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The genetic component in coronary heart disease – a review

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1. INTRODUCTION

The importance of atherosclerosis in public health has generated a vast literature relating to epidemiology of the disease, clinical descriptions of symptoms and records of variation of serum concentration of cholesterol, triglyceride or lipoprotein fractions in normal or affected persons. Such evidence has led to a number of hypotheses as to the origins of the disease, and although rival theories may be espoused with fervour or may fluctuate in popularity, a familial predisposition to coronary disease is a recurring theme. In this brief review, which does not aim to be exhaustive and is not concerned with hypertension, we shall focus attention on the more important aspects and attempt to set the conflicting evidence in perspective.

By atherosclerosis we refer to the development in the coronary arteries of fibrous plaques which may be already present in the early twenties (Enos, Holmes & Beyer, 1953). As often described, the typical plaque consists of a core of cellular debris accompanied by cholesterol and cholesteryl-esters, enclosed in a layer of smooth muscle cells and connective tissue. Increase in size is chiefly by proliferation of smooth muscle cells and/or platelet adhesion, accompanied by vascularization and calcification of the debris. Such changes result in increasing stenosis of the artery and/or thrombotic occlusion with consequent risk of myocardial infarction. An alternative possibility is sudden cardiac death for which the immediate causes remain obscure.

Similar events in other arteries, e.g. cerebral, iliac, femoral, abdominal, etc., result in such clinical conditions as stroke, gangrene, aneurism, etc. Hypotheses about the origin of coronary heart disease (CHD) have focused attention on different aspects of the primary lesion. The more important concepts may be summarized as follows:

(i) The most widely held view is that the entry and retention of plasma lipids, especially cholesterol, either by endocytosis or more specific routes, stimulate smooth muscle cell multiplication and hence plaque formation.

(ii) An alternative view (Ross & Glomset, 1976) regards the stimulation of smooth muscle cell proliferation as a consequence of endothelial injury by abrasion, excess serum lipid concentration, immune reactions etc. Intimal connective tissue exposed by injured epithelium allows platelet adhesion which in turn gives

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rise to various compounds which stimulate smooth muscle cells to divide and hence lead to plaque formation. On this hypothesis platelets carry a special responsibility in the origin of plaques.

(iii) A rather different approach was proposed by Benditt & Benditt (1973), who provided evidence that plaques may be of monoclonal origin by an ingenious demonstration of apparently non-random inactivation of the *X* chromosome in comparisons between cell samples from plaques and adjacent non-plaque tissue in women heterozygous for electrophoretically distinct forms of glucose-6-phosphate dehydrogenase. Needless to say there is a rival view that the apparent monoclonal condition is a secondary consequence of selection in an initially polyclonal cell population. On the Benditt hypothesis, plaques represent a species of 'benign neoplasm' thus inviting speculation about what might induce individual smooth muscle cells to undergo a cycle of proliferation.

At present there seems little prospect of a clear verdict in favour of one or other of these hypotheses; indeed it may turn out that the prime causes are heterogeneous. Quantitative estimation of differences between individuals in plaque development is perhaps the single most important piece of missing information we need for assessing coronary risk in populations and groups of relatives. Although an elevated serum lipid level, especially of cholesterol, has long been regarded as a risk factor in atherosclerosis, a substantial proportion of persons who suffer from myocardial infarction display serum concentrations of triglyceride and cholesterol similar to those presented by healthy members of the community.

Although a great deal of the earlier biochemical evidence refers to concentrations of total serum cholesterol and/or triglyceride in relation to the risk of CHD, it is more informative to record the concentration of the alternative serum lipoprotein fractions which refer to the array of molecules made up of characteristic ratios of cholesterol and its esters, triglyceride, phospholipid and protein. Differences in the relative amounts of these constituents confer differences in molecule size and density and the latter attribute provides the basis for separating them into more or less functionally distinct categories by preparative density gradient centrifugation. Table 1 summarizes the main features of the populations of molecules falling within the accepted density limits. The fractions are referred to, in order of increasing density, as very low (VLDL), intermediate (IDL), low (LDL) and high (HDL) density lipoproteins. VLDL is the chief vehicle of triglyceride and LDL of cholesterol.

