Alkaline Phosphatase in Human Sera and Placentae

Starch gel electrophoresis reveals many phosphatase components including a polymorphism in placentae.

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Heterogeneity of nonspecific human serum alkaline phosphatase has been demonstrated by electrophoresis on starch granules (*1*), by cellulose chromatography (*2*), and by immunologic means (*3*). No more than three types of phosphatase have been observed by any one method. Our observations indicate that at least 16 bands of nonspecific alkaline phosphatase activity are evident in human sera following electrophoresis on hydrolyzed starch gel (*4*), although not all occur in a single individual. The purposes of this investigation have been, firstly, to describe the influence of disease, pregnancy, and ethnic origin upon the appearance of certain zones of enzyme activity; secondly, to explore by other methods the validity of the observed heterogeneity; and thirdly, to test the hypotheses that heterogeneity of the serum phosphatases peculiar to pregnancy reflects diversity of placental phosphatase and that such diversity is under simple genetic control.

Vertical starch gel electrophoresis was performed with a discontinuous buffer system (*5*) at 4°C. Zones of enzyme activity were developed directly on a longitudinal gel slice by using a solution containing 0.05-percent beta-naphthyl sodium phosphate, 0.005*M* $MgSO_4$, 0.05-percent fast blue RR salt, and 0.06*M* *p*H 9.7 sodium borate-boric acid buffer (*6*).

The nonspecific alkaline phosphatase activity observed after electrophoresis has been arbitrarily divided into A, B, C, D, E, and F zones. The most rapidly migrating zone, A, lies immediately cathodic to the transferrin C band while the D zone centers about the area of the haptoglobin 1-1 band. Examples of all except the uncommon E zone are shown in Fig. 1. Further diversity exists within zones such that two distinct bands have been observed in the A zone, two in the B zone, three in the C zone, five in the D zone, and three in the F zone. Among more than 700 sera examined, no one serum exhibited more than four zones with a total of eight distinct bands. Normal adults possess one or two C components and occasionally a faint F band. The single C component of childhood is uniformly slower than the C bands of adults.

With one exception, the A, B, and the more slowly migrating D components have been observed only in pregnancy. Such D components as are seen in pregnancy are uncommon and limited to Negroes. West African Negroes present much variation in the D zone. None of the phosphatase components of pregnancy have been seen prior to the 15th week of gestation. One or both of A and B zones are evident in almost all women by the 28th week of pregnancy. Once present these zones persist throughout gestation and disappear by the sixth postpartum week. The relative proportion of total serum alkaline phosphatase represented by the A and B components is approximately 0.50 and is sufficient to account for the major portion of serum alkaline phosphatase elevation developing during pregnancy. No evidence of A and B components appeared in the umbilical cord serum of more than 30 infants whose mothers possessed one or both these components. Components equivalent to A and B were not present in the serum of five pregnant rhesus monkeys.

The relative proportion of women with A-zone activity was found to differ between American whites and American Negroes as shown in Table 1. Although numerous individuals with both A- and B-zone activity were detected, the only accurate distinction that could be simply made in every case was between A and "not-A." All "not-A" persons have B-zone activity. The differences between the two ethnic groups suggested the existence of a genetic polymorphism. Some confirmation of this possibility was provided by examination of sera from Nigerian women in the last trimester of pregnancy (Table 1). With the assumption that the presence of A-zone activity is determined by a dominant or co-dominant gene (the allele being responsible for zone B), gene frequencies can be computed with the further assumption of a Hardy-Weinberg equilibrium (Table 1). An independent estimate of American Negro hypothetical allele frequency can then be realized from the knowledge that the American Negro is approximately 70 percent West African and approximately 30 percent European in genetic origin (*7*). The frequency of the hypothetical A allele in American Negroes thus computed is

$$(0.7)(0.063) + (0.3)(0.319) = 0.140,$$

a figure in excellent agreement with the directly computed value of 0.134. Such argument suggests, although it does not prove, that the presence of A-zone alkaline phosphatase activity is simply inherited.

