Enzyme Polymorphisms in Man
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C. GENETICS OF MAN

Enzyme polymorphisms in man

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There are a large number of different enzymes synthesized in the human organism, and many of these probably contain more than one structurally distinct polypeptide chain. If current theories about genes and proteins are correct we must suppose that the primary structure of each of these different polypeptides is determined by a separate gene locus, and that there are probably also other loci which are specifically concerned with regulating the rate of synthesis of particular polypeptides or groups of polypeptides. Furthermore, we may expect that genetical diversity in a human population will to a considerable extent be reflected in enzymic diversity. That is to say, in differences between individuals either in the qualitative characteristics of the enzymes they synthesize, or in differences in rates of synthesis.

The work I am going to discuss was largely aimed at trying to get some idea of the extent and character of such genetically determined enzyme diversity among what may be regarded as normal individuals. When my colleagues and I started on this line of work about three years ago the information available about this aspect of the subject was very limited. It had of course been recognized for quite a long time that there are many rare metabolic disorders, the so-called 'inborn errors of metabolism', which are due to genetically determined deficiencies of specific enzymes (Harris 1963). These conditions can in general be attributed to mutant genes which result either in the synthesis of an abnormal enzyme protein with defective catalytic properties, or in a gross reduction in rate of synthesis of a specific enzyme protein. By and large such genes appear to be relatively uncommon and have frequencies of between 0·01 and 0·001 in the general population. Heterozygotes often show a partial enzyme deficiency though they are usually in other respects quite healthy. A few cases are also known where a specific enzyme deficiency occurs quite commonly in certain populations. The most extensively studied example of this is glucose 6-phosphate dehydrogenase deficiency, and it seems likely that in this particular case the relatively high incidence in certain populations is attributable to a specific selective advantage which the deficiency may confer in situations where endemic malaria is an important selective agent (Motulsky 1964).

Virtually all these enzyme deficiencies have been identified in the first instance because of some more or less striking clinical or metabolic disturbance of which they were the cause. They therefore represent a highly selected group of mutants,

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and cannot be expected *per se* to provide us with any clear picture of how extensively genetically determined enzyme variation which does not result in overt pathological manifestations may occur in the general population. Nor can they provide us with any precise indication of what the general character of such 'concealed' variation might be. Whether it is, for example, mainly a matter of minor quantitative differences in rates of synthesis, attributable to genes at so-called 'regulator' or 'operator' loci, or whether qualitative differences involving enzyme structure are an important feature.

In attempting to tackle this rather general problem we have adopted a quite empirical and perhaps somewhat simple-minded approach. Our idea was to see whether, if we examined a series of arbitrarily chosen enzymes in normal individuals in sufficient detail, we would find genetically determined differences, and if so whether such differences were common or rare, and whether they were peculiar to one class of enzyme rather than another.

Because we would need to examine the selected enzymes in quite a large number of different people, and because we wished to carry out family studies on any enzyme differences that turned up, we were in the first instance largely forced to confine our attention to enzymes present in blood. We had, of course, also to make some decision about the kind of techniques we would utilize in looking for such differences. A wide variety of methods suitable for examining the many different properties of enzyme proteins are available, and it would have been impractical to attempt to utilize more than a few of these. In practice we have mainly relied in the first instance on the technique of starch gel electrophoresis. This is known to be capable, if one gets the conditions right, of detecting quite subtle differences in molecular charge and molecular size. It is however not designed to pick up other sorts of molecular differences, and it is also not very sensitive in the detection of small quantitative differences. Thus we could expect to detect at best only a proportion of all possible forms of enzyme variation.

Despite these limitations, we have found, during the course of examining in varying degrees of detail some ten arbitrarily chosen enzymes, three quite striking examples of genetically determined polymorphism.

Red cell acid phosphatase

The first enzyme we selected for study on this arbitrary basis was red cell acid phosphatase (Hopkinson, Spencer & Harris 1963, 1964). This enzyme is known to differ both in its pattern of substrate specificity and in its inhibition characteristics from the acid phosphatase present in other tissues, and it is thought to occur only in the erythrocyte. Its precise function, however, is not known.