The lipoproteins are further distinguished by the particular types of protein or Apoprotein present, as noted in the Table. Apo-B is the major protein constituent of LDL. In HDL the major constituents are Apo-AI and Apo-AII, while the minor constituents include Apo-E, Apo-D and also Apo-CI, CII, and CIII which exchange with VLDL in which the C-apoproteins are major constituents along with Apo-B and Apo-E. The lipoproteins are dynamically inter-related although the pathways have been only partially resolved. It is established that an important route of metabolism is from VLDL via IDL to LDL by reactions probably located in the liver. The metabolism of HDL is still obscure.

It is hardly surprising that modification of rates of conversion and other reactions by either genetic or environmental means should lead to unusually elevated concentrations of one or other lipoprotein fraction, referred to as hyperlipidaemia, and the recognition of characteristic profiles of serum lipoprotein composition has provided a useful analytical approach to different biochemical situations associated with CHD. Some years ago Fredrickson, Levy & Lees (1967) established a series of so-called types of hyperlipidaemia: I, IIa, IIb, III, IV, V according to the particular lipoprotein fraction(s) elevated in the serum. However, experience has demonstrated that such types do not correspond to biological entities (Hazzard *et al.* 1973); several may occur in the same sibship and even the same individual may be classified differently on separate occasions.

Table 1. *Properties of plasma lipoprotein in man*

Major fraction	Density range	Percent average composition				Major apoproteins
		Protein	Cholesterol	Triglyceride	Phospholipid	
Chylomicrons	<0·95	2	7	84	7	B C-I, C-II C-III, A-I
VLDL	0·95–1·006	8	20	51	19	B C-I, C-II, C-III, E
IDL+LDL	1·006–1·063	21	45	11	22	B
HDL	1·063–1·21	50	22	4	24	A-I, A-II

Note: The density range of IDL is 1·006–1·02. The information is drawn from the paper by Kwiterovich *et al.* (1979).

An additional case for separate estimation of lipoprotein concentrations has appeared more recently. Whereas abnormally elevated concentrations of LDL and/or VLDL are potentially unwelcome, the converse is true for elevated levels of HDL (Miller & Miller, 1975; Gordon *et al.* 1977). It is widely believed that coronary risk is inversely related to HDL serum concentration, and clinicians now refer to the 'protective' role of HDL in atherosclerosis, stroke and allied conditions. Since HDL accounts for about a quarter of it, attention merely to total serum cholesterol concentration is no longer adequate.

The potential scope for genetic intervention in the heterogeneous processes which affect the development of atherosclerosis is obviously considerable. Claims for genetic or familial effects are of several kinds, especially:

(i) Familial evidence of risk of CHD without reference to cause.

(ii) Hypotheses of monogenic effects associated with alternative combinations of abnormally high lipid values which show clustering in families.

(iii) Correlation between risk of CHD and the serum concentration of par-

ticular lipoprotein fractions whose variation is suspected to be partly polygenic in origin.

Before considering the evidence one general qualification is worth noting. Communities living in different parts of the world differ greatly in the incidence of CHD, which is highest in affluent populations with a western life style. On the other hand, aboriginal populations, e.g. in Malaysia (Burns-Cox, Chong & Gilman, 1972), or in Africa (Tobias, 1966) are not or only rarely subject to CHD and have low serum concentrations of cholesterol but not necessarily of triglyceride. Urbanization of such people is associated with increased risk of CHD. Thus non-genetic causes compounded of differences of diet, levels of activity, life style, etc., are prime determinants of such major differences in the occurrence of atherosclerosis. Even in a small country like Britain significant regional differences in risk of CHD occur between the relatively low values of south east England compared with the high values of the west of Scotland, where the incidence of CHD exceeds that of the east coast of Scotland. The reason for such differences is unknown and although environmental circumstances doubtless play a major role it would be premature to exclude some effect of gene frequency differences.

