Genetic Studies of Blood Group-Associated Variations in a Human Serum Alkaline Phosphatase

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THE OCCURRENCE OF alkaline phosphatase activity in the blood plasma has been recognized for many years (Kay, 1930), and it has become clear that large quantitative variations occur in this activity at different ages and in certain pathological conditions (Hess, 1963). It has also been shown that serum alkaline phosphatase is heterogeneous, composed of distinct molecular types apparently deriving from bone, liver, intestine, and placenta (Boyer, 1961; Cunningham and Rimer, 1963; Gutman, 1959; Hodson, Latner, and Raine, 1962; Schlamowitz, 1958). Several authors have suggested that genetic factors might control certain observed variations in phosphatase activity. Boyer (1961) studied serum alkaline phosphatases separated by starch gel electrophoresis, observing multiple electrophoretic components in normal sera. He presented evidence for a possible polymorphism involving an alkaline phosphatase, apparently of placental origin, in the sera of pregnant women.

Hodson, Latner, and Raine (1962) noted that a slowly migrating phosphatase component, detected by starch gel electrophoresis and thought to originate from the intestinal mucosa, showed variable levels of activity in different individuals. Cunningham and Rimer (1963) noted similar variation on starch gel in a slowly migrating component, probably the same as that seen by Hodson, Latner, and Raine. They found that in a sample of 50 sera, 60% had a detectable slow-moving component, while the remainder did not. They suggested that genetic factors might be involved but presented no data.

Arfors, Beckman, and Lundin (1963a) also noted variation on starch gels in a slow phosphatase band, presumably the same as that seen by Hodson, Latner, and Raine and Cunningham and Rimer. They classified individual serum samples into Types 1 and 2 on the basis of the absence or presence of this phosphatase band "B." Studies of a group of twins suggested that this variation was under genetic control. A very striking association between phosphatase type and the ABO blood groups was noted. Later Arfors, Beckman, and Lundin (1963b) presented results on a small sample indicating an association with the Lewis blood groups and suggested that the phosphatase component might be complexed in some way with blood group substances.

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Gahne (1963) observed similar variation in a serum phosphatase of cattle and presented evidence that the presence or absence of the component was genetically determined. Rendel and Stormont (1964) found the same type of variation in sheep serum and showed this to be associated with the R blood group system. These authors also refer to unpublished evidence that the phosphatase variation in cattle is related to the J blood group system. Since the human ABO, the bovine J, and the ovine R blood groups are thought to be homologous systems (Rendel and Stormont, 1964), this serum phosphatase association in the three different species takes on added significance.

This paper presents the results of further studies of the phosphatase variation in human serum. These data corroborate the association between phosphatase type and the ABO blood groups and reveal an association with the ABH Secretor trait (and thus indirectly with the Lewis blood groups as previously reported). The data also show that the phosphatase variation is of a quantitative nature and is influenced by genetic factors in addition to the ABO and Secretor loci, as well as by temporal and age factors.

MATERIALS AND METHODS

Serum Specimens

For the study of population aspects of this variation, a sample of 676 Caucasian individuals was drawn from a larger group being surveyed by the Department of Epidemiology of the University of Michigan in the Tecumseh Community Health Study (Epstein, 1960; Napier, 1962). The Department of Human Genetics is collaborating in this long-term health and morbidity survey of an entire town of population about 10,000. The sample used for these studies contained a number of family groups, but these were random and unselected, so the sample may be considered an unbiased cross-section of the total population. Each individual was typed for ABO, Rh, MNSs, Kell, Duffy, Kidd, Lewis, and P blood groups, for secretion of ABH and Le^a substances in saliva, and for the serum haptoglobin, transferrin, and Gc variant systems.

For family studies of phosphatase variation among group B and group O secretors, 56 families having one or more children were selected from among the many hundreds available in the Tecumseh population. In every family selected, the parents were ABH secretors and were either group O or group B. Only the ABH secretor children of these couples were included in the family studies. In most instances, the family groups were composed of parents in the 30–50 year age group, with children of ages 5–20 years.

Sera from a group of 125 dizygotic and 101 monozygotic adult male twin pairs were generously supplied by Drs. J. V. Neel and H. Gershowitz. This group was composed entirely of World War II veterans. Ages ranged from approximately 35 to 45 years at the time specimens were collected. Zygosity determinations were based upon ABO, MN, Rh, Kell, Duffy, P, Secretor, Gm, and haptoglobin types, as well as upon morphological characteristics (Gershowitz and Neel, unpublished results).

Forty-eight serum specimens, composed of three samples from each of 16

individuals, were kindly supplied by Dr. H. Gershowitz. The three samples were collected at different times at intervals varying from 11 to 50 days. All individuals were adult male members of the U. S. Navy. All were of blood group B. Secretor types were unknown.