A fairly simple method was developed for detecting the enzyme after electrophoresis in starch gel. The surface of the gel is incubated in a reaction mixture containing phenolphthalein diphosphate at pH 6·0, so that at any site of acid phosphatase activity free phenolphthalein is liberated. This can then be detected by making the surface of the gel alkaline, so that the sites of enzyme activity appear as bright red zones.

When haemolysates from a series of normal individuals were examined using this procedure, it was found that every sample showed more than one zone of enzyme activity. We were evidently dealing with what is now generally called a set of isoenzymes. Furthermore, there were clear-cut person-to-person differences in the number, the mobilities, and the relative activities of these isoenzyme components (figure 65). Five distinct phenotypes were soon identified. They are now referred to as A, BA, B, CA and CB, and in the British population occur with frequencies of about 0·13, 0·43, 0·36, 0·03 and 0·05 respectively. The initial family studies showed that these phenotypes are genetically determined and led to the hypothesis that three allelic genes (P^a, P^b and P^c) at an autosomal locus are involved (phenotypes A and B being produced by the homozygous genotypes P^aP^a and P^bP^b respectively, and phenotypes, BA, CA and CB by the heterozygous

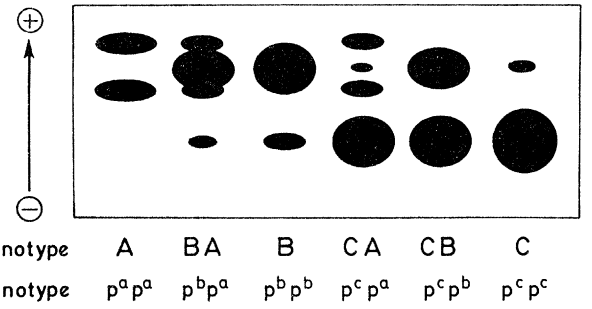

FIGURE 65. Diagram of isoenzyme components seen in the various red cell acid phosphatase phenotypes after electrophoresis at pH 6·0.

genotypes P^aP^b, P^aP^c and P^bP^c). The hypothesis predicted the occurrence of a sixth phenotype corresponding to the genotype P^cP^c. Gene frequency considerations indicated that this would be fairly uncommon (about 1 in 625 of the general population), and indeed a few examples of what is probably this phenotype have now been observed (Lai, Nevo & Steinberg 1964).

With the five common phenotypes fifteen different mating types are possible and the segregation pattern in most of these has now been studied (table 7). These quite extensive family data have proved to be fully consistent with the hypothesis and the findings have also been confirmed by several other groups working with different populations. There seems little doubt therefore that these acid phosphatase variations reflect a polymorphism involving at least three alleles, and a variety of studies on the properties of the isoenzymes in the different phenotypes makes it appear reasonably certain that these alleles determine the synthesis of structurally different forms of the enzyme.

A particularly interesting feature of the polymorphism is that the qualitative differences between the phenotypes are reflected quantitatively by differences in the levels of enzyme activity (Spencer, Hopkinson & Harris 1964*a*). Levels of total acid phosphatase activity were determined by a standard method in a series of haemolysates from individuals of the different phenotypes, using *p*-nitrophenyl phosphate as substrate. Although there was considerable variation in activity

between individuals of any one phenotype, nevertheless quite marked differences between the mean values for different phenotypes could be demonstrated (table 8). Using these values one may examine the question as to whether the quantitative

