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12 May 1964

Acid Phosphatases of Human Red Cells: Predicted Phenotype Conforms to a Genetic Hypothesis

Abstract. *Analysis of data from 80 families and 369 unrelated individuals confirms the hypothesis that human red cell acid phosphatase phenotypes are determined by three codominant alleles at a single autosomal locus. Further confirmation is afforded by the finding of the predicted sixth phenotype.*

Hopkinson, Spencer, and Harris (*1, 2*) demonstrated that the acid phosphatase of human red cells may occur in different forms which are genetically determined. They proposed that the acid phosphatase phenotypes are determined by three alleles, P^a, P^b, and P^c, at an autosomal locus. They observed five phenotypes, two homozygous (called A and B) and three heterozygous (called BA, CA, and CB) types, and predicted a sixth type, homozygous C (*1, 2*). We report here data confirming their genetic hypothesis and the discovery of the C homozygote (*3*).

The phenotypes were determined by starch-gel electrophoresis. We followed the procedure of Hopkinson *et al.* (*2*), except that we obtained better separations with an 8 percent starch concentration rather than the 10 percent recommended by the makers (*4*).

Acid phosphatases from the red cells of members of families from a genetic isolate (a religious group—the H sect) composed of people of Swiss and German ancestry, and of members of families of mixed ancestry from Brazil, were studied. The cells obtained from the persons of sect H were fresh (less than 4 days old); the cells of the persons from Brazil had been stored in glycerol at −40°C for at least 6 months before use. No effects of storage were noted.

The family data are presented in Table 1. The samples from family members were not tested in family groups. The data are adequately explained by the hypothesis advanced by Hopkinson *et al.* (*1, 2*) that the phenotypes are due to three alleles P^a, P^b, P^c, and to that extent confirm it. A significant deficit of BA offspring from matings of B × BA (to a greater degree in set A than in set B) occurs in our sample. Such a deficit does not appear in the data of Hopkinson *et al.* (*1, 2*). It is possible that experimental errors entered into our determinations of phenotype during the earlier stages of our work (set A and the first part of set B) because the ratios seemed to improve as our work proceeded.

At this point, since we had confirmed the hypothesis of three alleles, we set out to find matings which could have C offspring. Therefore, only samples from the parents of the Brazilian families were tested. Two hundred and sixty-three individuals were examined before we found a mating (CB × CB) with both parents having the P^c allele. These 263 parents, plus those of the 53 Brazilian families tested completely (Table 1), provide a sample of 369 unrelated individuals, which may be used to test the hypothesis of three alleles by comparison with the Hardy-Weinberg equilibrium. The data are presented in Table 2. The agreement between the observed and expected values computed on the basis of the Hardy-Weinberg law is clearly satisfactory. The frequency of allele P^c is essentially the same in this sample as it is in the English sample of Hopkinson *et al.* (*2*) (0.03 compared to 0.04), but there are marked differences between the frequencies of P^a and P^b in the two samples (0.20 compared to 0.36, and 0.77 compared to 0.60, respectively). This suggests that the gene frequencies may vary among different populations as well as among smaller isolates, and thus be useful for population studies.

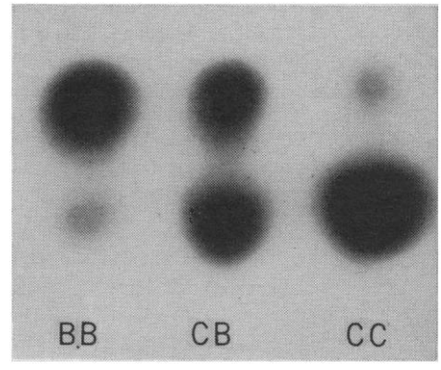

Fig. 1. Starch-gel electrophoretic pattern of acid phosphatase phenotypes of red cells, *BB*, *CB*, and *CC* (from left to right). Insertion is at the base of the figure and migration is toward the anode at the top of the figure. The conditions of electrophoresis and staining are essentially those described by Hopkinson *et al.* (*2*).

The CB × CB mating is of particular interest, because two of the seven children showed a new phenotype, presumably the missing C phenotype, three of the remainder were B, and two were CB. Figure 1 shows one of the homozygous CC samples with a homozygous BB and a CB sample for comparison. It is striking that the CC phenotype is almost the mirror image of the BB phenotype. The starch-gel electrophoretic pattern of each has two zones; in B the faster zone shows greater activity, while in C the slower zone shows more activity. There is much greater activity in the slower zone of C, however, than there is in the faster zone of B. This is in accordance with the prediction of Hopkinson *et al.* (*2*) based on quan-

Table 1. Phenotypes of acid phosphatase in red cells in families from two populations.

Matings		Offspring					
			Phenotypes				
Phenotype*	No.	Total	A	BA	B	CB	CA
A. An inbred group isolated by religious customs living in the U.S.							
A × BA	5	30	15	15			
A × B	2	14		14			
B × B	2	13			13		
B × BA	11	64		23	41		
BA × BA	7	35	12	13	10		
B. Families of mixed Caucasian, Negro, and South American Indian ancestry							
A × BA	3	13	6	7			
A × B	3	7		7			
B × B	17	83			83		
B × BA	23	104		47	57		
B × CB	5	24			13	11	
BA × CB	2	8		2	2	1	3

* Matings are listed according to the phenotypes of the parents without regard to sex.

Table 2. Phenotypes of acid phosphatase of red cells of 369 individuals from a Brazilian population.