Electrophoresis and Classification

Starch gel electrophoresis was performed by the horizontal method of Smithies (1955) in a borate buffer at pH 9.0. Starch was obtained from Connaught Medical Research Laboratories. The starch and buffer concentrations used varied somewhat with each particular lot of starch but were in general approximately 12 g starch per 100 ml buffer at 0.02 M H_3BO_3 and 0.008 M NaOH. The electrophoretic separation time was 17 hours at 6 V/cm, at 4° C.

The alkaline phosphatase components were visualized by the staining method of Lawrence, Melnick, and Weimer (1960) employing sodium α-naphthyl acid phosphate and Blue RR Salt in a Tris buffer at pH 8.8. Gels were sliced horizontally and incubated in the reaction mixture for five hours at 37°C. They were then transferred to destaining solution (methanol 5 vols., water 5 vols., acetic acid 1 vol.) for 18 hours. After destaining, the intensity of the slowly migrating band was evaluated visually and classified into one of five classes: 0—no detectable phosphatase activity in the position of the slowly migrating band; 1—very weak or questionable band; 2—definite but weak band; 3—band of moderate intensity; 4—strong band.

No significant loss of activity in the slowly migrating band due to storage could be discerned. Samples stored as long as three years yielded a distribution of phosphatase classes similar to that in samples collected more recently.

RESULTS

Quantitative Nature of Variation

Although they report some variation in strength of the slowly moving phosphatase band when present, Arfors, Beckman, and Lundin (1963a) have classified the variation simply as Type 2 (positive) or Type 1 (negative). These authors state (Arfors, Beckman, and Lundin, 1963b) that the band is an alkaline phosphatase, but they have detected it under staining conditions designed for detecting *acid* phosphatases. Under conditions more nearly optimal for alkaline phosphatases, we have been able to obtain more intense staining of the same band and have observed that the intensity of the band after staining is distributed as a continuous variable rather than in discrete positive or negative classes. For present purposes, therefore, it was felt that more information could be obtained by a semiquantitative classification. Consequently the variation was classified into five categories, as already described. Figure 1 shows the classes 0 through 4. The quantitatively variable component is the band labeled B. The more rapidly migrating band A is always present, although also of varying intensity, as will be described in a subsequent section. In some sera, a still slower band C is observed. The significance of this band is not understood at present. Also in some sera, band D, close to the origin,

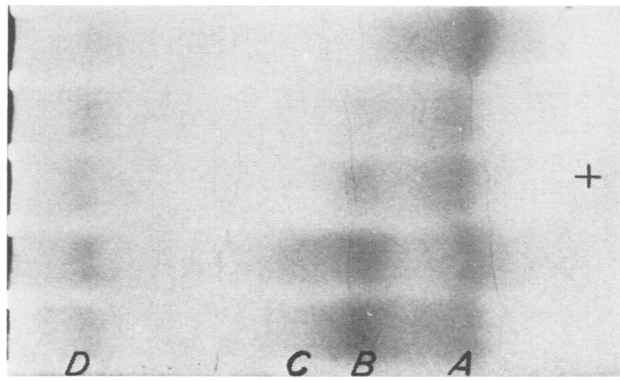

FIG. 1. Semiquantitative classes of activity in alkaline phosphatase band B after starch gel electrophoresis. Phosphatase classes are, from top to bottom: 0, 1, 2, 3, and 4. The bands A, B, C, and D, discussed in the text, are labeled at the bottom of the gel.

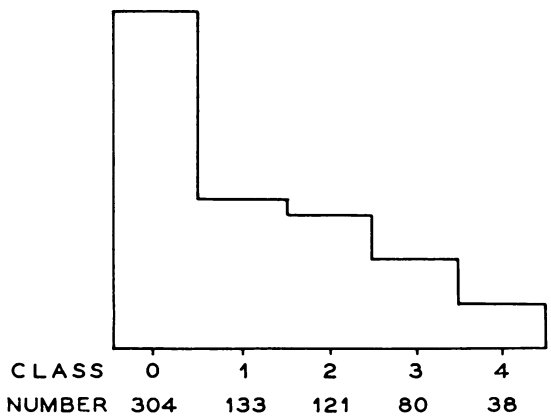

FIG. 2. Frequencies of the five phosphatase classes in a Caucasian population.

stains very intensely. This occurs only when a very intense band A is present. Its position coincides with that of the beta-lipoprotein and its presence may be due to nonspecific binding to beta-lipoprotein (cf. Boyer, 1961).

