HYPOPHOSPHATASIA: A GENETIC STUDY

BY

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Since hypophosphatasia was first described (Rathbun, 1948), other cases which have been recognized have raised the possibility that this defect of alkaline phosphatase is an inherited inborn error of metabolism. Schneider and Corcoran (1950) first suggested that this was an inherited disorder when two cases were found in one family, and this was corroborated by Sobel, Clark, Fox and Robinow (1953). McCance, Fairweather, Barrett and Morrison (1956) demonstrated reduced levels of serum alkaline phosphatase in three generations of one family, suggesting that this was a recessively inherited disorder in which the heterozygous state could be demonstrated by measuring the serum alkaline phosphatase or the phosphoethanolamine in the urine of carriers of the trait. Fraser (1957) then reviewed the literature and pointed out that 26% of reported siblings were affected: the expected incidence of a recessive characteristic. Kretchmer, Stone and Bauer (1958) tried unsuccessfully to identify heterozygous carriers by studies of alkaline phosphatase in leucocytes. Most recently, Harris and Robson (1959) have reported a study of 381 relatives of known cases by urinary chromatograms for phosphoethanolamine, demonstrating a manifestation rate of 0.58 with this method. Because of the importance in genetic counselling, it was felt worthwhile to study the family of two cases which have been under observation since 1954 and reported, in part, by Rathbun (1959).

The aim of the study was to determine, if possible, the definitive level of serum alkaline phosphatase indicative of the carrier state and the incidence of the carrier state in 30 relatives in three generations.

Material and Method

Serum alkaline phosphatase was measured using a modification of the method of Taussky and Shorr (1953).

Reagents. The following reagents were used:

Alkaline Phosphatase Substrate. 2.5 g. sodium glycerophosphate (beta); 2.12 g. sodium diethylbarbiturate.

Dissolve and dilute to 500 ml. with distilled water. The solution is kept under toluene in the refrigerator. The pH, when mixed with one-tenth its volume of serum, should be 8.5.

35% Trichloroacetic Acid Solution (wt./wt.). 35 g. TCA dissolved in 65 g. water.

Ammonium Molybdate Solution—10%. 50 g. of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ are weighed into a litre beaker and about 400 ml. of 10N sulphuric acid are added with constant stirring to prevent caking. When completely dissolved, the solution is transferred to a 500 ml. volumetric flask and washed in quantitatively with 10N sulphuric acid to 500 ml. mark.

Sulphuric Acid 10N. 278 ml. of concentrated sulphuric acid are slowly added to about 700 ml. distilled water. After cooling, the solution is further diluted to 1,000 ml.

Ferrous Sulphate—Ammonium Molybdate Reagent. This must be made up freshly before use. 10 ml. of ammonium molybdate stock solution are transferred to a 100 ml. amber volumetric flask and diluted to about 70 ml.; 5 g. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ are added and the solution is made up to volume and shaken until the crystals are dissolved.

Phosphate Stock—100 mg./100 ml. 450 mg. KH_2PO_4 are heated in an oven at 80 degrees centigrade for 24 hours and cooled in a desiccator over night. Of this, 438 mg. KH_2PO_4 are dissolved and diluted to 100 ml. with distilled water and kept under toluene.

Phosphate Working Reagent. 2 ml. stock solution is diluted to 100 ml. with distilled water. This keeps for one week.

Method. Start with enough tubes (12 × 100 mm.) to take a water blank, two phosphorus standards and four tubes for each serum sample (two of these will be for incubated and two for non-incubated serum). Into each tube measure 1 ml. substrate solution and place all the tubes in 37° C. water bath for five minutes. To two of the tubes add 0.1 ml. serum, mix well, close with 'Parafilm' and return to the 37° C. water bath for exactly 60 minutes. After one hour, the tubes are placed in an ice bath for five minutes, during which time 0.5 ml. of 35% TCA solution is added to each tube; mix well. The tubes are removed and placed at room temperature for five minutes, during which time 0.1 ml. water, standard phosphate working solution and serum are added, respectively, to the remaining tubes; mix well.

All tubes are centrifuged for five minutes at 2,000 r.p.m. in groups starting with incubated serum. Supernatant (1 ml.) from each tube is placed in 12 × 75 mm. cuvettes and 1 ml. ferrous sulphate ammonium molybdate reagent is added to each cuvette. Mix well. After five minutes, the cuvettes are read in the Coleman spectrophotometer at 680 mμ.

Blood Collection. Members of the family group and a control subject with a known normal range of serum alkaline phosphatase were tested simultaneously to eliminate false values due to technique or reagent variation. Tests were performed in groups of three family members and one control subject. Blood samples were drawn from the four subjects within a total period of about 15 minutes and the determinations were performed on the serum as soon as it was separated by centrifugation. Because serum values change if the determination is not performed at once, family members in out-of-town hospitals, all the tests were performed using the same technique, equipment and reagents as in the War Memorial Children's Hospital Research Laboratory.

