Genetical Studies on Human Red Cell Acid Phosphatase[1]

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WE HAVE RECENTLY DESCRIBED (Hopkinson, Spencer and Harris, 1963) a new human polymorphism involving differences in the acid phosphatase which occurs in red cells. The differences were detected by starch gel electrophoresis, and so far five distinct phenotypes have been recognized. They have been referred to as A, BA, B, CA, and CB. They each show at least two electrophoretically distinct components.

The five phenotypes differ from one another both in the electrophoretic mobilities and in the relative intensities of the components present. Furthermore, there are significant differences between the phenotypes in the over-all level of red cell acid phosphatase activity (Spencer, Hopkinson and Harris, 1964). It has been suggested that these differences may be accounted for by postulating the occurrence of three allelic genes, P^a, P^b, *and* P^c.

In the present paper, we summarize the data which we have collected to date on the different aspects of this polymorphism and discuss a number of problems which these results pose.

METHODS

Electrophoresis and Staining

Red cell hemolyzates are subjected to electrophoresis in starch gel at pH 6.0. The zones of acid phosphatase are then detected using phenolphthalein diphosphate as substrate. The details of the method at present in use are as follows.

Red cells are washed three times in 0.9 per cent NaCl, centrifuging for five minutes at 3000 rpm. Hemolyzates are prepared by the addition of two volumes of distilled water to one volume of washed packed red cells.

The starch gels are made from Connaught Laboratory hydrolyzed starch using the starch concentration recommended by the makers for the particular batch. The gel is made using a succinic acid/tris buffer (0.0025 M in succinic acid, 0.0046 M in tris, pH 6.0). A citric acid/NaOH buffer (0.41 M in citric acid, pH 6.0) is used as a bridge solution. The dimensions of the gel usually made are 19 cm long × 8 cm wide × 0.7 cm thick; this is adequate for at least 4 sample insertions. The hemolyzates are inserted into the gel

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on very thick filter paper (Whatman No. 17). Electrophoresis is carried out horizontally at 6 volts/cm for 17 hours in a cold room at +5°C. The gels are then sliced horizontally and placed in perspex boxes. Fifty ml of 0.005 M phenolphthalein diphosphate sodium or calcium salt in 0.05 M citrate buffer, pH 6.0 (readjusted to pH 6.0 after dissolving the phenolphthalein salt) is poured on to the cut surface of the gel, which is then incubated at 37°C for 3 hours. After this, the reagent mixture is poured off, and about 2 ml of concentrated ammonia is added to the box and the lid replaced. The ammoniacal atmosphere makes the surface of the gel alkaline, and the areas where phenolphthalein has been liberated by acid phosphatase are revealed as red zones. Sharp definition of the various zones only persists for about half an hour because of the diffusion of the free phenolphthalein.

Hemolyzates to which 2-mercaptoethanol has been added (1 μl/ml) will still give satisfactory patterns after having been stored at +5° C for up to ten days. Longer storage however results in a marked loss of activity and also in modification of the electrophoretic pattern, with the appearance of one or more rapidly moving zones. Whole red cells kept in ACD at +5°C will give satisfactory patterns after much longer storage.

Acid Phosphatase Assay

The assay is performed with *p*–nitrophenyl phosphate as substrate (Bessey *et al.*, 1946). Hemolyzates used for acid phosphatase typing in the starch gels are diluted 1 in 6 with distilled water so that the hemoglobin concentration is about 1.2 g/100 ml; this dilute hemolyzate is used in the assay system.

The substrate solution is prepared fresh daily by dissolving 263 mg disodium *p*–nitrophenyl phosphate in 25 ml 0.05 M citrate buffer, pH 6.0. The pH is adjusted to 6.0 with 0.05 M citric acid and the solution is made up to 50 ml with 0.05 M citrate buffer, pH 6.0, to give a 0.02 M solution of the substrate. The assay is carried out in centrifuge tubes, and for each hemolyzate three tubes are prepared, each containing 0.5 ml substrate solution and 0.4 ml water. The reaction is started by the addition of 0.1 ml hemolyzate. In the first tube, the reaction is stopped immedately by the addition of 2 ml 10 per cent trichloroacetic acid (TCA) while the remaining tubes are incubated in a water bath at 37°C for 15 and 30 minutes, respectively, before 2 ml TCA is added. The precipitated proteins are centrifuged down at 3000 rpm for 5 minutes, and 1 ml aliquots of the clear supernatants are transferred to clean tubes. With each batch of assays a standard curve is prepared using aliquots of a 1×10^{-4} M solution of pure *p*–nitrophenol diluted to a final volume of 1 ml with distilled water.