The frequencies of the five phosphatase classes in the Caucasian Tecumseh population are shown in Fig. 2. This shows the continuous nature of the distribution and the relatively high frequency of phosphatase Class 0 individuals. In this sample, 46% fell in Class 0, compared with 72.5% Type 1 (negative) found by Arfors, Beckman, and Lundin (1963a) in a group of 200 twin pairs. This lower percentage of negatives probably reflects the greater sensitivity of the present staining method. The 54% positive (Classes 1 through 4) compares favorably with the 60% who had a detectable slow-moving band in the sample of fifty normal and pathological sera studied by Cunningham and Rimer (1963).

Factors Influencing Variation

ABO type. Table 1 shows the distribution of phosphatase classes by ABO and Secretor types in the Tecumseh population. Note that the majority of

TABLE 1. DISTRIBUTION OF PHOSPHATASE CLASSES BY ABO AND SECRETOR TYPE

Secretor	ABO	Phosphatase class 0	1	2	3	4	Total	Mean
Pooled	A_1	162	59	16	0	0	237	0.38
	A_2	40	18	8	1	1	68	0.54
	B	15	5	20	10	7	57	1.81
	O	74	44	67	69	30	284	1.78
	A_1B	10	5	3	0	0	18	0.61
	A_2B	3	2	7	0	0	12	1.33
		304	133	121	80	38	676	
Secretor	A_1	110	47	15	0	0	172	0.45
	A_2	20	16	7	1	1	45	0.82
	B	3	3	20	10	7	43	2.35
	O	13	29	65	69	30	206	2.36
	A_1B	6	5	3	0	0	14	0.79
	A_2B	2	1	7	0	0	10	1.50
		154	101	117	80	38	490	
Nonsecretor	A_1	52	12	1	0	0	65	0.22
	A_2	20	2	1	0	0	23	0.17
	B	12	2	0	0	0	14	0.14
	O	61	15	2	0	0	78	0.24
	A_1B	4	0	0	0	0	4	0.00
	A_2B	1	1	0	0	0	2	0.50
		150	32	4	0	0	186	

χ^2 (ABO) = 227.7, 20 df, $P < 0.001$
χ^2 (Secr) = 149.6, 4 df, $P < 0.001$

group A_1 individuals fall into Class 0, but a number were also placed in Classes 1 and 2, indicating that the association between the two traits is not absolute. There is a relatively high frequency of blood group O in all five phosphatase classes. These findings are basically in agreement with the type of association observed by Arfors, Beckman, and Lundin (1963a). The present data show, however, the wide range of phosphatase levels among blood group O individuals and the relatively low levels occurring in those group A_1 individuals who have a detectable phosphatase B band. This sample is also large enough to define the association with respect to blood groups A_2 and B. Among A_2 individuals the distribution is similar to that among A_1 individuals. There is a greater frequency of individuals having a detectable phosphatase band B, as shown by the higher mean value for phosphatase level, particularly among secretors. There is no significant difference between groups A_1 and A_2 in phosphatase distribution, however. Nor is there a significant difference between groups B and O in phosphatase distribution. Apparently, the basic distinction is that between group A and non-A. The A_1B and A_2B individuals fall between the group A and non-A extremes. There is no significant difference between homozygotes and heterozygotes in group A_1, however. Among fifty group A_1 individuals from the Tecumseh sample genetically proved to be A_1O heterozygotes, the mean phosphatase level was 0.28 compared with 0.41 for the remainder of the group A_1 individuals. This difference is not

TABLE 2. RELATIONSHIP OF PHOSPHATASE CLASS TO LEWIS TYPE

	Phosphatase class						
	0	1	2	3	4	Total	Mean
Le(a−b−)	0	3	7	6	1	17	2.29
Control (See text)	16	32	85	79	37	249	2.35

$\chi^2 = 2.02$, 4 df, $0.8 > P > 0.7$

significant, nor is there a significant difference between the two groups in the distribution of phosphatase levels.

Secretor type. Among ABH nonsecretors, no individual was found with a phosphatase classification greater than 2, regardless of ABO type (Table 1). The majority of individuals (81%) were classified as Class 0. Probably with respect to nonsecretor type, as with the group A_1 effect, the association is not absolute, although this is not so definite as it was in the case of group A_1; only four nonsecretor individuals were classified in Class 2, and those in Class 1 are, by definition, of a questionable nature. Further studies with a more sensitive assay will be required to determine with certainty whether the ABH nonsecretor individual truly lacks the phosphatase B component completely. It seems probable that the nonsecretor effect is a more basic one than the ABO, since no significant differences in distribution of phosphatase classes occur between different ABO types among nonsecretors ($\chi^2 = 3.44$, 10 df, $0.95 > P > 0.90$).