TABLE 7. SEGREGATION OF RED CELL ACID PHOSPHATASE PHENOTYPES IN 216 FAMILIES

parents	number of matings	children						total
		A	BA	B	CA	CB	C	
A × A	4	8	—	—	—	—	—	8
A × BA	25	31	25	—	—	—	—	56
A × B	11	—	20	—	—	—	—	20
A × CA	4	3	—	—	2	—	—	5
A × CB	5	—	2	—	5	—	—	7
BA × BA	51	24	52	21	—	—	—	97
BA × B	50	—	52	44	—	—	—	96
BA × CA	7	6	3	—	3	2	—	14
BA × CB	8	—	5	5	2	6	—	18
B × B	24	—	—	58	—	—	—	58
B × CA	9	—	7	—	—	16	—	23
B × CB	16	—	—	23	—	16	—	39
CA × CA	—	—	—	—	—	—	—	—
CA × CB	1	—	1	—	1	—	—	2
CB × CB	1	—	—	—	—	1	1	2
totals	216	72	167	151	13	41	1	445

TABLE 8. MEANS AND STANDARD DEVIATIONS OF RED CELL ACID PHOSPHATASE ACTIVITY IN INDIVIDUALS OF KNOWN PHENOTYPES

The activity is expressed as μM *p*-nitrophenol liberated in $\frac{1}{2}$ h at 37 °C/g haemoglobin.

phenotype	number of individuals	mean activity	standard deviation
A	33	122·4	16·8
BA	124	153·9	17·3
B	81	188·3	19·5
CA	11	183·8	19·8
CB	26	212·3	23·1

effects of the three postulated alleles are additive in a simple way or not. If they are additive one could expect the following relationships to be true:

$$(a) \quad \tfrac{1}{2}\overline{\mathrm{A}} + \tfrac{1}{2}\overline{\mathrm{B}} = \overline{\mathrm{BA}},$$
$$(b) \quad \overline{\mathrm{CA}} - \tfrac{1}{2}\overline{\mathrm{A}} = \overline{\mathrm{CB}} - \tfrac{1}{2}\overline{\mathrm{B}},$$

where $\overline{\mathrm{A}}$, $\overline{\mathrm{BA}}$, $\overline{\mathrm{B}}$, etc., are the mean values for the various phenotypes. It will be seen that the results support the idea of simple additivity rather well ($\frac{1}{2}\overline{\mathrm{A}} + \frac{1}{2}\overline{\mathrm{B}} = 155{\cdot}35$, $\overline{\mathrm{BA}} = 153{\cdot}9$, $\overline{\mathrm{CA}} - \frac{1}{2}\overline{\mathrm{A}} = 122{\cdot}6$ and $\overline{\mathrm{CB}} - \frac{1}{2}\overline{\mathrm{B}} = 118{\cdot}15$). Estimates from these data of the average activity attributable to each allele are:

$$P^a \rightarrow \ 60{\cdot}7 \pm 1{\cdot}1 \text{ units},$$
$$P^b \rightarrow \ 93{\cdot}7 \pm 1{\cdot}0 \text{ units},$$
$$P^c \rightarrow 120{\cdot}3 \pm 3{\cdot}7 \text{ units}.$$

Somewhat unexpectedly the three values turn out to be very close to the simple ratio 2:3:4, and it is tempting to think that this may have some special significance in terms of enzyme structure.

It is of interest to note that if one determines red cell acid phosphatase activities in a series of randomly selected individuals one obtains a continuous unimodal distribution which, in fact, is not dissimilar in form to the distributions usually obtained when other enzymes in man are examined quantitatively in randomly selected populations. In particular the variance when related to the mean is of the same order of magnitude as is found with many other enzymes. In the present case however it is clear that the overall distribution represents a summation of a