The *p*–nitrophenol color is developed in the assay and standard tubes by the addition of 4 ml 0.5 N NaOH. The intensity of the color is then measured at 415 mμ against a blank of 1 ml distilled water and 4 ml 0.05 N NaOH. The optical density at 30 minutes and at 15 minutes give a check on linearity. Both values are then combined to obtain an estimate of the quantity of *p*–nitrophenol liberated in one half hour.

Duplicate hemoglobin determinations are carried out using 0.1 ml of the assay hemolyzates in 4 ml of Drabkin's solution (Crosby *et al.,* 1954). The resulting colors and that of a standard hemoglobin solution are read against a blank of Drabkin's solution at 540 mμ.

The acid phosphatase activity is expressed as micromoles of *p*-nitrophenol liberated in one half hour at 37°C per gram of hemoglobin.

RESULTS

The Red Cell Acid Phosphatase Phenotypes

Figure 1 shows the patterns observed after starch gel electrophoresis at pH 6.0 of hemolyzates of the five phenotypes so far identified. In type A, two zones of roughly equal intensity are seen. In type B, two zones also are found, the faster of these being markedly slower than the fast zone of type A and slightly faster than the slow zone of type A. Furthermore, in type B the fast zone is relatively more intense than the slow zone. In type BA, a more complex pattern is seen. This can be reproduced by the electrophoresis of a mixture of hemolyzates of type A and type B in roughly equal amounts. However, it should be noted that the part of the type BA pattern which apparently corresponds to the fast zone of type A is not sharply defined but appears characteristically as a smear running forward from a relatively more intense zone corresponding to the fast zone of type B. Types CA and CB are both characterized by an extremely intense slow zone. In type CA, this is associated with faster moving zones similar to the two zones observed in type A and much weaker than the slow zone. In type CB, the intense slow zone is associated with a somewhat weaker faster zone which is similar to the main zone in type B. Examination of a number of blood samples from given individuals over a period of several months has shown the patterns to be reproducible and to be individual characteristics.

It is well established that the acid phosphatase activity found in red cells differs in certain of its properties from the acid phosphatase present in a number of other tissues. Of particular importance in the present context are the differences in substrate specificity. Thus, although red cell acid phosphatase readily hydrolyzes phenolphthalein diphosphate and *p*-nitrophenyl phosphate, it fails to produce any appreciable hydrolysis of α- or β-naphthyl phosphate. These latter substrates have been widely used for the histochemical demonstration of acid phosphatase in other tissues because of the ready formation of stable colored compounds by the coupling of the liberated α- or β-naphthol with an appropriate diazo reagent. This specificity has been demonstrated here by comparing the behavior of red cell hemolyzates, prepared as described above, with concentrated white cell preparations obtained from the same individual and disrupted either by sonication or by freezing and thawing. After starch gel electrophoresis at pH 6.0, it was found that the white cell preparations showed at least two zones of acid phosphatase activity which could be detected using both α- and β-naphthyl phosphate as substrate. However, no activity could be demonstrated using phenolphthalein diphos-