Since the ABH nonsecretor individual is usually Le(a+b−), these results support the findings of Arfors, Beckman, and Lundin (1963b), who reported that Le(a+b−) individuals lack the B phosphatase band. However, the primary effect seems to be due to Secretor type rather than Lewis type. Evidence for this is shown in Table 2. The 17 individuals tabulated were drawn from the Tecumseh random sample and from the family groups to be discussed later. All individuals were group B or O and Le(a−b−) on the red cells, ABH secretors, and nonsecretors of Le^a. They are, therefore, in all probability homozygous for the recessive *le* allele, as postulated by Ceppellini (1959) in his explanation of the inheritance of the Lewis blood groups. Such individuals have little, if any, Le^a or Le^b substance in their saliva and plasma. The distribution of phosphatase classes is the same as that in the control, which is composed of all group B plus group O ABH secretors from the Tecumseh sample. Thus the important factor is the ABH secretor type, not the Lewis type.

No effect on phosphatase level of dosage for Secretor type could be detected. The mean phosphatase classification among 48 proved *Se/se* heterozygotes from the Tecumseh sample (all group O or group B) was 2.38 compared with 2.35 among the 201 remaining group B plus group O secretors in the sample. The difference in the means is not significant, and no significant difference occurs between the phosphatase distributions in the two groups.

Other known genetic systems. Tests for association between phosphatase variation and other genetic markers were also carried out. Negative results

TABLE 3. VARIATION AMONG DIFFERENT SPECIMENS FROM SAME INDIVIDUAL

Source	df	S.S.	M.S.	F	P
Among individuals	15	115.5	7.70	5.75	<0.005
Among samples from same individual	32	43.0	1.34	4.06	<0.005
Between determinations on same sample	48	16.0	0.33		
Total	95	174.5			

were obtained for the MNSs, Rh, Kell, Duffy, Kidd, and P blood group systems, and for the Gm, Gc, and haptoglobin serum types. No dependence of phosphatase distribution upon sex was apparent in this sample. No association with nine B_2C or five CD_1 transferrin variants in the random sample of 676 could be discerned; however, the small numbers involved would only be useful for the detection of a very close association.

Temporal effect. To test the reproducibility of the method for determining the phosphatase class of an individual and to determine the persistence of an individual's phosphatase level over a period of time, a series of 48 serum specimens, composed of three samples taken at different times from each of 16 individuals, was examined. The intervals between sampling varied from two to seven weeks. Each specimen was subjected to electrophoresis and phosphatase classification on two separate occasions. An analysis of variance on the results of these classifications is shown in Table 3. This reveals that, although the variance between determinations on the same sample is relatively high, as would be expected with so crude a classification procedure, the differences in phosphatase classes among different samples from the same individual are significant at the 0.005 level of probability. As expected, a highly significant difference occurs among individuals.

This analysis clearly demonstrates that the level of phosphatase B does not remain constant with time. Variations occur between samples taken at different times from the same individual, beyond those attributable simply to lack of reproducibility of the classification procedure. The reasons for these temporal fluctuations are not clear. They could result from seasonal effects, diet, physiological state, or diurnal variation. Further studies will be necessary to determine the origin of this variation.

Age effect. Table 4 shows the distribution of phosphatase levels by age for all ABO and Secretor types and for group B and O secretors only. The total in Table 4(a) is larger by six than the total in Table 1 because it includes six individuals who were not secretor-typed and therefore were excluded from Table 1. Table 4(a) contains data from the Tecumseh sample. These data have been grouped into four age spans within which the mean phosphatase levels are quite homogeneous. The only statistically significant difference is between groups greater and less than 16 years of age. In order to test whether this age effect was related to blood groups, classifications by age and ABO and Secretor type were done. In Table 4(b) are included all group B and O secretor individuals from the Tecumseh random sample, plus all of

TABLE 4. DISTRIBUTION OF PHOSPHATASE CLASSES BY AGE AND BLOOD TYPE

Age group	Phosphatase class 0	1	2	3	4	Total	Mean
(a) All ABO and Secretor types							
5–15	97	41	28	17	5	188	0.89
16–20	20	16	11	9	4	60	1.35
21–40	92	35	35	25	11	198	1.13
above 40	96	44	45	31	20	236	1.30
Total	305	136	119	82	40	682	1.14

$\chi^2 = 16.80$, 12 df, $0.2 > P > 0.1$

χ^2 (5–15 vs. >15) $= 10.84$, 4 df, $0.05 > P > 0.02$

(b) Group B and O secretors only							
5–15	23	45	39	24	7	138	1.62
16–20	1	7	18	12	8	46	2.41
21–30	5	2	19	12	4	42	2.19
above 30	14	23	72	64	42	215	2.45
Total	43	77	148	112	61	441	2.16