Possibly relevant in this context is the report of comparisons by Vlodaver, Kahn & Neufeld (1969) of the coronary artery in early life, i.e. under 10 years of age, in three different ethnic groups in Israel, i.e. Ashkenazis, Yemenites and Bedouins. The intima and muscular-elastic layers were more developed in Ashkenazi males than in males of the other groups or Ashkenazi females. Such a sex difference was not apparent in Yemenites and held for only one age group of Bedouins. These distinctions, for which the authors favoured a genetic origin, are correlated with the differences between groups in the occurrence of CHD. Also Pesonen, Norio & Sarua (1975), from autopsies on infants under one year of age in Finland, claimed that the vascular layer of the coronary artery was thicker in babies whose grandparents came from the eastern part of the country than in those whose ancestors came from the west, where the mortality from CHD is lower than in the east. Although there is the usual environmental caveat such contrasts could be related to ethnic differences between two groups of settlers who originally colonized the different regions and were responsible for the present dialect differences.

Apart from the well defined genetic forms of CHD like familial hypercholesterolaemia, risks associated with a genetic predisposition to higher serum concentrations of LDL or VLDL will depend on the prevailing environment which determines the average serum concentration of a given population. In less affluent communities the risk associated with such variation of polygenic origin may be nil, and this may have been true of the comparatively recent ancestors of contemporary populations with a high CHD risk. Given such gene-environment interaction, a changing environment creates problems in estimating a genetic component, as in the United States or some western countries, where, quite recently there has been a decline in the incidence of coronary heart disease, apparently not confined to particular social categories. As in many other aspects

of human variation the path to estimation of genetic effects in CHD is strewn with minefields of uncertainty and beset with many false turnings due to spurious correlations.

2. GENETIC EVIDENCE

(i) *Familial incidence*

This refers to estimates of the risk of coronary disease in first degree relatives of male or female patients with CHD. Slack & Evans (1966) reported for a London population a five-fold greater risk for early coronary death in first degree male relatives of male index patients under age 55 with less risk ($2\frac{1}{2}$ times) to the corresponding female relatives. For first degree relatives of either sex of female index patients under 65 the increase was nearly seven-fold. The estimated risk for first degree relatives will be influenced by the choice of age and sex of index patient, since the earlier the age of attack the greater the influence of predisposing factors while the frequency of the disease is much lower in women than men. Rissanen & Nikkila (1977), in studies on a Finnish population, noted at least a five-fold greater risk of death from coronary disease before age 65 for fathers of index males under 56 years of age and five and a half times greater risk for brothers of index males than for brothers of controls. The risk for sisters of index patients was two and a half times greater than for sisters of controls.

Thordarson & Fridriksson (1979) compared first and second degree relatives of persons in Iceland who had experienced myocardial infarction before age 61 in males and before 70 in women. The risk of death due to ischaemic heart disease was about four-fold higher in first degree relatives of male index patients and over seven-fold higher for fathers and brothers. Mothers and sisters of either sex showed a four to five-fold increase in risk.

Nora *et al.* (1980) made a case for increased recognition of the genetic contribution to ischaemic heart disease. They studied 207 patients who had a myocardial infarction before age 55 years and 621 matched controls, and assessed the risk of ischaemic heart disease associated with nineteen variables commonly believed to be associated with the disease, e.g. family history, elevated serum cholesterol or triglyceride concentration, smoking, exercise, blood pressure, etc., and of these a positive family history was clearly pre-eminent. Heritability for the disease on the entire data worked out at 63%.

Such evidence of familial association is compatible with several twin studies, e.g. Harvald & Hauge (1970) which show a higher degree of concordance in CHD among like-sexed monozygous than dizygous twins. Although there is the usual reservation about how far such correlations between relatives are genetic or environmental in origin, the authors have favoured a genetic contribution. Following Falconer's (1965) procedure for treating the incidence of such diseases as a threshold character, Slack (1974) estimated heritability for total liability to coronary disease in men under age 55 as 60% and in women under 65 as nearly 70%, while Rissanen & Nikkila (1977) estimated an even higher value of 80% in men. Thordarson & Fridriksson (1979) arrived at lower estimates of respectively

20 % in males and 30 to 35 % in females. The very high estimated values probably include an environmental component or perhaps bias arising from less than perfect applicability of the model.