Among more than 120 patients, with a variety of disease and elevated serum alkaline phosphatase activity, only two had activity in other than C and F zones. Simple starch gel electrophoresis of nonspecific serum alkaline phosphatase does not, therefore, offer promise as a tool for differential diagnosis of disease.

The F zones may be α_1-, α_2-, or α_2-β-globulin in conventional mobility. Several F components may be present after two-dimensional (paper-starch gel) (*8*) electrophoresis, although only one appears after simple migration on starch gel. The F zones appear to be related to the more rapidly migrating zones, since the appearance of D components is often accompanied by very slow F components. Tissue alkaline phosphatases are often associated with lipoproteins (*9*). Binding of certain serum alkaline phosphatases to large molecule lipids might account for the scant mobility of the F zone; however, neither F nor other zones of serum

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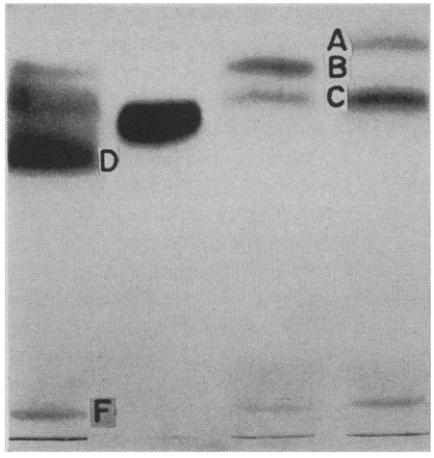

Fig. 1. Electrophoretic migration on starch gel of nonspecific alkaline phosphatase in four different sera. Migration was toward the top of page. The slots of sample insertion can be seen at the bottom of illustration. From left to right the serum patterns are BCD_2, C-childhood, BC, and AC. F components are present, but poorly displayed, in all sera.

alkaline phosphatase behave as lipoproteins during ultracentrifugation in dense solution. Furthermore, *n*-butanol, which breaks the lipid-protein association (*9*), had no influence on serum-band or tissue-band alkaline phosphatase migration. A further characteristic of the F zone is its disappearance in concentrations of Rivanol (2-ethoxy-6,9-diaminoacridine lactate) which leave A, B, and C components unaffected.

Additional evidence for heterogeneity of serum nonspecific alkaline phosphatases in normal subjects is provided by cellulose chromatography. Sera from eight individuals with BC components and from one with AC components were pooled. Alkaline phosphatase was separated from the bulk of serum proteins by fractionation with Rivanol. Rivanol was removed by passage through G-75 Sephadex, and phosphatase was then concentrated on a 0.01*M* NaCl diethylaminoethanol-cellulose column. Phosphatase was eluted as a concentrate, dialyzed, applied to a triethylaminoethanol-cellulose column, and then eluted with a linear NaCl gradient. Eluted fractions from triethylaminoethanol cellulose were characterized by enzymatic assay and by their behavior during starch gel electrophoresis. The small amount of A-zone activity as well as previously undetected D-zone activity formed a single peak distinct from the remainder, and in addition some separation of B and C zones occurred. Complete chromatographic separation of human placental and intestinal alkaline phosphatase was also obtained.