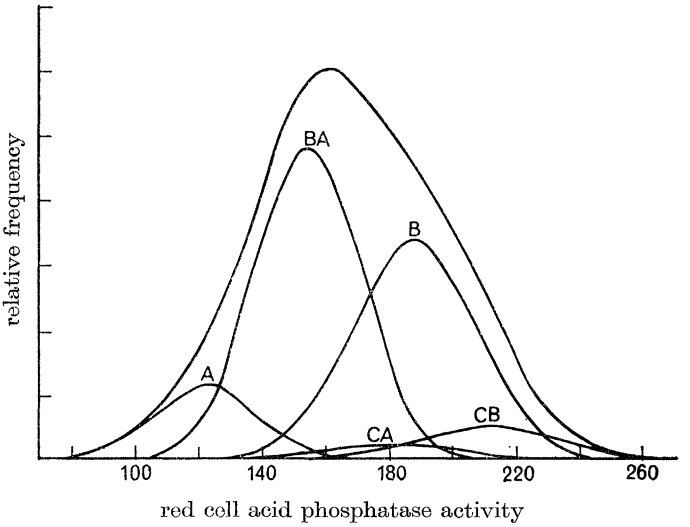

FIGURE 66. Distribution of red cell acid phosphatase activities in the general population (top line) and in the separate phenotypes. The curves are constructed from the values given in table 8 and from the relative frequencies of the phenotypes observed in a randomly selected population.

series of separate but overlapping distributions corresponding to each of the qualitatively different phenotypes (figure 66). Furthermore, the genetical component of the variance of the overall distribution can be largely if not entirely attributed simply to the effects of the three alleles. It is not unreasonable to suppose that the genetical component of other examples of continuous variation in enzyme levels may have a similar simple underlying basis.

PHOSPHOGLUCOMUTASE

One of the main problems in studying enzymes by starch gel electrophoresis is the development of sensitive and specific methods for the detection of the zones of enzyme activity. The general approach has been to utilize substrates which will yield coloured products or products capable of reacting rapidly with some chemical included in the reaction mixture to give a coloured compound. Phenolphthalein

diphosphate, for example, proved to be a useful substrate for red cell acid phosphatase, and naphthyl phosphates have been widely utilized for the study of other phosphatases. However, for the majority of enzymes this approach is not feasible because of their very restricted range of substrate specificity.

One way round this difficulty is to utilize other enzymes in the reaction mixture so as to build up a sequence of reactions culminating in the formation of some detectable substance. Such reaction mixtures are usually complex and often include six or more different interacting components whose relative concentrations require careful adjustment. However, the general method is proving extremely valuable and has opened up the possibility of examining many previously inaccessible enzymes. Our first successful application of this idea was with phosphoglucomutase, and it led to the discovery of another example of enzyme polymorphism (Spencer, Hopkinson & Harris 1964*b*).

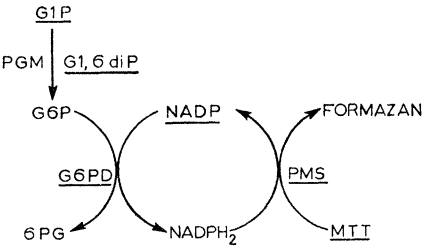

FIGURE 67. Sequence of reactions in the detection of phosphoglucomutase (PGM) after starch gel electrophoresis. The underlined components are contained in the reaction mixture. [*Key*: G1P, glucose 1-phosphate; G6P, glucose 6-phosphate; G1,6diP, glucose 1,6-diphosphate; 6PG, 6-phosphogluconate; G6PD, glucose 6-phosphate dehydrogenase; NADP and $NADPH_2$, oxidized and reduced nicotinamide adenine dinucleotide phosphate; PMS, phenazine methosulphate; MTT, tetrazolium salt.]

Phosphoglucomutase catalyses the reversible transfer of phosphate between glucose 1-phosphate and glucose 6-phosphate, and it has an important role in carbohydrate metabolism. Its detection following starch gel electrophoresis was accomplished via the sequence of reactions shown in figure 67. The underlined components are included in the reaction mixture and the sites of phosphoglucomutase activity are located by the deposition of a blue-coloured formazan formed by the reduction of the tetrazolium salt *MTT*.

When haemolysates from different individuals are subjected to starch gel electrophoresis and this reaction system is applied, one obtains the rather complex isoenzyme patterns shown in figure 68. At least seven different zones of activity (*a–g*) may be detected, and three quite distinct types of pattern can be identified in different individuals. These are referred to as PGM1, PGM2-1 and PGM2. The phenotypes differ in the occurrence of components a, b, c and d; a and c being present in PGM1 and PGM2-1, but not PGM2, while b and d are present in PGM2-1 and PGM2 but not PGM1. Components e, f and g are present in all the three phenotypes.