$\chi^2 = 62.08$, $P < 0.001$

(c) Means for group B and O secretors by age and sex

Age group	Males No.	Males Mean	Females No.	Females Mean
5–15	74	1.82	64	1.53
16–20	14	2.64	32	2.31
21–30	22	2.50	20	1.85
above 30	108	2.49	107	2.41
Total	218	2.23	223	2.09

the individuals included in the family studies to be discussed in a later section. The two groups were homogeneous, so were pooled in this table. Again, a very striking difference between the 5–15 and the above 15 age groups is evident. The distribution in the 21–30 age group is not significantly different from that of the 16–20 or above 30 groups. The data in Table 4(b) were further subdivided by sex, and the means by age and sex are presented in Table 4(c). The mean for the age group 21–30 is strikingly lower in females; however, when phosphatase distributions among males and females for each of the four age groups are compared, the differences are not statistically significant. A much larger sample would be required to test whether these differences are meaningful. Analysis of phosphatase levels by age among (1) only group A_1 individuals, (2) only nonsecretor individuals, and (3) the total Tecumseh sample minus group B and O secretors reveals no significant age effect in these groups. Thus it appears that the age effect demonstrated in Table 4(a) is attributable to differences among group B and O secretors, with the principal effect being a significantly lower phosphatase level before the age of approximately 16 years. This, it will be noted, is exactly opposite the effect noted in this age group for total serum alkaline phosphatase activity (Bodansky, 1933).

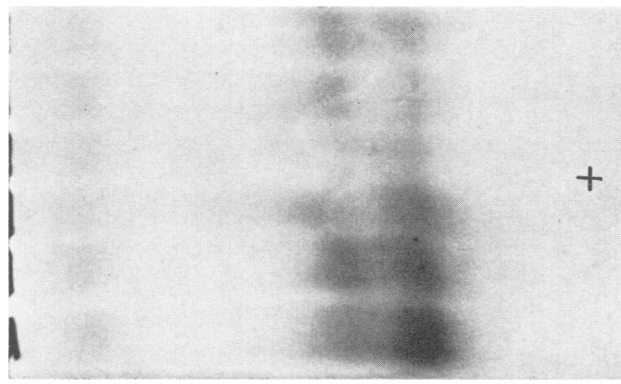

FIG. 3. Variation in activity of alkaline phosphatase band A after starch gel electrophoresis. Band intensities were classified from top to bottom: 2, 1, 2, 3, 4, and 4.

Wide variation in the intensity of the rapidly migrating band A was also observed. This variation was classified into Classes 1 through 4, on the basis of band intensity, in a manner similar to that used for the B component. Figure 3 shows the different classes. Analyses of these groups by age leads to the very striking association shown in Table 5. The concentration of component A in the 5–16 age group is significantly and uniformly higher than in the group older than 16 years. It seems quite probable that the high total serum alkaline phosphatase values found in children below age 16 (Bodansky, 1933) are due primarily, if not entirely, to higher concentrations of component A.

Other genetic factors. The data presented here leave no doubt that the ABO and Secretor loci have a significant association with level of phosphatase B. If an individual is group A or a nonsecretor, his phosphatase B level is low or zero, whereas among group B or O secretors the levels may range from zero to very high values and the mean level is high. Figure 4 presents the distribution among all of the randomly ascertained B and O secretors tested thus far. The distribution approximates a normal distribution. A number of analyses have been carried out to determine whether there is any genetic basis for this variation.

As a first approach to this problem, a twin analysis was conducted. Serum specimens from 125 pairs of dizygotic and 101 pairs of monozygotic adult male twins of all ABO and Secretor types were available from another study (H. Gershowitz and J. V. Neel, unpublished). All of these sera were analyzed for phosphatase B levels. An analysis of variance was performed and intraclass correlations were calculated, first on the entire group. These are shown in Table 6(a). The correlation between monozygotic twins is more than twice that for dizygotic twins. The variance ratio indicates a highly significant difference in the within pair variances. This is consistent with the findings of Arfors, Beckman, and Lundin (1963a) and indicates significant genetic factors controlling the phosphatase B variation. These authors found complete concordance among monozygotic twins for phosphatase types but 35% discordance among dizygotic twins. Our quantitative analysis does not support

TABLE 5. ASSOCIATION OF PHOSPHATASE COMPONENT A CONCENTRATION WITH AGE

Age group	Band A phosphatase class				Total
	1	2	3	4	
5–16	0	2	20	24	46
Above 16	13	37	2	0	52
Total	13	39	22	24	98

$\chi^2 = 76.92$, 3 df, $P < 0.001$

complete concordance among monozygotic twins but does show significantly greater similarity than exists in dizygotic twins. The variance within monozygotic pairs is probably due to temporal variation and to technical error.