A	B	BA	CA	CB	C
		Observed			
15	220	111	4	19	0
		*Expected**			
14.4	219.9	112.2	4.4	17.7	0.4

* On the basis of Hardy-Weinberg law with allele frequencies: P^a=0.197, P^b=0.772, and P^c=0.031.

titative estimates of enzyme activity of each of the five phenotypes they observed. Exchange of photographs has established that the sample classified as C by Harris and his colleagues (*3*) shows the same pattern as the samples we have classified as C.

The finding of the C phenotype completes the verification of the genetic hypothesis previously offered to explain the genetic data (*1, 2*). This polymorphism promises to be a useful tool for the study of problems ranging from population genetics to the genetic control of enzyme structure.

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References and Notes

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5. Supported in part by NIH grants HE 03708, GM 07214, and GM 10424.
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Irradiation of the Blood: Method for Reducing Lymphocytes in Blood and Spleen

Abstract. *Insertion of a beta-emitting source into the right atrium of the heart permits intensive irradiation of the circulating blood, with subsequent depletion of lymphocytes in the peripheral blood and lymphoid organs.*

The lymphocyte, because of its relation to the immunologic response, is the subject of experiments designed to test and define its critical role. McGregor and Gowans (*1*), by creating external thoracic duct fistulas of several days' duration, demonstrated that in the rat such a fistula produces a peripheral lymphopenia and, significantly, a depletion of lymphocytes from the lymph nodes and the spleen. Such animals are altered in their immunologic response to foreign proteins and to skin homografts. Reduction in the peripheral circulating lymphocytes, described by Cronkite *et al.* (*2*), followed extracorporeal irradiation of calf's blood that had been pumped through a spiral of Tygon tubing; after irradiation for 5 hours 90 percent of the blood received more than 1000 rad by random mixing. The lymphocyte population was reduced for several weeks, judging by the decline in lymphocyte counts in the peripheral blood. Moreover, immunologic responses in the calf were altered.

Those studies, attaining much the same end with dissimilar techniques, suggested the advantages of a method capable of modifying the lymphocyte population for more extended periods; an internal radioapplicator appeared to be a logical refinement. Circumferential vascular radioapplicators have been replaced by small cylindrical applicators 20 mm long and 3 mm in diameter containing 300 mc Sr^{90}-Y^{90}, a beta-emitter of 2.18 Mev maximum energy with a half-life of 28 years. The radioisotope is in the form of microspheres and is sealed in the applicator (*3*). The half-value layer of aqueous solutions to this radiation is approximately 1 mm. The stainless steel casing, 0.025 mm thick, results in conversion of less than 1 percent of the radiant energy to bremsstrahlung in the steel. The applicator is suspended in the blood stream within the right atrium where its position depends on the posture of the experimental animal. More often than not it lies touching the walls of the atrium, although the motion of the heart and the concavity of the interior of the heart chamber prevent broad areal contact with the endocardium. The radioapplicator is readily introduced into a branch of the right jugular vein and is advanced into the atrium at the end of a silicone-coated, barium-impregnated, plastic catheter. For protection of the operators, when the applicator is in the operative field, the area of dissection is covered with saline to a depth of several centimeters to absorb the beta rays. Since positioning is not critical, it is determined by dead reckoning; the catheter is advanced in the jugular vein caudad from the angle of the mandible a distance equal to a fixed proportion of the length of the spine,

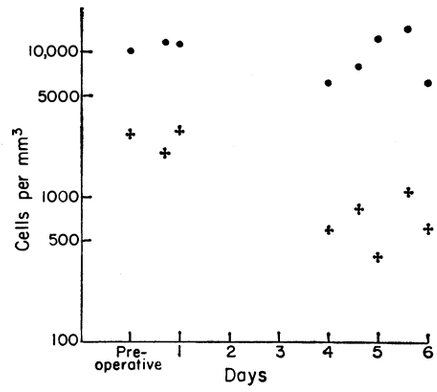

Fig. 1. Leukocyte counts in the peripheral blood of a dog after irradiation of the circulating blood with an intracardiac radioapplicator. Dots = total count; crosses = lymphocytes.

depending on the species. Positioning is verified by x-ray.

The dosimetry, approximate because of the uncontrolled positioning of the applicator relative to the surrounding blood volume, is based on viscosity degradation in vitro and thermal luminescence techniques. Determinations by two independent laboratories were within reasonable agreement. The output of the applicator is expressed as an integral dose-rate in gram-rads per hour; from this datum, the total circulating blood in a dog weighing 10 kg may receive a maximum dose of 300 rads per hour from the intracardiac applicator. The myocardium of the right atrium receives considerable beta-radiation, but this tissue is known to be resistant to larger doses of radia-

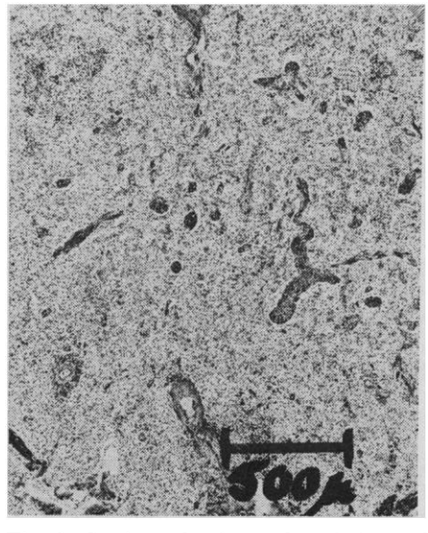

Fig. 2. Section of spleen after 6 days of irradiation of the circulating blood, showing very marked reduction of the lymphoid elements in the white pulp.