In the British population the incidence of the three phenotypes has been found to be PGM1 0·58, PGM2-1 0·36 and PGM2 0·06. Studies on the segregation of

these phenotypes in more than 150 different families involving all the possible mating types make it clear that two autosomal alleles (*PGM*^{1} and *PGM*^{2}) determine these differences. Phenotypes PGM 1 and PGM 2 represent the homozygotes *PGM*^{1} *PGM*^{1} and *PGM*^{2} *PGM*^{2}, and phenotypes PGM 2-1 the heterozygote *PGM*^{1} *PGM*^{2}.

This suggests that the isoenzyme components a and c determined by *PGM*^{1} may be molecular alternatives of components b and d determined by *PGM*^{2}. Possibly these isoenzymes contain a common polypeptide chain, and the difference between the two homozygous phenotypes depends on a small structural difference in this, which involves perhaps a single amino acid substitution. If this is so then one would presume that this polypeptide chain is not present in the isoenzyme components e, f and g, as they appear to be uninfluenced by this gene substitution, and

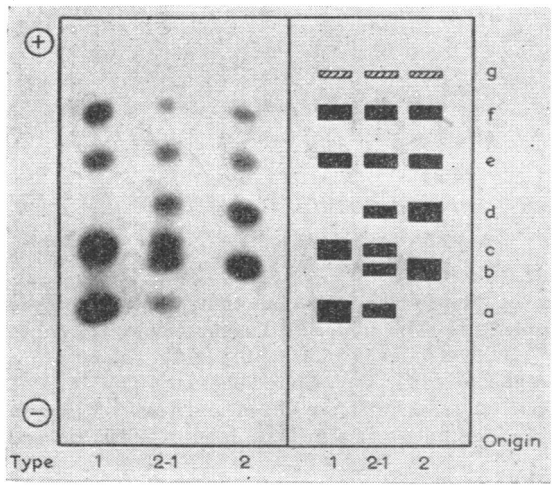

FIGURE 68. Photograph and diagram of phosphoglucomutase isoenzyme patterns obtained by starch gel electrophoresis at pH 7·4.

their structures are presumably therefore determined by other loci. Some support for this idea has recently been obtained by the discovery of an uncommon variant involving components e and f but not affecting a, b, c or d. This variant was found to segregate independently from phenotypes 1, 2-1 and 2, and the family study indicated that it was determined at a separate and not closely linked locus. It is also possible that other structurally distinct polypeptide chains may be contained in these isoenzyme components. These might, for instance, account for the mobility differences between isoenzymes a and c, or between e and f, and would if present imply the existence of further loci involved in the determination of this enzyme. No doubt structural studies on the isolated isoenzyme components will enable these questions to be resolved.

Unlike red cell acid phosphatase, phosphoglucomutase occurs in many different tissues, and it was therefore of some importance to see whether the isoenzyme components and the polymorphism found in erythrocytes also occurred elsewhere. It has in fact been possible to demonstrate that this is the case. The tissues studied

included liver, kidney, muscle, brain, skin and placenta. The phosphoglucomutase isoenzymes have also been demonstrated in tissue culture cells grown *in vitro*. The tissue cultures were started from small skin biopsies from different individuals and were kept going for up to ten passages for more than three months. The cells were harvested at different times and the phosphoglucomutase examined. In each case the PGM phenotype found was the same as that originally observed in the red cells of the donor whose skin was used to start the culture.

Adenylate kinase

The third polymorphism discovered in the screening programme of arbitrarily selected enzymes involves the enzyme adenylate kinase (Fildes & Harris 1965). This catalyses the reversible reaction

$$2\,ADP \rightleftarrows ATP + AMP$$

and two procedures for detecting the enzyme after starch gel electrophoresis have been developed. Both of these require complex and different multienzyme reaction mixtures, but reveal the same pattern of zones of activity. It has been found that the enzyme as it occurs in erythrocytes, and also in skeletal muscle, includes several distinct isoenzyme components, and so far two discrete phenotypes have been recognized. One of these occurs in about 1 in 10 of the general population, and family studies indicate that the individuals showing it are heterozygous for two autosomal alleles. This work is still in its preliminary stages and it has not yet been possible to determine whether all or only some of the isoenzymes present are involved in the polymorphism.