Since the ABO and Secretor effects on phosphatase level are quite marked and since approximately 40% of dizygotic twin pairs in this sample were discordant for group A versus non-A and/or secretor versus nonsecretor types, it would be quite remarkable if a significant difference were not observed. The important question is whether the difference is due solely to ABO and Secretor effects. To test this, only those twin pairs who were of group B or O and also ABH secretors were analyzed. Twenty-seven pairs of dizygotic and 40 pairs of monozygotic twins were available for this analysis. The results are presented in Table 6(b). While the correlations are somewhat lower, the relative magnitudes are approximately the same as those for the entire group. The variance ratio is about the same and is again significant. These results strongly suggest that significant genetic determinants are operating in the control of the variation in phosphatase levels among group B and O secretors.

To obtain further evidence on this point, a series of 56 group B and O secretor couples with 121 group O or B secretor offspring was taken from the Tecumseh material, and phosphatase classifications were done on their sera. The distribution of phosphatase values among offspring of the various mating types is given in Table 7. The mean values show a general correlation of the children's phosphatase levels with those of their parents, although the levels in the children are usually lower, due to the age effect. No discrete

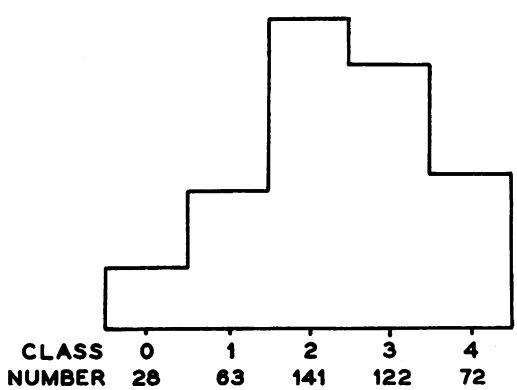

FIG. 4. Distribution of phosphatase classes among group B and O secretors.

TABLE 6. ANALYSIS OF TWIN DATA

Source	df	S.S.	M.S.	F	P
(a) All ABO and Secretor types					
Dizygotic twins					
Between pairs	124	290.58	2.34	1.98	<0.005
Within pairs	125	147.00	1.18		
Total	249	437.58			
Monozygotic twins					
Between pairs	100	342.42	3.42	6.58	<0.005
Within pairs	101	52.50	0.52		
Total	201	394.92			
Variance ratio $\frac{W_{DZ}}{W_{MZ}} = \frac{1.18}{0.52} = 2.27\ (P < 0.005)$					
Intraclass correlations $r_{DZ} = 0.332$, $r_{MZ} = 0.736$					
(b) B and O Secretors only					
Dizygotic twins					
Between pairs	26	51.37	1.98	1.72	>0.10
Within pairs	27	31.00	1.15		
Total	53	82.37			
Monozygotic twins					
Between pairs	39	87.01	2.23	4.13	<0.005
Within pairs	40	21.48	0.54		
Total	79	108.49			
Variance ratio $\frac{W_{DZ}}{W_{MZ}} = \frac{1.15}{0.54} = 2.13\ (0.025 > P > 0.01)$					
Intraclass correlations $r_{DZ} = 0.265$, $r_{MZ} = 0.612$					

pattern of segregation can be discerned. Table 8(a) shows the results of an analysis of variance of these data. There are significant differences between mating types and between families within mating types. The intraclass correlation is 0.598. These are the results expected if genetic factors play a significant role in determination of the phosphatase B variation, and they support and complement the results of the twin studies.

It is also possible, however, that these effects could be due to common environmental factors in families. As a test of this, the within and between variances of the parental pairs were determined and compared, as shown in Table 8(b). The variance ratio of 1.37 indicates no significant association of phosphatase B level among parents. The correlation coefficient of 0.154 is not significant. This argues against common environment as the principal factor contributing to the significant family and mating type effects shown by the analysis of variance of family data.