Immunologic evidence for serum nonspecific alkaline phosphatase heterogeneity was provided by the successive use of rabbit antihuman alkaline phosphatase and starch gel electrophoresis. Rabbit antiplacental and anti-bone human alkaline phosphatase were prepared by the subcutaneous injection of partially purified enzyme (*10*) in company with a complete Freund adjuvant. Individual sera with various phosphatase patterns were reacted with an antienzyme for several days. Thereafter the supernatant fluid was examined electrophoretically in parallel with a nonreacted serum sample. A, B, D, and certain F bands were uniformly removed by rabbit antiplacental phosphatase, while C-zone activity was undisturbed. Unfortunately, a certain amount of cross reaction occurred with partially purified human intestinal and liver alkaline phosphatase, and accordingly, by this technique, the identity of A, B, and D zones of activity with placental phosphatase remains in doubt. Rabbit anti-bone phosphatase removed only C-zone activity and did so completely in normal and pregnant adults, children, and a patient with Paget's disease. Rabbit anti-bone phosphatase did not cross react with partially purified alkaline phosphatases from placenta and intestine. Immunologic studies with kidney and liver alkaline phosphatase are in progress. Electrophoretically separable tissue alkaline phosphatase components can also be resolved chromatographically. Consequently the problem of cross reactions can possibly be resolved in the future by the use of antigens which are chromatographically pure. The use of anti-bone phosphatase, unlike antiplacental phosphatase, resulted in the formation of a distinct ladder of newly formed slowly migrating zones. Such zones may represent soluble antigen-antibody complexes in the states of antigen excess and antibody excess. In the case of antibody excess visible evidence is possibly provided for the existence of such complexes (*11*). Enzyme precipitates failed to appear in titrations between purified human bone alkaline phosphatase and its antienzyme.

Nonspecific alkaline phosphatases obtained from various human organs have been compared with serum components. Purified bone alkaline phosphatase, prepared from an osteogenic sarcoma (*10*), and milk alkaline phosphatase (*12*) exhibit migration similar to the C zone. Intestinal alkaline phosphatase (*10*), obtained from three individuals, migrated, in each case, in a fashion similar to D zone. Several F zone type components of varying mobility were also present in each organ extract. Two-dimensional electrophoresis (paper-starch gel) indicated no precise agreement of any organ alkaline phosphatase migration with any of the known major serum components. However, among 20 unselected women, the appearance of serum A and B components was in every case precisely reproduced by *n*-butanol extracts of the serum donor's placenta. Contamination of placenta with the donor's blood as a source of agreement was excluded by the failure to observe in placental extracts any of the C components present in serum. Furthermore, the phosphatase activity of placental extracts is considerable and necessitates dilution prior to electrophoretic analysis, thereby minimizing the importance of contamination by the relatively low levels of serum phosphatase. Three placental nonspecific alkaline phosphatase types in the A and B zones were observed (Fig. 2). If the unproved assumptions concerning simple genetic control of A and B zones are correct, then by a co-dominant hypothesis there should be three phenotypes,

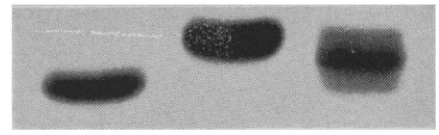

Fig. 2. Electrophoretic migration on starch gel of nonspecific alkaline phosphatase in *n*-butanol extracts of three different human placentae. The origin has been removed and only the relevant portion of analysis is shown. Migration was toward the top of page. From left to right the patterns are B, A, and AB.

Table 1. Proportion of women in the last trimester of pregnancy with serum alkaline phosphatase A-zone activity. Test for homogeneity of A-zone proportion among ethnic groups: $\chi^2 = 28.9$; D.F. = 2; $P < 0.001$.

Number in group	Proportion with A-zone activity	Calculated hypothetical dominant or co-dominant A allele frequency
	U.S. whites	
97	0.536	0.319
	U.S. Negroes	
100	0.250	0.134
	Nigerians	
98	0.122	0.063

namely, *AA*, *AB*, and *BB*. The *AB* type possesses an *A*, a *B*, and an intense intermediate band. Sex of child had no influence on the appearance of placental phosphatase type. The relative proportions of *AA* and *AB*, which were unknown after examination of serum samples, may be projected from the hypothetical allele frequency given in Table 1, being thus approximately 1 (*AA*) : 4 (*AB*) in individuals of European ancestry. Among 12 placentae from such persons three *AA* and nine *AB* patterns appeared. The numbers involved are small; however, the observed ratio tends to support the inference of simple genetic control. Since the placenta has fetal origin, any genetically determined variation in the alkaline phosphatase of this organ would be determined by fetal genotype. The absence in umbilical cord serum of appreciable placental A and B components presumably results from a selective barrier.