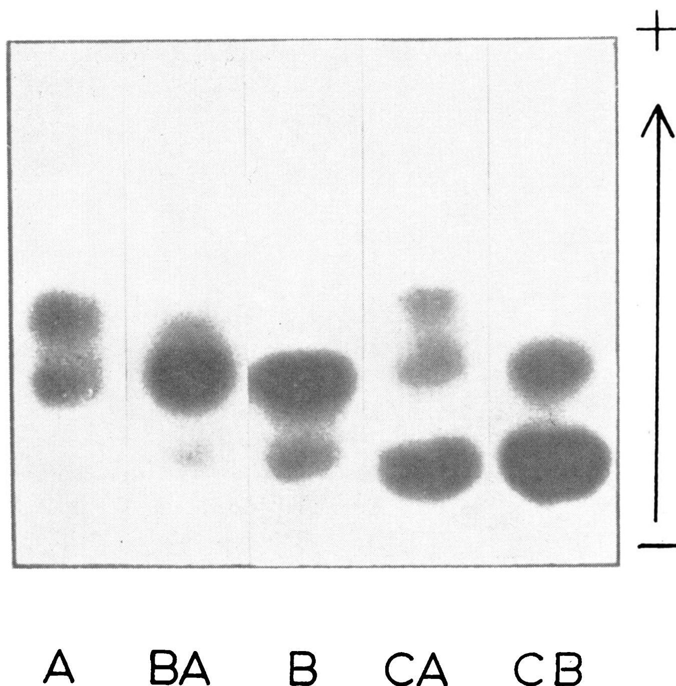

FIG. 1. The five red cell phosphatase patterns obtained by starch gel electrophoresis of hemolyzates. The lower edge of the photograph is the start line. The hemoglobin moves down toward the cathode and is not shown.

phate. In the case of the red cell preparations, the zones of activity could be readily detected using phenolphthalein diphosphate, but not with α– or β–naphthyl phosphate. Furthermore, the white cell acid phosphatase did not correspond in mobility to the red cell zones, nor did they show the characteristic individual differences in pattern.

Evidence for heterogeneity of red cell acid phosphatase has been previously reported by Abul–Fadl and King (1949) from the study of pH activity curves and by Angeletti and Gayle (1962) from the elution patterns obtained by chromatography on DEAE cellulose. It has not been possible as yet, however, to relate these observations to the multiple components observed after starch gel electrophoresis.

Population Incidence

Table 1 gives the number of individuals of the different phenotypes observed in 274 unrelated healthy adult British men and women and in 93 individuals with a variety of hematological abnormalities. No gross differences between the frequencies in the two groups or between the two sexes were observed, and the total data have been combined to obtain estimates of the phenotype frequencies in the British population. It will be noted that more than 80 per cent of the population are of types BA and B, and the other types are relatively uncommon.

TABLE 1. NUMBERS OF INDIVIDUALS OF THE DIFFERENT ACID PHOSPHATASE PHENOTYPES IN A RANDOM SERIES OF BRITISH ADULT MALES AND FEMALES AND IN A SERIES OF UNRELATED PATIENTS WITH VARIOUS HEMATOLOGICAL DISORDERS

Acid phosphatase phenotype	Healthy adults		Patients with hematological disorders		Total	Incidence
	Male	Female	Male	Female		
A	17	12	4	8	41	0.112
BA	90	42	16	27	175	0.477
B		56	62	118		0.843
CA	2	5	1	1	9	0.025
CB	8	6	5	-	19	0.052
Total	176	98	41	52	367	1.001

The only other population that we have been able to examine in any detail is that of the Tristan da Cunhan Islanders. This is a highly inbred population known to have been derived from only 15 ancestors (mainly European) during the last century. The whole population comprising nearly 300 individuals was evacuated to England after a recent volcanic eruption. It was possible to examine the acid phosphatase in 140 of them, and the frequencies of the different phenotypes are shown in table 2. There is a much higher incidence of type B than observed in the British population.

A small group of Negroes living in London have also been examined. The results are given in table 3.

Gene Frequencies

It has been suggested (Hopkinson, Spencer and Harris, 1963) that these phenotypic differences are determined by three allelic autosomal genes, P^a, P^b, and P^c. The relation between the observed phenotypes and the postulated genotypes is shown in the first two columns of table 4. It will be seen that the hypothesis predicts the occurrence of a genotype P^cP^c, for which a phenotype has not yet been identified.

If this hypothesis is correct then one may obtain estimates of the frequencies p, q, and r of the genes P^a, P^b, and P^c in a given population by counting (e.g., p = frequency of type A + ½ frequency of type BA + ½ frequency of type CA). For the sample of 367 British adults given in table 1, $p = 0.36$, $q = 0.60$, and $r = 0.04$. From these gene frequency estimates, one can determine the expected frequencies of the various phenotypes assuming random mating and absence of appreciable selection. These then may be compared with the observed frequencies. The appropriate calculations are given in table 4, and it will be seen that there is good agreement between the observed numbers of the various phenotypes and the numbers expected. The results are thus consistent with the hypothesis. For the British population, the calculations suggest that the predicted, but not yet observed, phenotype corresponding to the genotype P^cP^c may be expected to occur in about one in 600 randomly selected individuals.