Placental alkaline phosphatase

The various enzymes studied in this screening programme were selected because among other reasons they were known to occur in the erythrocyte, and this is obviously convenient if one wishes to carry out population and family investigations. However, the erythrocyte is a rather specialized cell type, and even though it is often possible to demonstrate that many red cell enzymes occur in essentially the same form in other tissues, it might be considered that a survey restricted to one cell type could present a somewhat biased picture of human enzyme variation in general. Analogous studies on enzymes localized to other tissues are for obvious reasons very much more difficult to pursue, and so far have not been carried out in any systematic way. However, it has been possible to investigate in some detail one particular example of a polymorphism involving what can be regarded as an organ-specific enzyme, and the results illustrate something of the possibilities and problems which such enzymes may present.

The enzyme is an alkaline phosphatase present in quite large amounts in the human placenta. It appears to be peculiar to this organ and to be different from the alkaline phosphatases present in other tissues such as liver, kidney and bone.

Following earlier work by Boyer (1961), it has now been possible to demonstrate that placentae may be classified into at least six distinct phenotypes according to

the electrophoretic behaviour of the alkaline phosphatase they contain (Robson & Harris 1965). The electrophoretic patterns are illustrated in figure 69, and one may note that complete discrimination of the six phenotypes requires electrophoresis at two different pH's. The six phenotypes are referred to as S, F, I, SF, SI and FI. In types S, F and I most of the alkaline phosphatase activity is present in a single rapidly moving component, which however has a different mobility in each type. In the I phenotype, the characteristic component has a mobility very close to that of the F phenotype at pH 8·6, but is indistinguishable from that of the S phenotype at pH 6·0. In phenotypes SF, SI and FI, three such components are found, two of them in each case having mobilities similar to the component present in S, F or I,

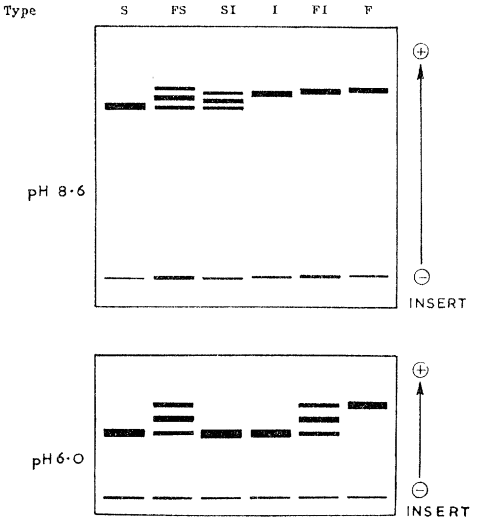

FIGURE 69. Placental alkaline phosphatase patterns obtained by electrophoresis at pH 8·6 and pH 6·0.

while the third has an intermediate mobility. There are reasons for thinking that this third component in these three phenotypes may represent a 'hybrid' enzyme containing polypeptide chains characteristic of the two other components present. In each of the six phenotypes at least one other component which migrates only very slowly may be seen. These slow components do in fact exhibit slight differences in mobility in the different phenotypes, and these can be shown to correlate with the more striking differences observed in the major and more rapidly moving components.

