, involves not only careful planning but some soul-searching. The tools for detecting the subjects and classifying them according to lipoprotein disorder are adequate. The means for establishing the presence of ischaemic heart disease are about as good as we shall have for some time. The ability to maintain a large difference in lipoprotein concentrations between control and experimental populations is also present. Here, two principal choices exist. One is to afford neither diet nor drug to the controls and both to the experimental group. The second is to recommend diet to both groups, the drug to an experimental and a placebo to a control

group. The latter has the advantage of permitting double-blind design. Its disadvantage is a somewhat lesser difference between LDL concentrations to be achieved between the two groups. One may still expect, however, to realize a 25 to 30% difference in LDL concentrations by drug alone, and in the light of general opinion, even before the Intersociety Commission report, it will doubtless be easier to have the drug or placebo as the only difference between the two groups.

Randomization of subjects into control and treatment groups is highly desirable; probably it should be considered essential. Ethical objection to this procedure in volunteers cannot rest on the argument that "essential" treatment is being deprived the controls. After all, that is the question being asked.

The most difficult decisions hinge on end points. These always determine the number of subjects judged necessary for the study. Estimates have been made of the number of subjects needed in various dietary studies among men not selected on the basis of their cholesterol levels, and in which the cholesterol (mainly LDL) level in the experimental group will average about 10% lower than that in controls. Where death or the definite appearance of ischaemic heart disease is used as the end point, the estimated number of subjects required over a five-year observation period would be no fewer than 50,000 (National Heart Institute, 1969).

Theoretically, at least, the increased incidence of ischaemic heart disease in familial type II patients might cut any requirement from a fifth to a tenth of that needed in "normal" men. More precise figures will be very useful in this regard, and more exact data of both prevalence and incidence of ischaemic heart disease in type II patients must be collected without delay.

In every real sense morbidity and mortality are the questions that must be answered ultimately. There is a distinct possibility, however, that different information of great usefulness could be obtained in a much smaller group of patients in an observation period of two to three years. The end point would be measurement of progression or regression of coronary artery involvement by angiography. It might be augmented by the quantification of maximal work required to produce angina or electrocardiographic abnormalities or similar noninvasive techniques.

There are no data to allow an accurate prediction of how much alteration in arterial bore can be expected from lowering LDL. Dr. John Brensike, of the National Heart and Lung Institute, has informed me that, after a review of angiograms and discussion with Dr. Mason Sones at the Cleveland Clinic, he has learnt that one should be able to detect a change no greater than 20% in calibre of the coronary arteriograms in repeat studies of the high quality performed by Sones.

The hazard of coronary angiography is the major factor to be weighed in a study that will require at least two such procedures per subject. It will be necessary to decide whether the steadily increasing use of angiography, particularly in the assessment of operability for surgical bypass, now affords sufficient safety and potential benefit to patients who have either clear clinical evidence or great likelihood of ischaemic heart disease to begin such a study.

It should be possible for one large centre to initiate a study involving 200 or more type II patients if angiographic end points are used. Whether an observation period longer than two years will be required cannot be determined except by conducting the experiment.

If mortality and morbidity are to be the end points in a five-year or ten-year study the subjects required will probably number several thousand. Partially in anticipation of the need for such an experiment, a programme will be initiated next year in America that will help identify prospective participants. The National Heart and Lung Institute will support special lipid clinics or laboratories. The goals will include determination of the prevalence of different types of hyperlipoproteinaemia and promotion of research on the

numerous and special problems of diagnosis and management these disorders present to physicians.

It cannot be assumed a priori that familial type II is a perfect model for test of the theory that cholesterol-lowering diets will benefit the general population. Regardless, the question must now be answered for the benefit of type II patients themselves. It is quite likely that they may be the first to tell us whether the effects of a "prudent diet" are as good as is supposed.

Type III Hyperlipoproteinaemia

One other mutant or group of mutants is also of special interest in the problem of determining the effect on vascular disease of treating hyperlipoproteinaemia. This is familial type III hyperlipoproteinaemia. It, too, represents a problem in LDL metabolism, but of a quite different sort from type II. It is much rarer, but easier to treat. It is also associated with premature vascular disease, especially of peripheral vessels, though the frequency of such involvement is not the same in available studies (Borrie, 1969; Fredrickson and Levy, 1971) and needs to be determined in larger, unbiased samples.

It is not so easy to establish a diagnosis of type III, and probably there are errors attending its identification in numerous published case reports. The phenotype rests solely on the lipoprotein pattern (Fig. 2) in the absence of diseases to which hyperlipoproteinaemia might be secondary. For prac-

FIG. 2—Type III.

tical purpose the latter consists almost solely of diabetic acidosis.

The pattern requires demonstration of qualitatively abnormal LDL in the circulation. The definitive test is an ultracentrifuge separation showing that β -migrating lipoproteins (shown to be LDL with an abnormally high content of triglyceride) float at a density <1.006 (Fredrickson *et al.*, 1967). Normally only VLDL or chylomicrons float at this density. In the absence of this test, one has until recently had to fall back on clinical criteria: the presence of variable increases in both cholesterol and glycerides, usually in a ratio of 1:1, or the palmar xanthomas seen in about two-thirds of patients, many of whom have diabetes and claudication.