TABLE 2. NUMBERS OF INDIVIDUALS OF THE DIFFERENT ACID PHOSPHATASE PHENOTYPES IN 140 TRISTAN DA CUNHAN ISLANDERS

Acid phosphatase phenotype	No. of individuals			Incidence
	Male	Female	Total	
A	1	1	2	0.014
BA	8	12	20	0.143
B	56	62	118	0.843
CA	—	—	—	—
CB	—	—	—	—
Total	65	75	140	1.000

TABLE 3. NUMBERS OF INDIVIDUALS OF THE DIFFERENT ACID PHOSPHATASE PHENOTYPES IN 23 NEGROES

Acid phophatase phenotype	No. of individuals	Incidence
A	1	0.05
BA	7	0.30
B	15	0.65
CA	—	—
CB	—	—
Total	23	1.00

The gene frequencies for the Tristan da Cunhan Islanders were $p = 0.09$ and $q = 0.91$. For the small samples of Negroes, they were $p = 0.2$ and $q = 0.80$.

Cord Bloods

The acid phosphatase patterns appear to be fully developed at birth. Twenty-three cord bloods have been examined, and all could be classified as readily as adult bloods. There were no obvious peculiarities or extra components. The numbers of the different types in these 23 cord bloods are given in table 5. It will be seen that the distribution is in reasonable agreement with that observed in adults, although the total sample is small.

Family Data

A total of 107 families have been studied in which samples of blood from both parents and one or more of their children could be obtained. In all individuals, blood groups were determined, and families in which evidence for illegitimacy was obtained were excluded from the series. With five phenotypes, 15 parental combinations may occur. Examples of only 11 of these have so far been observed. The others must be relatively rare. The findings are summarized in table 6a, b, and c.

Table 6a gives the results in 71 British families which were unselected as far as red cell acid phosphatase type was concerned. They are not completely random because several of the families were related to each other and also because a number of the families were collected because of other inherited peculiarities which were being studied at the same time. Table 6b

TABLE 4. EXPECTED AND OBSERVED NUMBERS OF THE DIFFERENT PHENOTYPES IN A SAMPLE OF 367 BRITISH ADULTS

Expected numbers are obtained using the gene frequencies $p = 0.36$, $q = 0.60$, $r = 0.04$.

Postulated genotype	Phenotype	Expected frequency		Expected no. in random sample of adults	Observed no. in sample
$p^a p^a$	A	p^2	0.1296	47.6	41
$p^a p^b$	BA	$2pq$	0.4320	158.5	175
$p^b p^b$	B	q^2	0.3600	132.1	123
$p^a p^c$	CA	$2pr$	0.0288	10.6	9
$p^b p^c$	CB	$2qr$	0.0480	17.6	19
$p^c p^c$	[C]	r^2	0.0016	0.6	0
			1.0000	367.0	367

TABLE 5. DISTRIBUTION OF ACID PHOSPHATASE PHENOTYPES IN 23 CORD BLOODS

Acid phosphatase phenotype	Observed no.	Expected no. (from adult frequencies)
A	2	2.6
BA	12	11.0
B	8	7.7
CA	–	0.6
CB	1	1.2
Total	23	23.1

is based on 15 families, each of which was selected through a child with a particular acid phosphatase type. These families were mainly collected in the early stages of the investigation when we wished to get some idea of the mode of inheritance of the less common types. Table 6c consists of 21 Tristan da Cunhan families.