On the basis of the phenotypic patterns and their relative frequencies, it is possible to construct a simple genetical hypothesis which will account for these variations. This suggests that three autosomal allelic genes are concerned, phenotypes F, I and S representing the three homozygous genotypes, and phenotypes SF, SI and FI the corresponding heterozygotes. From the observed incidence of the six phenotypes in the British population, one may readily obtain values for the frequencies of the three postulated genes. They are 0·27, 0·09 and 0·64, and using these values one finds a very good agreement between the observed incidence of

the phenotypes and those expected assuming a Hardy–Weinberg equilibrium (table 9)

It is of course obvious that family studies of the ordinary kind are impracticable in the case of a characteristic peculiar to the placenta. However it occurred to us

TABLE 9. OBSERVED AND EXPECTED NUMBERS OF PLACENTAL ALKALINE PHOSPHATASE TYPES IN A POPULATION SAMPLE ASSUMING A HARDY–WEINBERG EQUILIBRIUM

($p = 0{\cdot}27$, $q = 0{\cdot}09$ and $r = 0{\cdot}64$)

placental alkaline phosphatase type	expected incidence		expected numbers in population sample	observed numbers in population sample
S	r^2	0·410	135·9	141
SF	$2pr$	0·346	114·7	111
F	p^2	0·073	24·2	28
SI	$2qr$	0·115	38·2	32
FI	$2pq$	0·049	16·1	15
I	q^2	0·008	2·7	5
totals	$(p+q+r)^2$	1·001	331·8	332

TABLE 10. PLACENTAL ALKALINE PHOSPHATASE PHENOTYPES IN 130 DIZYGOTIC TWIN PAIRS

dizygotic twins		expected incidence assuming three alleles with frequencies p, q and r	expected incidence $p = 0{\cdot}27$ $q = 0{\cdot}09$ $r = 0{\cdot}64$	observed incidence
like pairs				
S	S	$\frac{1}{4}r^2(1+r)^2$	35·80	39
SF	SF	$\frac{1}{2}pr[pr+(1+p)\,(1+r)]$	25·34	27
F	F	$\frac{1}{4}p^2(1+p)^2$	3·82	6
SI	SI	$\frac{1}{2}qr[qr+(1+q)\,(1+r)]$	6·90	6
I	I	$\frac{1}{4}q^2(1+q)^2$	0·31	1
FI	FI	$\frac{1}{2}pq[pq+(1+p)\,(1+q)]$	2·24	3
			74·41	82
unlike pairs				
S	SF	$pr^2(1+r)$	23·58	26
S	F	$\frac{1}{2}p^2q^2$	1·95	0
S	SI	$qr^2(1+r)$	7·85	9
S	I	$\frac{1}{2}q^2r^2$	0·21	0
S	FI	pqr^2	1·30	1
SF	F	$p^2r(1+p)$	7·70	3
SF	SI	$pqr(1+2r)$	4·60	4
SF	I	pq^2r	0·18	0
SF	FI	$pqr(1+2p)$	3·12	1
F	SI	p^2qr	0·55	2
F	I	$\frac{1}{2}p^2q^2$	0·03	0
F	FI	$p^2q(1+p)$	1·09	0
SI	I	$q^2r(1+q)$	0·73	1
SI	FI	$pqr(1+2q)$	2·39	1
I	FI	$pq^2(1+q)$	0·31	0
			55·59	48

that we might be able to test the hypothesis by studying a series of pairs of placentae from dizygotic twins, because such twin pairs can be regarded as pairs of sibs. We were fortunate in being able to obtain such material from an extensive investigation of twin births which is being carried out in the Birmingham area under the general direction of Dr John Edwards. So far we have been able to examine the alkaline phosphatase types in 260 placentae from 130 dizygotic twin pairs. The findings are summarized in table 10. In 82 pairs the alkaline phosphatase phenotypes were the same, and in 48 pairs they were different. This result excludes the possibility that these placental phenotypes are determined by the maternal genotypes, because if this were so none of the pairs should have shown any differences.

If the phenotypes depend on the foetal genotype then one may test the hypothesis by calculating the expected incidence of the different sorts of sib pair using the gene frequencies previously obtained. This is shown in table 10, and it will be seen that there is quite good agreement between the numbers of the different sorts of twin pair observed, and those expected according to the hypothesis.