(ii) *Monogenic effects*

The evidence here refers to the occurrence, in particular families, of relatives who display a particular kind of unusual lipid profile. The serum cholesterol concentration may be very high due to elevated LDL, or serum triglyceride may be raised due to increased concentration of VLDL, or relatives may occur with increased concentrations of both lipoprotein fractions. Clinical evidence, e.g. associated presence or absence of xanthomata, localized lipid deposits in the skin, and greater risk of CHD has led to hypotheses of segregation at a single locus to account for such variation. Since lipoprotein scores are continuously distributed it is arbitrary as to what is considered an abnormally high value. It has become conventional to take concentrations at or above the 95 or sometimes the 90 percentile of a single tailed distribution as a criterion to classify serum records as normal or abnormal and thereby generate an apparently bimodal distribution. In familial hypercholesterolaemia, in which the LDL concentration is grossly elevated, the case for such a simple interpretation is supported by convincing biochemical evidence, whereas in other kinds of lipid abnormality this is not so and the simple interpretation may be criticized.

Familial hypercholesterolaemia has long been recognized as simply inherited. In the very rare homozygotes the serum concentration of cholesterol is extraordinarly high, there is extensive occurrence of xanthomata and early coronary death. Heterozygotes also manifest higher levels of serum cholesterol, variable manifestation of xanthomata and increased risk of coronary disease. The origin of this type of lipoprotein abnormality has been elucidated in an impressive series of studies, e.g. Goldstein & Brown (1973), Brown & Goldstein (1976), who used cultured skin fibroblasts to show that homozygosity of a particular gene prevents the development of cell membrane receptors. Normally these bind LDL molecules which are then internalized and degraded, thereby releasing cholesterol to inhibit 3-hydroxy-3-methylglutaryl Co-enzyme A reductase (HMG CoA reductase), the rate limiting enzyme of cholesterol synthesis. Heterozygotes are roughly midway between the alternative homozygotes in degree of binding, inhibition of HMG CoA reductase, etc. The abnormal elevation of serum LDL appears to be due to breakdown of catabolism via this receptor route, and although, given the very low concentrations required to inhibit HMG CoA reductase compared with serum concentrations, it might seem difficult to accept an *in vivo* role for the reaction, the LDL concentrations are probably low enough at the sites of action for the feed-back mechanism to be effective.

Within the general framework of receptor mediated binding and internalization of LDL there is clear evidence of genetic heterogeneity in which either binding is nil (receptor-negative) or there is a low level of binding and internalization (receptor-defective), or LDL molecules may bind normally to membrane receptors but are not internalized. In the latter case the electron microscope reveals a

spatial difference in the distribution of receptors on membranes since they do not occur in coated pits as in normal cells (Goldstein, Anderson & Brown, 1979). Such heterogeneity in the primary reactions invites speculation about receptor structure and function.

These differences do not exhaust the evidence for genetic heterogeneity in familial hypercholesterolaemia. Breslow *et al.* (1975) classified homozygous familial hypercholesterolaemic individuals into two categories who either do or do not respond to diet and drug therapy. HMG CoA reductase activity was not inhibited by the presence of LDL in fibroblasts from the latter but was reduced to 41 % of the control, LDL-free activity in the former. Although specific binding of LDL to fibroblasts was substantially reduced in both categories it appeared lower in the treatment-resistant patients. Prevention of LDL degradation and hence hypercholesterolaemia may also arise by alterations in the LDL molecule which suppress binding to apparently normal receptors, demonstrated in a father and daughter belonging to a family with a history of hypercholesterolaemia (Higgins, Lecamwasam & Galton, 1975). More systematic study of variation between individuals in the binding capacity of LDL molecules is called for; it could turn up a few surprises. Evidently there is still some way to go before the genetic heterogeneity of familial hypercholesterolaemia is resolved between allelic and/or non-allelic differences.