The results in general are in reasonable agreement with those which would be expected from the hypothesis that three allelic genes are concerned in determining these variations. From the hypothesis, one may readily determine the phenotypes which would be expected to occur among the offspring of particular parental combinations. It will be seen from table 6 that among the 251 children in the 107 families so far studied, only the phenotypes expected on the hypothesis actually occurred. In particular, one may note that all the 64 children of B × B matings were also of type B, and that all 24 children of the relatively uncommon types CA and CB were derived from matings in which at least one parent was CA or CB. The only anomalous feature of the data is the occurrence of a somewhat higher than expected proportion of BA children and somewhat lower proportion of B children from BA × BA matings. Whether this is a real phenomenon or due to chance will not become clear until more data is assembled.

Levels of Acid Phosphatase Activity in the Different Phenotypes

It was noticed from inspection of the gels that the average level of activity, as indicated by the intensity of staining, appeared to vary from type

TABLE 6A. DISTRIBUTION OF ACID PHOSPHATASE PHENOTYPES IN 71 ENGLISH FAMILIES, UNSELECTED AS FAR AS ACID PHOSPHATASE PHENOTYPE IS CONCERNED

		Children					
Parents	No. of matings	A	BA	B	CA	CB	Total
A × BA	8	9	10	–	–	–	19
A × B	6	–	12	–	–	–	12
BA × BA	23	10	32	6	–	–	48
BA × B	19	–	20	16	–	–	36
BA × CA	1	–	–	–	–	1	1
BA × CB	2	–	2	2	1	3	8
B × B	7	–	–	19	–	–	19
B × CA	2	–	2	–	–	3	5
B × CB	3	–	–	6	–	2	8
	71	19	78	49	1	9	156

TABLE 6B. DISTRIBUTION OF ACID PHOSPHATASE TYPES IN 15 ENGLISH FAMILIES SELECTED THROUGH A CHILD WITH A PARTICULAR PHENOTYPE

			Children					
Phenotype of child selected	Parents	No. of matings	A	BA	B	CA	CB	Total
A	A × BA	5	6	5	–	–	–	11
CA	A × CA	1	–	–	–	2	–	2
CA	A × CB	2	–	2	–	4	–	6
A	BA × BA	2	2	3	–	–	–	5
CB	BA × CA	2	1	–	–	–	3	4
CA	BA × CB	1	–	–	–	1	1	2
CB	B × CA	1	–	–	–	–	2	2
CB	B × CB	1	–	–	1	–	1	2
	Total	15	9	10	1	7	7	34

TABLE 6C. DISTRIBUTION OF ACID PHOSPHATASE PHENOTYPES IN 21 TRISTAN DA CUNHAN FAMILIES

		Children					
Parents	No. of matings	A	BA	B	CA	CB	Total
BA × BA	1	–	1	1	–	–	2
BA × B	6	–	6	8	–	–	14
B × B	14	–	–	45	–	–	45
Totals	21	–	7	54	–	–	61

to type roughly in the order A < BA < B, CA < CB. In order to examine this in more detail, we have assayed the level of activity of the enzyme in whole hemolyzates from a series of individuals whose acid phosphatase type was separately determined by starch gel electrophoresis. The assay was carried out using *p*-nitrophenyl phosphate as substrate. The distributions of the activities in the individual hemolyzates of the different acid phosphatase types are shown in Fig. 2.

There is considerable variation in level of activity among individuals of any given type. However, this does not obscure some highly significant differences among the types. Thus, it is apparent that the average level of ac-