It remains that a direct Mendelian proof of genetic control is unattainable at the moment. Nonetheless, if the A and B zones of serum reflect placental phosphatase heterogeneity, as seems likely, and if this diversity has a simple genetic basis, as suggested by the different proportions of A and B zones in several ethnic groups, then the differences in electrophoretic mobility of A and B zones may be due to a single amino acid substitution in placental alkaline phosphatase molecules. Alkaline phosphatase is abundant in the placenta (*13*), and preliminary observations suggest that considerable enzyme purification with good yield is possible. Consequently, it may be practicable to delineate further the differences between A and B placental phosphatase by peptide analysis utilizing the fingerprint technique of Ingram (*14*).

The multiple procedures employed in this investigation, when supplemented by examination of substrate preference, *p*H optimum, and response to inhibitors, may permit identification of the tissue of origin of the divers serum alkaline phosphatases observed after starch gel electrophoresis (*15*).

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Relative Effectiveness of Document Titles and Abstracts for Determining Relevance of Documents

Abstract. Individuals who received documents through a selective dissemination of information system were asked to determine the relevance of documents to their work interests on the basis of titles and of abstracts. The results indicate that there was no significant difference between the usefulness of titles and of abstracts for this purpose.

In two previous experiments, conducted by me and my associates, results seemed to indicate that titles were as useful as various forms of abstracts for determining the relevance of a specific article for a given purpose. But when titles were compared with abstracts as means of obtaining specific information on specific questions, it was found that abstracts were significantly better (*1*). The purpose of the experiment reported here was to compare the effectiveness of titles with that of abstracts when they were used for the purpose of notifying research workers of the availability of documents which might be relevant to their work interests.

During 1960, a selective dissemination of information system was in operation in the Advanced Systems Development Division of the International Business Machines Corporation. One of its purposes was to notify scientists, engineers, managers, and technicians in the organization of the availability of documents which might be relevant to their work interests and to give them the opportunity to order individual copies. A general description of a system of this kind is given by H. P. Luhn (*2*). The system discussed here and an earlier version of it have been described in detail (*3*).

Briefly, the system consists of matching key words that state the interests of users with key words selected from documents processed by the system. When a predetermined percentage *p* of matches occurs, a notification is sent to the user. In the experiment under discussion this percentage was set at between 0.20 and 0.28 (for 93 percent of the documents processed, *p* was at 0.20).

The notification consists of the IBM card printed with the title of the article, the name of the author or authors, the abstract, the source of the document, and the number of pages. Upon receiving the notice, the user determines, among other things, whether or not he is interested in receiving a copy of the document (*4*); if he is, he responds appropriately and is sent a copy. These responses to notices are called "first responses." After reading the document, the user again responds, indicating whether or not the document was in fact relevant to his interests. These are called "second responses." From the results of previous experiments it appeared that utilizing the title only would be as effective for the purpose of ordering documents as utilizing both the title and the abstract. Two hypotheses were formulated to test this assumption.

1) There is no significant difference between the ordering rate for documents when titles are used for notification and the rate when a combination of titles and abstracts is used. ("Hard copy orders on first responses.")

Table 1. Rates of ordering and accepting documents on the basis of notification by title and by title and abstract.

First response (%)		Second response: Judgments of relevance of hard copies received (%)
Hard copy orders	Accepted notifications*	
Notification by title only		
24.6	55.8	61.8
Notification by title and abstract		
24.5	58.1	58.9

* Any of the following responses constitutes an accepted notification: (i) A hard copy is ordered; (ii) the document is of interest, though a hard copy is not ordered; (iii) the document is of interest and the user already has a copy.