Thus it appears that there is genetically determined variation in the capability of each individual to produce the phosphatase B component. The group A and nonsecretor types apparently mask or inhibit the expression of the phosphatase B. If this is true, the group B or O secretor offspring of group

TABLE 7. SUMMARY OF GROUP B AND O SECRETOR FAMILY DATA

Mating type*	No. families	No. children	No. children in class					Mean
			0	1	2	3	4	
0×1	1	2	1	1	0	0	0	0.50
0×2	3	6	5	1	0	0	0	0.17
0×3	2	4	2	1	1	0	0	0.75
1×1	2	5	1	2	2	0	0	1.20
1×2	9	22	5	7	7	2	1	1.41
1×3	1	4	0	0	0	2	2	3.50
1×4	2	4	1	1	0	1	1	2.00
2×2	6	22	7	9	5	1	0	1.00
2×3	10	18	0	3	7	7	1	2.33
2×4	7	12	0	5	3	2	2	2.08
3×3	4	7	0	0	4	2	1	2.57
3×4	8	14	1	4	6	2	1	1.86
4×4	1	1	0	0	1	0	0	2.00
Total	56	121	23	34	36	19	9	1.64

*Reciprocal matings pooled.

TABLE 8. ANALYSIS OF VARIANCE FOR GROUP B AND O SECRETOR FAMILIES

Source	df	S.S.	M.S.	F	P
(a) *Families*					
Among families	55	129.47	2.35	4.20	<0.005
Among mating types	12	62.12	5.18	3.30	<0.005
Among families within mating types	43	67.35	1.57	2.80	<0.005
Within families	65	36.43	0.56		
Total	120	165.90			
		Intraclass correlation = 0.598			
(b) *Parental couples*					
Between couples	57	80.7	1.42	1.37	>0.10
Within couples	58	60.5	1.04		
Total	115	141.2			
		Intraclass correlation = 0.154			

A or nonsecretor parents should show a normal distribution of phosphatase B levels. Although such families occur infrequently, a number have been analyzed and are tabulated in Table 9. The distribution of levels among the group B and O secretor offspring of such matings is similar to that among the children in the group B and O secretor families. Thus, the ABO and secretor types of the parents do not directly affect the distribution of phosphatase B levels in their children. It would appear that the group A and secretor effects are superimposed on a background of additional genetic variations which are independent of ABO and secretor type.

DISCUSSION

These data support the findings of Arfors, Beckman, and Lundin (1963a), with respect to the ABO effect on phosphatase B expression, and extend them

TABLE 9. SUMMARY OF FAMILIES PRODUCING GROUP B OR O SECRETOR OFFSPRING IN WHOM ONE OR BOTH PARENTS WERE GROUP A_1 OR NONSECRETORS

S = ABH Secretor. NS = ABH Nonsecretor. Phosphatase class in parentheses.

Mating (Male × Female)	Offspring			
	A_1 S	A_1 NS	B or O S	B or O NS
(a) *Both parents group A or nonsecretor*				
1. $A_1S(1) \times A_1S(0)$	2–(0)	—	1–(3)	—
	1–(1)	—	—	—
2. $ONS(0) \times A_1BS(1)$	—	—	1–(2)	—
	—	—	1–(3)	—
3. $A_1S(0) \times ONS(0)$	—	—	1–(3)	1–(0)
4. $ONS(0) \times A_1S(0)$	1–(0)	—	1–(2)	—
5. $A_1S(0) \times BNS(0)$	1–(0)	—	1–(2)	—
6. $A_1S(0) \times A_1NS(1)$	1–(0)	—	1–(1)	—
7. $A_2S(2) \times A_1NS(1)$	1–(0)	—	1–(2)	—
8. $A_1S(1) \times ONS(0)$	2–(1)	1–(0)	1–(2)	—
9. $A_2S(1) \times ONS(1)$	—	—	1–(1)	—

Summary of Group B and O Secretor Offspring

	Phosphatase Class				
	0	1	2	3	4
Above matings	0	2	5	3	0
Control (Family data)	23	34	36	19	9

$\chi^2 = 2.57$, 4 df, $0.7 > P > 0.5$

	A_1 S	A_1 NS	B or O S	B or O NS
(b) *One parent Group B or O Secretor, other Group A_1 or Nonsecretor*				
1. $ONS(0) \times OS(4)$	—	—	1–(2)	2–(0)
2. $ONS(0) \times OS(3)$	—	—	1–(1)	—
	—	—	1–(3)	—
3. $ONS(0) \times OS(2)$	—	—	1–(2)	3–(0)
4. $OS(4) \times ONS(0)$	—	—	1–(2)	—
5. $OS(3) \times ONS(0)$	—	—	1–(2)	1–(0)
6. $OS(2) \times BNS(1)$	—	—	2–(2)	—
7. $BS(3) \times ONS(1)$	—	—	1–(0)	—
	—	—	2–(3)	—
8. $OS(3) \times A_1NS(0)$	—	—	1–(2)	—
9. $OS(3) \times A_1NS(0)$	—	1–(1)	1–(3)	—
10. $OS(4) \times A_1NS(0)$	1–(0)	—	1–(1)	1–(1)
11. $A_1NS(0) \times OS(2)$	—	—	1–(3)	—
12. $OS(4) \times A_1NS(0)$	1–(0)	—	2–(2)	—
	1–(1)	—	—	—
13. $OS(2) \times A_1S(0)$	—	—	2–(1)	—