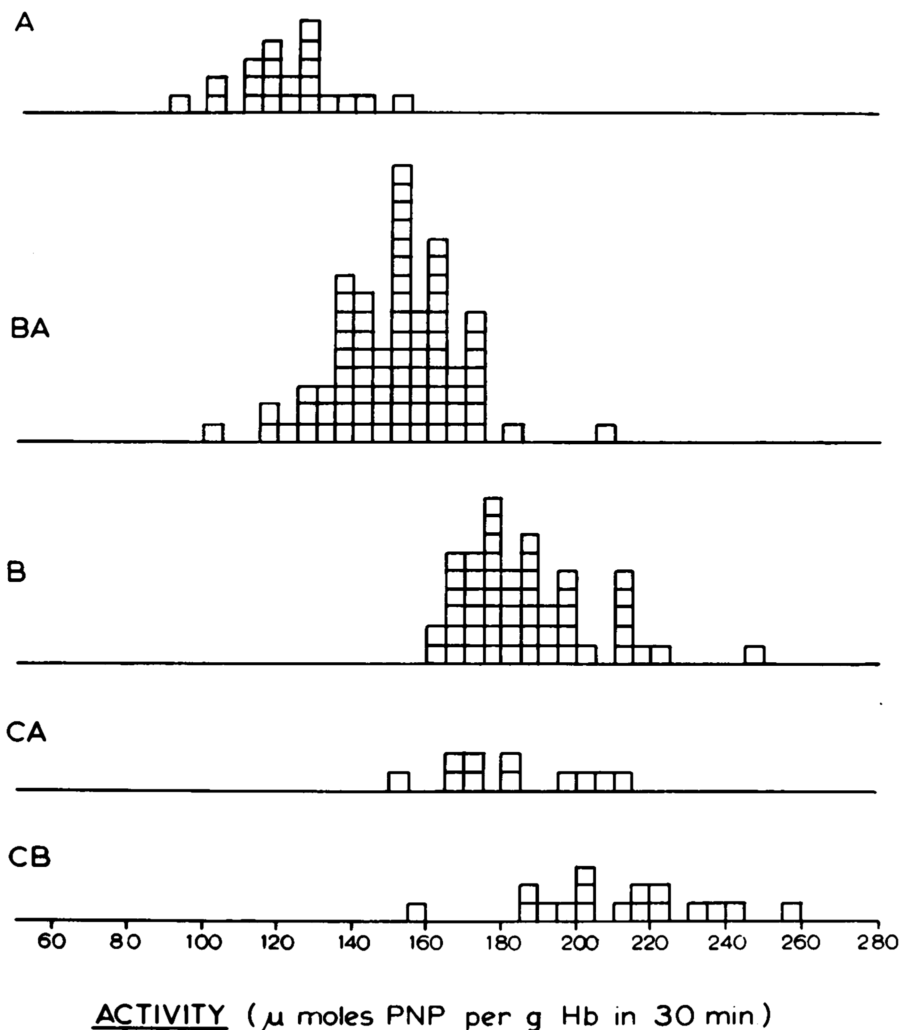

FIG. 2. Distribution of red cell acid phosphatase activities in hemolyzates from individuals of different phenotypes. Each square represents one individual. Activities are expressed as micromoles p-nitrophenol liberated in one-half hour at 37°C per g hemoglobin.

tivity in type B is greater than that in type A, and the average level in type BA is intermediate between the two. Similarly, individuals of type CB show on the average higher activity than individuals of type CA. The means and standard deviations for the various distributions are given in table 7. Using these values for the means, it is possible to make some interesting, if speculative, calculations.

Thus, if we accept the genetical hypothesis outlined above and make the further assumption that all the acid phosphatase activity observed in the various types is determined by the three genes, P^a, P^b, and P^c, then we can ask whether the quantitative effect of these genes is additive in a simple way or whether some more complex dosage relationships occur.

TABLE 7. MEANS AND STANDARD DEVIATIONS OF RED CELL ACID PHOSPHATASE ACTIVITY IN INDIVIDUALS OF KNOWN PHENOTYPES

The activity is expressed as micromoles *p*-nitrophenol liberated in one-half hour at 37°C per g hemoglobin.

Type	No. 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

We will refer to the mean level of activity in the different phenotypes as $\overline{A}$, $\overline{BA}$, $\overline{B}$, $\overline{CA}$, and $\overline{CB}$. If the effects of the three alleles P^a, P^b, *and* P^c are additive, then the following two relationships should occur:

(1) $\frac{1}{2}\overline{A} + \frac{1}{2}\overline{B} = \overline{BA}$,

(2) $\overline{CA} - \frac{1}{2}\overline{A} = \overline{CB} - \frac{1}{2}\overline{B}$.

From table 7, it can be seen that

$\frac{1}{2}\overline{A} + \frac{1}{2}\overline{B} = 155.35$,

and that $\overline{BA} = 153.9$,

also $\overline{CA} - \frac{1}{2}\overline{A} = 122.6$,

and $\overline{CB} - \frac{1}{2}\overline{B} = 118.15$.

Thus, perhaps somewhat surprisingly, the mean values observed support the hypothesis of simple additiveness rather well.