In the studies of Goldstein *et al.* (1973*a*, *b*), who have made the most positive claim for monogenic segregation in a study of 500 survivors of myocardial infarction and their relatives, families were classified according to putative segregating lipoprotein phenotype, i.e. elevated serum cholesterol alone, elevated tryglyceride alone or elevation of both, if at least one relative, as well as the index survivor, exceeded the relevant 99th percentile if of age 20 or over, or the 95th percentile if of younger age. For both hypercholesterolaemia and hypertryglyceridaemia this procedure led to the appearance of bimodality which was interpreted as evidence of single gene segregation in the families concerned.

For hypercholesterolaemia, the subsequently discovered biochemical correlations, derived from the study of fibroblasts from obligate heterozygotes, supports a monogenic interpretation. But until putatively heterozygous individuals in affected families are identified by the appropriate biochemical tests there must be doubt as to how far the raised serum cholesterol concentrations are due to a common origin as distinct from polygenic determination.

The same authors made a parallel case for monogenic origin of hypertriglyceridaemia, while Murphy & Kwiterovich (1977) and Fredrickson, Goldstein & Brown (1978) have also provided evidence of bimodal distribution for high triglyceride levels. Although the association between CHD and elevated cholesterol levels is well established, the relationship with hypertriglyceridaemia is less well defined and reports vary in this respect, possibly due to genetic heterogeneity as well as the extent to which risk depends on interaction between elevated VLDL and other unidentified variables, including the serum concentrations of other lipoprotein fractions (Kwiterovich, Bachorik & Chatterjee, 1979).

Goldstein *et al.* (1973*a*, *b*) also made a claim for simple inheritance of another

condition – so called 'combined hyperlipidaemia', typically defined by abnormally elevated levels of both cholesterol and triglyceride. However, the hypothesis of monogenic determination required the assumption of variable gene expression to allow for elevation of either only one or other of the lipids or various intermediate states and this makes the case for simple inheritance unconvincing. Glueck *et al.* (1973) also reported families in which the index cases showed elevated levels of both cholesterol and triglyceride, with variable concentrations in first degree relatives.

Elston *et al.* (1975) from a study of a large pedigree of 195 persons, found clear evidence of monogenic segregation for high serum cholesterol values and also found apparent bimodality of triglyceride levels, independent of the cholesterol concentrations and of uncertain status. Glueck *et al.* (1975) have also described the clustering of abnormally high values of HDL cholesterol in a number of families, but although the ratio of normal to high HDL values suggested monogenic segregation, bimodality was not evident and the authors preferred an environmental origin of the individual differences in sib resemblance.

Thus, apart from hypercholesterolaemia, especially that associated with xanthomatosis (Heiberg & Berg, 1976), we have to suspend judgement as to the true interpretation of familial clustering, pending further biochemical evidence. Lack of a biochemical marker creates a problem in accepting the appearance of segregation in lipoprotein profiles at their face value and prevents us from knowing whether lipoprotein profiles in other kindreds which resemble or overlap those in the reference series have a similar origin. It is often difficult, if not impossible, to discriminate between a monogenic and polygenic origin of such differences by statistical tests (Smith, 1976), especially when environmental variation is present, while the procedure for selecting families for comparison may generate the appearance of bimodality for reasons other than monogenic segregation (cf. Slack, 1975; Murphy & Kwiterovich, 1977). Most of the evidence refers to a single determination of a person's serum lipid concentration but individual variability of triglyceride concentration is several times greater than of cholesterol, when compared on a log scale, and such differences are also evident in repeat measurements at different times on the same individual (Robertson & Cumming, 1979; Robertson *et al.* 1980).

The statistical problem of discriminating between alternative models, monogenic versus polygenic or partly monogenic and partly polygenic, has given rise to theoretical discussion: see the reports of a recent Symposium edited by Sing & Skolnick (1979) in which there are a series of papers on this topic by Karlin, Carmelli *et al.*, Moll *et al.*, Elston, Morton & Rao and other authors. This plethora of advice indicates *inter alia*, the most appropriate categories of related and unrelated subject for analysis, conditions rarely encountered in practice. No doubt such procedures would improve the discrimination between models in particular sets of data but would not affect the problem of extrapolation in the absence of biochemical criteria. For practical purposes, progress is more likely through a better understanding of lipoprotein metabolism and determination of

the consequences of different kinds of environmental change or genetic influence on the serum lipoprotein profile, keeping a look out for loci which are segregating for allelic differences with measurable effects.