Thus the evidence strongly suggests that these placental alkaline phosphatase phenotypes are determined by the foetal genotype and that at least three autosomal alleles are concerned. The biological significance of the polymorphism is still quite obscure, but it might well be of importance in problems concerned with maternal–foetal interaction. It may also perhaps be worth considering other enzyme polymorphisms from this point of view. The phosphoglucomutase polymorphism, for example, can also be readily demonstrated in the placenta and here again it has been shown that the placental phenotype is determined by the foetal and not the maternal genotype.

Discussion

Although this work is still in its early stages, an interesting and perhaps in some ways an unexpected picture of enzyme variation in human populations is beginning to emerge.

In the course of examining some ten arbitrarily chosen enzymes, in none of which we had any particular reason to expect any degree of variation, and not all of which have been examined in great detail or by perhaps the most suitable methods, we have come across three quite striking examples of enzyme polymorphism. Although one can hardly draw firm numerical conclusions from such a small series, it seems likely, unless we have been excessively lucky in our choice of enzymes, that polymorphism to a similar degree may be a fairly common phenomenon among the very large number of enzymes that occur in the human organism.

Some idea of how extensive this diversity might be can be obtained by considering together the various enzymes which have been shown to exhibit some degree of polymorphism in our own population. Relevant data on seven such enzymes are given in table 11. For the present purpose only those variations where two or more allelic genes have been found to have frequencies greater than 0·01 have been included. In the case of one enzyme, serum cholinesterase, variation at

two different loci fall into this category, so that eight loci are represented in all. Of these at least six can be regarded as 'structural' loci since the variation produced appears to involve qualitative differences. In the other two cases (serum cholinesterase E_2 and acetyl transferase) only quantitative differences in enzyme level have so far been identified, but it is possible that these may also reflect structural differences in the enzyme protein present.

TABLE 11. ENZYME POLYMORPHISM IN THE ENGLISH POPULATION

enzyme	number of alleles with frequency greater than 0·01	frequency of commonest phenotype	probability of two randomly selected individuals being of the same phenotype	reference
red cell acid phosphatase	3	0·43	0·34	Hopkinson, Spencer & Harris (1963)
phosphoglucomutase	2	0·58	0·47	Spencer, Hopkinson & Harris (1964*b*)
placental alkaline phosphatase	3	0·41	0·31	Boyer (1961) Robson & Harris (1965)
acetyl transferase	2	0·50	0·50	Price Evans & White (1964)
adenylate kinase	2	0·90	0·82	Fildes & Harris (1965)
serum cholinesterase				
locus E_1	2	0·96	0·92	Kalow & Staron (1957)
locus E_2	2	0·90	0·82	Harris, Hopkinson, Robson & Whittaker (1963)
6-phosphogluconate dehydrogenase	2	0·96	0·92	Fildes & Parr (1963)
combined	—	0·037	0·014	—

Each of these variations occurs independently of the others so that quite a large number of different phenotypic combinations may be found in the general population. Indeed, the commonest of these will occur in less than 4% of people and the probability that two randomly selected individuals would be found to have the same combination of phenotypes is less than 1 in 70. Thus just taking into account this very limited series of examples, quite a high degree of individual differentiation in enzymic make-up is demonstrable, and it is of interest that most of this is probably attributable to variation in enzyme structure.

These different polymorphisms pose a variety of intriguing problems both in biochemistry and in genetics. One would like to know, for example, what is the precise nature of the structural differences between the variant forms of a given enzyme, and whether these are reflected in kinetic differences and in differences in functional activity. It is notable that several of these enzymes apparently occur in multiple molecular forms even in homozygous individuals. The recognition of these so-called isoenzyme systems is a fairly new development in enzymology and the further investigation of these particular examples, and of their variant forms, may well help to throw light on the general biological significance of this phenomenon.

One would also like to know why these different enzyme phenotypes occur with the particular frequencies that we observe, and why, as is the case, for example,

with red cell acid phosphatase and placental alkaline phosphatase, the gene frequencies may vary quite widely from one population to another. Presumably selective differences are important here but at present we have virtually no idea what these might be. However, one may reasonably hope that, if the metabolic and functional differences which presumably derive from the various enzyme differences can be elucidated, this may provide us with some indication of what selective factors may be important.

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