If these simple assumptions are correct, the average activity attributable to each of the three postulated genes will be approximately:

$$P^a \longrightarrow 61 \text{ units}$$
$$P^b \longrightarrow 94 \text{ units}$$
$$P^c \longrightarrow 120 \text{ units}$$

This suggests that the average activity of the predicted type C (P^cP^c) should be about 240 units. It will be noted that the ratio of the activities P^a:P^b:P^c is very close to 2:3:4. It is tempting to think that this might have some special significance in terms of enzyme structure.

The findings are consistent with the patterns of activity in the different phenotypes as observed after starch gel electrophoresis. Thus, as has been mentioned, a pattern very similar to that observed in type BA may be obtained by running a mixture of aproximately equal amounts of hemolyzates of types A and B. Also the patterns in types CA and CB can be plausibly regarded as equivalent to mixtures of a hypothetical type C with types A and B, respectively.

One aspect of these quantitative results of general significance is the following: If one took a random sample of individuals and determined their red cell acid phosphatase activities, one would obtain a continuous unimodal distribution. Furthermore, if a random series of families were studied in the same way and the results subjected to classical correlation and regression

analysis, one would no doubt obtain convincing evidence that the variation in acid phosphatase levels was in large part genetically determined. Thus, the situation would be formally analogous to many other examples of what has been called quantitative inheritance in man, where it has been usually concluded that a polygenic multifactorial system was involved and a large number of different genes were concerned. However, in the present situation, it looks as if a large part of the genetical variance in acid phosphatase levels can be accounted for in terms of only three alleles. It is possible that other examples of quantitative multifactorial inheritance may have a similar simple underlying basis.

Pathological Conditions

Although red cell acid phosphatase has not been very widely studied in pathological conditions, two rather striking abnormalities have been reported. Valentine *et al.* (1961) found a significantly increased level of activity in megaloblastic anemia, and Oski *et al.* (1963) observed a markedly decreased level of activity in red cells of individuals with the Caucasian type of glucose-6-phosphate dehydrogenase deficiency. Our findings suggest that an increase or decrease in the average level of activity in a particular condition could arise in two different ways. It might result from an association of the particular condition with one or another of the less common phenotypes. Alternatively, it might be a consequence of the pathological process and occur indiscriminately in any of the phenotypes. In the former situation, there would not necessarily be any true increase or decrease in activity if the values in the affected individuals are compared with the normal values of the appropriate type. In the other situation, however, the increase or decrease in the levels ought to be seen even when comparisons are made with normals of the corresponding type.

Some information on this question has been obtained with respect to megaloblastic anemia. In a series of 27 cases, the following numbers of the different phenotypes were found: 2 type A, 12 type BA, 9 type B, 2 type CA, and 2 type CB. Thus, all the five phenotypes so far recognized were present, and despite a slightly increased frequency of types CA and CB in this small series, there is no indication of any close association between megaloblastic anemia and any specific acid phosphatase phenotype. Furthermore, the patterns obtained were not obviously different from those found in other individuals. Quantitative studies confirmed the increased levels in this condition. The effect was, as might be expected, more striking in the untreated cases, and the values obtained in these are given in table 8. The increase in acid phosphatase level appeared to occur in each of the phenotypes, but more extensive studies will be required before one can decide whether it differs in degree in the different types. Similar studies on glucose-6-phosphate dehydrogenase deficiency would also be of interest, but so far we have not had any suitable case material available.

A number of other examples of hematological conditions have also been

TABLE 8. RED CELL ACID PHOSPHATASE ACTIVITIES IN 17 INDIVIDUALS WITH UNTREATED MEGALOBLASTIC ANEMIA

Activity expressed as micromoles *p*-nitrophenol liberated in one-half hour at 37°C per g hemoglobin.

Acid phosphatase phenotype	No. of patients	Individual activities	Mean	Normal controls M ± SD
A	1	193	193	122 ± 17
BA	7	220, 214, 185, 185, 238, 211, 179	205	154 ± 17
B	7	286, 290, 197, 208, 219, 253, 211	238	188 ± 20
CA	2	254, 228	241	184 ± 20

examined by these methods without as yet revealing any striking peculiarities in the frequencies of the phenotypes affected, the patterns observed or the levels of activity.