In this connexion a search for polymorphism in particular Apo-proteins may prove rewarding as in the recent study of the alternative forms into which the arginine-rich Apoprotein-E is resolved by isoelectric focusing in polyacrylamide gels. Utermann, Pruin & Steinmetz (1978) have shown that this protein is genetically polymorphic. In the direction of increasing pI, Apo-E appears as a series of bands designated Apo-EI, Apo-EII, Apo-EIII and, when present, Apo-EIV. Alternative genotypes can be identified by the relative intensity of staining of particular bands. Utermann *et al.* (1978) favoured a two allele model, with presence or absence of protein at the E-IV position as probably due to segregation at a different locus. But, more recently, Zannis & Breslow (1980) have used two-dimensional electrophoresis to reveal a basic pattern of a major band and a 'tail' of minor bands. The molecules which make up this pattern are subject to charge differences which move the whole complex along the gel to occupy three alternative positions. We have confirmed this finding (Cumming & Robertson, in preparation) and, from pedigree analysis, favour a three allele model, which can also account for variation at the E-IV position. Preliminary observations suggest the occurrence in our population in North East Scotland of a common allele of frequency of 0·7–0·8 and the less common alleles with frequencies of about 0·1. Thus heterozygotes are common in the population.

The Apo-E polymorphism is bound to become important in the study of variation in lipoproteins. Thus, so far all typical hyperlipidaemic Type III patients characterized by abnormally high cholesterol content of VLDL, unusually high triglyceride values and altered electrophoretic mobility of VLDL, have turned out to be homozygous for one of the alleles but not all such homozygotes are hyperlipidaemic. In the latter case the homozygotes show a substantial lowering of low density lipoprotein and hence approximately 20% reduction in the average level of total serum cholesterol, and also an increase in the concentration of VLDL and IDL and hence higher levels of serum triglyceride, although the relatively higher cholesterol content of VLDL is particularly noticeable. Heterozygotes are on average intermediate between the homozygotes in these respects. When this particular combination is combined with independently acting genes predisposing to hyperlipidaemia or perhaps environmental circumstances which tend in the same direction there appears to be an interaction which leads to substantial increase in concentration of both VLDL and IDL. A similar type of interaction may occur in the corresponding heterozygotes so that the effect of a single dose of the allele may indicate the presence of other genes or conditions which tend to raise serum lipid concentration. To clarify these relations we need comparisons of the serum concentrations in genetically different sibs. We are collecting such information in a general study of Apo-E polymorphism which offers a promising approach to analysis of the effects of quantitative variation in lipoproteins and the clinical consequences. It is important to know whether the Apo-E genotype affects

the risk of coronary disease and, if so, whether differences in gene frequency could play a role in regional differences in the incidence of CHD.

Another rather different kind of genetically determined lipoprotein difference has been identified in the so-called Lp (a) antigen. Lp (a), originally considered a genetic variety of LDL whose presence was determined by a single dominant allele (Berg, 1963), now appears to be distinct from most of the LDL on the basis of immunological, chemical and physico-chemical criteria, although its hydrated density (1·050–1·10) overlaps the LDL range. Also, by increasingly sensitive assay methods, Albers, Adolphson & Hazzard (1977) detected the presence of Lp (a) in all but one of a thousand individuals; the exception lacked the B Apoprotein. So Lp (a) must be regarded as a quantitative character with a very high heritability. There is good evidence that higher levels of Lp (a) are associated with increased risk of CHD, especially at an earlier age (Dahlen & Frick, 1974). A three-fold risk was suggested for higher concentrations of the antigen. Since the effects of even higher values of Lp (a) on total cholesterol are very slight, Berg suggested that the apparently increased risk of CHD could hardly be attributed to an unspecific elevation of cholesterol concentration but rather to specific properties of the Lp (a) molecules. Also of interest is the experiment reported by Albers, Cabana & Hazzard (1975) in which the diet of a number of subjects was supplemented with cholesterol over a 28 day period. Whereas the LDL concentration showed a striking increase Lp (a) remained unchanged, suggesting metabolic independence.