Dr. R. I. Levy and I have concocted a rule of thumb to assist in the diagnosis of type III. It takes advantage of the fact that in type III the triglycerides are accompanied by a much higher proportion of cholesterol than the 1:5 ratio found in the usual VLDL. As seen in Table II, a number

TABLE II—A Rule of Thumb for Differentiating Type III from Type IV Hyperlipoproteinaemia

Type III (18)		Type IV (189)
C 480		C 260
TG 750		TG 445
325	C—TG/5	170

C and TG represent plasma cholesterol and triglyceride concentrations, respectively, in mg/100 ml. In parentheses after each type are the numbers of patients for whom the values given represent a mean. See text for further explanation.

equivalent to a fifth of the plasma triglyceride concentration is subtracted from the total plasma cholesterol. This leaves a much greater number (usually over 250) in type III patients than in others with hyperglyceridaemia.

This test is crude and imperfect, and it now appears that the simultaneous electrophoresis on polyacrylamide gel (PGE) and on one other medium provides a still simple and better one (Masket, Levy, and Fredrickson, 1969). In type III plasma no lipoproteins having the usual migration of LDL are present on PGE. On paper or agarose electrophoresis of the same plasma, however, β -migrating lipoproteins are present. This combination is rarely seen in plasmas that do not also show abnormally floating LDL (type III) after ultracentrifugation.

In 36 families affected with primary type III that we have seen, vertical transmission has been observed in five (Fredrickson and Levy, 1971). The genetic mode is not established. An incompletely penetrant dominant mode seems somewhat more likely than an autosomal recessive. A more complicated mechanism, such as inheritance of more than one mutant, has not been excluded. The aberrant biochemical mechanism is unknown.

It has been shown (Levy and Fredrickson, 1970) and amply confirmed (Borrie, 1969) that the hyperlipidaemia in type III can be eliminated quickly with treatment consisting primarily of maintenance of ideal weight and clofibrate. Nicotinic acid or D-thyroxine are similarly effective.

One study has been reported indicating that, by plethysmographic measurement, peripheral blood flow improves with a few months of such treatment (Zelis, Mason, Braunwald, and Levy, 1970). The results are at best tentative evidence of the efficacy of treatment of hyperlipoproteinaemia. True relief of claudication and angina would be more ideal end points, but both are notoriously unreliable indices to the effect of a given therapy. Angiography could be used to seek firmer evidence of regression or retardation of the disease. It is noteworthy that in type III the incidence of involvement of leg vessels already seems to be so common that the increased hazard of invasive techniques, using the femoral canal for entry, must be considered.

A study of treatment of type III using mortality and morbidity is possible. The disorder is quite uncommon (we have examined about 85 examples); many hundreds of patients no doubt can be collected in a co-ordinated search for this disorder projected in the national programme alluded to above. As in type II, it seems inevitable that we must prepare for randomization of patients in order to test the efficacy of lipid-lowering treatment. If all the patients with type III in such an experiment were held to ideal weight it is possible that the use of clofibrate in one group and a placebo in another could still afford as much as a 50% difference in cholesterol and glyceride concentrations between the two.

There are several important differences between this disorder and type II that will force a separate comparison and limit the extension of any result in type III to the general problem of atherosclerosis. Firstly, the patients will perhaps all be adults since type III has not yet been seen in a child. Secondly, a large number of patients will probably have obvious vascular disease before entry, tending to make the experiment more one of "secondary prevention." Finally, and most importantly from the standpoint of extrapolating the results to the common problem, it is not certain how comparable is the vascular disorder in type III to the atherosclerosis seen in most ischaemic heart disease.

Thus far I know of necropsy findings in only one case, a female patient of ours who died at 56 years of age of her second myocardial infarct (Roberts *et al.*, 1970). On her endocardial surface were very superficial yellow plaques quite reminiscent of the smeared peanut-butter appearance of the lesions on the skin. In frozen sections such lesions consisted of lipid-filled foam cells beneath the endocardial surface. Such a vascular lesion in type III has the appearance of pos-

sible ready reversibility; however, one hesitates to consider it prototypical of atherosclerosis.

The possibility of extraordinary vascular disease in type III must also be suspected from the presence in plasma of a lipoprotein not usually detectable there. Some of the "floating LDL" characteristic of type III remains even when plasma cholesterol and triglyceride concentrations have been brought to levels far below the normal means.

Simple increase in VLDL or pre- β -lipoproteins forms yet another abnormal state—and probably at least one other mutant gene—that is different from types II and III. Increasing evidence suggests that type IV is associated with premature ischaemic heart disease. As such, it doubtless represents another test of the efficacy of treating hyperlipidaemia that will require a unique solution. In its untreated state type III also is always associated with increased amounts of VLDL that apparently is chemically normal. In this way, and to a greater extent than type II, type III affords some opportunity to gauge the value of treating hyperglyceridaemia rather than hypercholesterolaemia.

Perhaps it is the awareness of the amount of work yet to be done with these special groups of susceptibles. Perhaps it is the familiarity with the difficulties that they, despite their heightened jeopardy for vascular disease, have in following the strictures now recommended to all of the population. Perhaps it is simply an unwarranted insistence on statistical proofs. Whichever it may be, in considering the report of the Intersociety Commission I cannot help but feel a preference for more trial before general commitment to the ordeal.

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