Summary of Group B and O Secretor Offspring

	Phosphatase Class				
	0	1	2	3	4
Above matings	1	4	9	5	0
Control (Family data)	23	34	36	19	9

$\chi^2 = 3.26$, 4 df, $0.7 > P > 0.5$

by showing the continuous nature of the variation. They further show that the Secretor locus is involved in phosphatase B expression. There can be no question about the dependence of phosphatase B levels upon alleles at the ABO and Secretor loci, but it is also clear that these are not absolute effects. Some A's and some nonsecretors have detectable amounts of phosphatase B. Thus it seems probable that the ABO and Secretor effects are secondary rather than the result of direct gene action at these loci.

The ABO blood group effect appears to involve simply group A versus non-A. It may be significant that group A_2 individuals, who have quantitatively lower levels of A substance on their cells and possibly in their secretions (Race and Sanger, 1962), tend to have higher levels of phosphatase B. It would be of great interest to determine whether phosphatase B level in group A individuals is correlated with level of A substance on the erythrocytes or in the secretions. The Secretor effect is more difficult to interpret in terms of quantitative differences in blood group substances. All nonsecretors lack H substance, which might therefore be postulated as necessary for the expression of phosphatase B. However, group B secretor individuals, who secrete the least H substance relative to other ABO types, and group O secretor individuals, who secrete the largest amounts of H substance (Plato and Gershowitz, 1961), are indistinguishable in their phosphatase distributions. Le^a and Le^b substances do not seem implicated in any simple way, since the distribution of phosphatase B levels is the same among nonsecretors and secretors of Lewis substances. It would seem worthwhile, however, to attempt correlation of phosphatase levels with plasma and saliva levels of the various blood group substances.

The twin and family studies on group B and O secretors show that additional genetic factors also are operative in the control of variation in phosphatase B level. The present data are not compatible with control of this variation by a single genetic locus. The effect would appear to be polygenic. However, it is possible that the other sources of variation—age, temporal effects, and technical inadequacies—could obscure single gene control. Further definition of this effect will be dependent upon the development of more quantitative methods for scoring phosphatase B level, better control of age in family studies, and better definition of the temporal effects.

Since there are apparently many serum components showing alkaline phosphatase activity (Boyer, 1961), it would seem desirable to determine the tissue of origin of this component. Studies by Hodson, Latner, and Raine (1962) indicate that a slowly-migrating alkaline phosphatase component, with a mobility similar to that of the B band, has its origin in the intestine. The A band possibly contains two components, one derived from bone and one from liver (Hodson, Latner, and Raine, 1962). The very striking age effect on the A band correlates well with the period of active bone formation and the period of high total serum alkaline phosphatase activity thought to be due to high levels of osteoblastic alkaline phosphatase. Therefore, there seems a reasonable probability that the phosphatase B component may be derived from the intestine and the A component from bone.

Greatest interest in this system resides in the implications with respect to gene action at the ABO and Secretor loci. The findings of similar associations between specific serum alkaline phosphatase components and the homologous bovine J and ovine R blood group systems greatly strengthen the likelihood of some general and important functional relationship. The ABO polymorphism over the years has been implicated in a variety of biological phenomena—association with peptic ulcer, gastric cancer, and other diseases; prezygotic selection; maternal-fetal incompatibility, etc. In this instance the association is with an identifiable biochemical activity. Further elucidation on this association and definition of the specific function of the phosphatase component could contribute to the understanding of the mechanisms of gene action which control the structure and expression of the ABH blood group substances and of the selective factors which maintain the ABO blood group polymorphism.

SUMMARY

Studies of previously reported variation in a specific serum alkaline phosphatase component detected by starch gel electrophoresis show that the variation is quantitative in nature. Expression of the phosphatase is masked or inhibited in individuals of ABO group A and in nonsecretors of ABH blood group substances. The nonsecretor effect is apparently not directly related to the Lewis blood group system. No association with other blood group or serum protein variant systems nor with sex was detected. Significant temporal variation in the level of the specific phosphatase in the serum of a given individual has been demonstrated. Among group B and O secretors, in which phosphatase expression is not inhibited, a significant age effect has been defined. The mean level of the specific phosphatase in adults is approximately 50% higher than that in children below the age of 16. Among group B and O secretors, a wide range of phosphatase levels is observed. Twin studies and family studies of this variation strongly indicate that it is genetically controlled, apparently by multiple loci.

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