Although it may seem improbable, the possibility that an increase in Lp (a) is a consequence rather than a cause of infarction cannot yet be excluded in the absence of prospective evidence.

A further antigenically recognized genetic variant of serum LDL, Ag(x), originally discovered by Allison & Blumberg (1961), has been shown by Berg *et al.* (1976) to be associated with slightly different concentrations of serum cholesterol or triglyceride which is higher in Ag $(x)^-$ than Ag $(x)^+$ individuals, especially in older subjects.

(iii) *Polygenic variation*

A number of reports provide evidence of heritable variation especially in total serum cholesterol, e.g. Adlersberg, Schaeffer & Steinberg (1975); Schaeffer, Adlersberg & Steinberg (1958); Mayo, Frazer & Stamatoyannopoulos (1969) and Martin, Kurczynski & Steinberg (1972). Aro (1973) in a study of survivors of myocardial infarction under age 50 and approximately 80% of their living first degree relatives, reported that average serum cholesterol and triglyceride concentrations in the relatives significantly exceeded the controls, although they were less than the index cases averages. There was also evidence of familial aggregation of elevated lipid levels in one third of the young survivors, a familiar finding in such studies, and most of these presented several abnormal lipoprotein profiles among groups of relatives. The author favoured a polygenic contribution to the familial resemblances. Moll, Powsner & Sing (1979) using maximum likelihood

estimation of variance components as well as least squares analysis, estimated that 50 % of the non-fasting serum cholesterol variation in a large population (4000) was additively genetic This analysis also indicated a number of families which appeared to show segregation.

Against the general consensus of a genetic contribution to such familial resemblance there is a report of nil correlation in serum cholesterol between parents and 16–18 year old children living in Israeli kibbutzim in each of which a common diet is provided from a single kitchen (Brunner *et al.* 1971). We can only speculate as to what the situation might be in comparisons between adult relatives of the same degree.

There have been several studies of identical and non-identical twins, e.g. Osborne *et al.* (1959), Gedda & Poggi (1960); Meyer (1962), Pikkarainen, Takkunen & Kulonen (1966), Heiberg (1974); Feinleib (1976); Weinberg, Avet & Gardner (1976), and on balance they provide evidence of a genetic contribution to total serum cholesterol and/or triglyceride variation, although there are inconsistencies in the apparent degree of genetic control of cholesterol and triglyceride and the authors sometimes differ in what they think the data imply. Sistonen & Ehnholm (1980) in a twin study of the main Apoproteins of HDL (A-I and A-II) estimated the heritability of A-II as 0·30 and 0·35 for females and males respectively but found no evidence of a genetic contribution to the variance of A-I. Given the possible inherent correlation between degree of genetic resemblance and environmental similarity where environmental variation is important, the interpretation of comparisons between monozygotic and dizygotic twins calls for considerable caution.

In a population study of variation of serum lipoprotein fractions in fasting serum (Robertson & Cumming (1979), the estimated heritability of VLDL triglyceride, LDL and HDL cholesterol from parent/offspring regression was respectively $0{\cdot}23 \pm 0{\cdot}20$, $0{\cdot}36 \pm 0{\cdot}18$ and $0{\cdot}67 \pm 0{\cdot}21$, values which are in fair agreement with the corresponding sib correlations. Variation in repeat measurements on the same individual at different times and degree of correlation with measures of body fatness, such as relative weight and skinfold thickness with estimated zero heritability, supported the relative importance of non-genetic variation in individual VLDL differences compared with the high heritability of HDL. Unlike other lipoproteins HDL shows little evidence of age-related changes, implying considerable homeostatic control. This might appear to conflict with the negative correlation with VLDL, which varies so greatly, but, although consistently negative, the correlation was found to be low ($-0{\cdot}1$ to $-0{\cdot}4$) so that only a small fraction of the variance of HDL is thus accounted for.