DISCUSSION

During recent years, it has become increasingly clear that human diversity can be studied with advantage at the molecular level. It has been found that certain proteins (e.g., haptoglobin and γ–globulin) may occur in different people in qualitatively different forms, and that these differences are genetically determined. Furthermore, with respect to a particular protein, the different forms may each be common in a given population. Each of these polymorphisms present interesting and indeed exciting problems both to the biochemical geneticist and to the population geneticist. Together they also raise the general question of what proportion of the many proteins and enzymes known to be present in the human organism exhibit similar molecular diversities. So far, however, only a limited number of proteins and enzymes have been studied from this point of view, and until a wider range has been examined, it will be difficult to make generalizations. At present one can only guess whether such polymorphisms will be found to occur in many, or in only a few, human proteins and enzymes. It is also not possible to say whether molecular diversification of this sort will prove to be a more distinctive feature of some classes of proteins of enzymes rather than of others.

It was with these somewhat general considerations in mind that the present work on red cell acid phosphatase was started. The choice of this particular enzyme was rather arbitrary and was based largely on the following two considerations. It is present in the red cell and is therefore readily accessible to study in different individuals and families. It appeared to be the kind of enzyme which might be susceptible to examination by starch gel electrophoresis, and this technique has proved to be particularly potent in uncovering subtle molecular differences. The fact that this arbitrarily chosen enzyme does turn out to exhibit a rather striking genetical polymorphism may be regarded as providing some support for the view that such molecular polymorphisms may not be a rare or unusual phenomenon.

The results obtained raise a number of interesting genetical and biochemical problems and point the way to several different avenues for further work.

From the genetical point of view, it is clearly necessary to assemble much more extensive family and population data. This should show whether the simple genetical hypothesis which has been proposed is correct or whether some more complex explanation is required. It should also indicate whether a distinctive phenotype corresponding to the postulated genotype P^cP^c actually exists. The present results suggest that such a phenotype might occur in about one in 600 individuals in an English population, that on electrophoresis it would be found to be composed predominantly, or entirely, of a slow moving component, and that the average level of activity in this phenotype should be about 240 units. Further population data would also be of interest because they would indicate what variations in gene frequency exist and how these are distributed geographically. They might, with luck, give some indication of the biological significance of this polymorphism.

According to our hypothesis, both type A and type B individuals are homozygous. Yet, in each case, the pattern after starch gel electrophoresis shows two acid phosphatase components. These presumably are structurally different; yet they appear to be controlled by a single gene. It is of importance to find out the nature of the molecular relationships of these separate components within these genotypes. Are we dealing with differences in molecular size, in molecular charge, or in both these characteristics? Do the individual components consist of one, or more than one, polypeptide chain? Questions about molecular charge and size and the number of different polypeptide chains involved also arise when differences between the different phenotypes are considered. Related to these matters are the problems posed by the observed differences in levels of enzyme activity between the various phenotypes. Do these occur because of differences in the catalytic properties of the enzyme proteins, or are they due to differences in the actual amount of enzyme protein present?

Studies aimed at obtaining answers to some of these questions have been initiated. Also we have begun to accumulate data suitable for studies of linkage with other gene loci and with chromosomal morphological peculiarities.

SUMMARY

A method for distinguishing five distinct phenotypes of human red cell acid phosphatase by starch gel electrophoresis is described. Data are given on the incidence of the phenotypes in a British population sample, in a sample of Tristan da Cunhan Islanders, and in a small group of Negroes. The results are also given of studies on 107 families in each of which both parents and one or more children were tested. The findings are discussed in terms of the hypothesis that these phenotype differences are determined by three allelic autosomal genes, and it is shown that they are in general consistent with this hypothesis.

Quantitative data on the over-all levels of red cell acid phosphatase activity in individuals of the different phenotypes are presented. They show significant differences between the phenotypes and suggest that in terms of enzyme

activity the three alleles have different quantitative effects and that these are additive in a simple way.

Studies on patients with megaloblastic anemia confirmed a previous report of increased red cell acid phosphatase activity. This increased activity appears to occur in each of the phenotypes.

Some general problems arising from the results are discussed.

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