TABLE 1
MALE RELATIVES OF PARENTS

Maternal		Paternal	
Name	Alkaline Phosphatase (Bodansky units)	Name	Alkaline Phosphatase (Bodansky units)
J.B.	1.8	P.B.	1.4
C.B.	3.1	Ra.B.	1.6
V.B.	3.4	Ph.B.	1.6
		R.B.	1.9
		J.B.	2.2
		Gd.B.	2.7
		G.B.	3.5
		Re.B.	3.5

Normal mean 2.7 ± 0.58

TABLE 2
FEMALE RELATIVES OF PARENTS

Maternal		Paternal	
Name	Alkaline Phosphatase (Bodansky units)	Name	Alkaline Phosphatase (Bodansky units)
L.L.	1.1	C.R.	1.3
L.B.	1.4	B.B.	1.5
I.Q.	1.7	D.P.	1.6
B.	1.7	J.B.	1.6
N.V.	1.7	A.H.	1.8
R.L.	2.0	B.M.	2.4
D.M.	2.5	B.	2.4
B.M.	2.6		

Normal mean 2.4 ± 0.7

FIG. 1.

The supernatant fluid obtained after deproteinization was kept refrigerated overnight. The colour was then developed with ferrous sulphate ammonium molybdate reagent. Separate experiments have shown that the phosphorus values are not affected by the overnight storage. All members of the control group were apparently healthy nurses, medical students, doctors and other hospital employees. A scatter diagram revealed no trend of variation in values with age among the adult population sampled.

Results

Stern (1947) has shown that females have lower values than males and the results were therefore separated into sex groups.

Table 1 shows the results obtained in the male relatives of the known cases, while Table 2 shows the results of the female relatives.

Table 3 gives the results of the examination of the siblings and Fig. 1 illustrates the family pedigree where heterozygous carriers were considered to be all persons with levels of serum alkaline phosphatase below 2.0 Bodansky units, regardless of sex.

TABLE 3
SIBLINGS OF CASES

Siblings		
Name	Sex	Alkaline Phosphatase (Bodansky units)
R.	M	6.1
R.	F	3.5
J.	M	5.0

TABLE 4

MEAN SERUM ALKALINE PHOSPHATASE LEVELS
(BODANSKI UNITS): FAMILY MEMBERS vs. CONTROLS
BY SEX

	Family Members			Controls		
	No.	Mean	S.D.	No.	Mean	S.D.
Males ..	11	2.4	± 0.8	18	2.6	± 0.7
Females ..	15	1.8	± 0.4	24	2.4	± 0.7
Total ..	26	2.1	—	42	2.5	—

Statistical Analysis

The control group consisted of 18 males and 24 females, and therefore equated with the family group in terms of sex ratio (Table 4).

Statistical significance ($p < 0.05$) could be attached to a reduction of 0.4 units in the overall mean value for the family group, as well as to a tendency for females generally to have lower values than males. However, a further tendency for values in the family group to be reduced more for females than for males failed to attain statistical significance.

Discussion

When these figures of the family group are compared with normal controls, not separated for sex, using analysis of variance (Snedecor, 1946) the difference is significant (Table 5). Hence it appears feasible to separate the carriers by this method. As the father (R.B.) had a level of 1.9 B.U. and the mother (B) had a level of 1.7 B.U., it is reasonable to state that the heterozygous carrier level in the male and female exists at levels at, and below, 1 S.D. of the normal mean. These figures suggest that a level below 2 Bodanski units of serum alkaline phosphatase is indicative of the heterozygous carrier state for at this level 50% of the examined aunts, uncles and parents are shown to be carriers; this is the expected incidence. This level will include, however, about 15% of normal persons owing to the overlapping of the normals with heterozygous carriers. Further careful studies may allow a more precise evaluation.

It has been our experience that the assay of serum alkaline phosphatase is plagued with many difficulties, and if the technique is not well controlled the results may be valueless. Unfortunately, in the literature, there are a wide variety of methods of determining the serum alkaline phosphatase. The results of one observer cannot be compared directly with those of another because of different methods used, even though these results are qualitatively similar. This has produced chaos in this field.

The evidence put forward by Harris and Robson

TABLE 5

ANALYSIS OF VARIANCE ON DATA IN TABLE 4

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F	P
Experimental group	1	2.96	2.96	6.58	< 0.05
Sex ..	1	1.92	1.92	4.27	< 0.05
Groups vs. sex interaction ..	1	0.99	0.99	2.20	> 0.05
Residual ..	64	29.11	0.45	—	—
Total ..	67	34.98	—	—	—

(1959), together with the studies presented here, confirms the fact that hypophosphatasia is an inherited recessive disease in which the carrier state can be detected in most cases. The importance of this in genetic counselling needs no emphasis.

Conclusions

Hypophosphatasia is inherited as a recessive characteristic.

The carrier state is represented by adult persons with serum levels below 2 Bodanski units.

The normal level for males is 2.7 ± 0.58 Bodanski units and for females is 2.4 ± 0.7 Bodanski units.

A plea for uniformity in chemical determinations is made.

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