It may also be relevant that Lewis *et al.* (1978) compared the serum lipoprotein concentrations in population samples from London, Naples, Uppsala and Geneva and found that, although LDL and VLDL concentrations showed substantial inter-population differences, average HDL concentrations did not.

As noted above HDL has recently come into the limelight because of the evidence for an inverse relation between risk of CHD and serum concentration of

HDL. When serum triglyceride concentration is held constant by regression analysis, HDL concentration remains an important determinant of coronary risk (Gordon *et al.* 1977), while the relationship of HDL cholesterol with coronary risk holds for every clinical manifestation of the disease as well as mortality. Glueck *et al.* (1975) from the study of high levels of one of the constituents of HDL (A–I) found evidence of increased life expectancy in members of families with at least one relative with particularly high concentrations of HDL and also an apparent rarity of premature coronary disease. Taggart & Stout (1979) reported that vascular disease of both the cerebral and coronary arteries is associated with reduced serum HDL concentrations. Hence if the genetic determination of individual variation in serum HDL cholesterol concentration is comparatively high this could contribute to the familial association of coronary disease discussed earlier. It also follows that unless we know the HDL serum concentration, interpretation of the potential consequences of other lipid values may prove misleading, so that the CHD risk associated with say a given high LDL or triglyceride concentration could differ if the HDL concentrations are either relatively high or low.

3. GENERAL CONCLUSIONS

Apart from the rare, simply inherited forms of familial hypercholesterolaemia and associated risk of infarction, there is very considerable evidence pointing to parallelism between the familial risk of CHD and the familial resemblances in serum lipoprotein concentrations, especially of LDL and HDL. In studies carried out in different populations there are often inconsistencies in the strength of such an association, but given our ignorance about the relevant environmental differences little further can be said on this point.

In any estimate of heritability environmental contributions to the observed correlation between relatives is an ever-present possibility. Decision based on heritability estimates derived from differences in human attributes is an act of faith, since it involves a generally untestable extrapolation, unlike the situation in livestock or experimental animals where such estimates can be validated or otherwise by selection. Comparisons of genetic variation of corresponding physiological variables in other species may provide relevant comment on data derived from man. For example Weibust (1973) estimated the heritability of serum cholesterol concentration in mice as 50% and then proceeded by two-way selection, to produce differences consistent with this estimate.

To take another example with possible implications for man. In several reports Clarkson *et al.* (1971) and in later work – see Kwiterovich *et al.* (1979) – have shown that in the squirrel monkey, addition of cholesterol to the diet, at rates similar to those in the diets of western societies, reveals differences between individuals who either develop hypercholesterolaemia (hyper-responders) or do not (hypo-responders) and who maintain their serum cholesterol concentration at levels similar to those of the controls. There is good evidence from breeding experiments of major genetic control of these characteristic differences in response

to such dietary supplements. Since these were experimental animals it was possible to minimize the effects of age and environmental differences by studying progeny in the same breeding season and housed under similar conditions. Hyper-responding animals can be identified early in life, and in this group there was further evidence of individual differences in the sites of atherosclerosis, e.g. particularly in the coronaries, the carotids or more generally. Hence if there were a change in gene frequency, say in favour of hypo- or hyper-responders, there would be a corresponding change in reaction to cholesterol in the diet and also in risk of coronary disease.

These observations may prove relevant both to inter- and intra population differences in risk of coronary heart disease in man, and hence we need to know how far man and squirrel monkey share such genetically determined properties of physiology and metabolism. There is evidence that although persons vary in the extent to which their serum cholesterol levels are altered in response to change of diet, they retain their initial ranking (Keys, 1971).

Thus to the simple question – what is the genetic contribution to coronary heart disease? – there is a very complex answer. To make it less so, we need better understanding of lipoprotein metabolic pathways and inter-relations, and hence of the serum profile, and to what extent and in what way plaque development is influenced by the concentrations of particular molecular species in the alternative lipoprotein fractions. A clear appreciation of the extent to which individuals respond differently to a given environmental situation in this area of study may well have implications for other aspects of human variation in which there is even less direct access to the biochemical and physiological determinants of the phenotype.

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