A POPULATION STUDY IN HUMAN BIOCHEMICAL GENETICS: THE RELATIONSHIPS OF SEVERAL METABOLITES IN EPILEPSY AND MENTAL RETARDATION

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ABSTRACT

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By

Habibollah Fakhrai

Some mentally retarded individuals are presumed to be suffering from physiological abnormalities induced by their genetics, environment or both. If a number of these individuals have some particular condition in common, they might all have an elevated or a diminished level of a particular metabolite, leading to the discovery of a new syndrome.

Over 1,700 mentally retarded individuals with and without epilepsy and a normal control group of 230 individuals were tested for several metabolites: glucose, blood urea nitrogen, serum glutamate oxalacetate transaminase, uric acid, calcium, inorganic phosphate, total protein, albumin, and alkaline phosphatase. Means, standard deviations, and the number of high and low outliers have been determined for the control and for the retarded and epileptic populations.

Retarded sib pairs in an institution were also tested for the above metabolites in 1971. In 1973 the sib pairs and some individuals with idiopathic epilepsy in the institution were also tested for these serum metabolites. In addition, these individuals were tested for serum ammonia, intestinal and liver alkaline phosphatases, ABO and Lewis blood groups and their secretor status.

Alkaline phosphatase was found to be high and blood urea nitrogen was found to be low in the epileptics when compared with either the other mentally retarded or the normal population. The level of alkaline phosphatase was higher in all retarded populations than in the normal population.

The evidence suggests that many mentally retarded individuals classified as epileptics may actually be suffering from liver damage which has induced ammonia intoxication of the brain. This finding indicates that a more thorough evaluation of epileptics may be necessary.

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Habibollah Fakhrai

A DISSERTATION

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To:

my mother, Gohar Ghotb

my father, Mr. Mahmud Fakhrai

my wife, Farideh

my professor, Dr. Herman M. Slatis

and to Tonya T. who is very special to me.

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INTRODUCTION

Some mentally retarded individuals are presumed to be suffering from physiological abnormalities induced by their genetics, environment, or both. If a number of these individuals have some particular condition in common, they might all have an elevated or a diminished level of a particular metabolite, leading to the discovery of a new syndrome.

Several metabolites were tested in retarded populations with or without epilepsy, including alkaline phosphates and also several blood markers. Any abnormality related to these metabolites or the blood gene markers in any of the observed populations might manifest itself when compared with a normal population.

The results of this study could be useful in better understanding of the genetics of mental retardation and epilepsy, and perhaps would be helpful in working toward their prevention or treatment.

LITERATURE REVIEW

Alkaline phosphatase

The term alkaline phosphatase (International enzyme classification 3:1:3:1) is applied to a group of enzymes sharing the capacity to hydrolyze phosphate mono-esters in an alkaline medium [41,72,73]. The enzyme has been proven to be a glycoprotein because it releases its sialic acid when treated with crystaline neuraminidase [36]. In 1930, Kay [50] stated that he measured phosphatase of blood serum for the first time in humans by using β-glycerophosphate as the substrate. Many authors have credited him as the first man to discover alkaline phosphatase. However, in 1967 Posen [72] credited Suzuki, Yashimura, and Takaishi (Bull. Coll. Agriculture, Tokyo Imp. University 7:503, 1907) as having discovered alkaline phosphatase.

Function of alkaline phosphatase

Despite the many years since alkaline phosphatase was discovered, we still do not understand its function. Kay [50] observed that the enzyme will hydrolyze all the phosphoric esters that were presented to it, namely hexosediphosphate, α and β-glycerophosphate, and guanine nucleotide. Still others report transphosphorylation activity for

alkaline phosphates [1,60,91]. Two types of reaction, therefore, are possible. In one the enzyme acts as a hydrolase, with cleavage of the P-O bond [50,60,91]:

$$RO - PO_3H_2 + HOH \xrightarrow{\text{Alk. } PO_4 \text{ ase}} ROH + H_3PO_4$$

The second type of reaction, a transphosphorylation in which the enzyme acts as a phosphotransferase, does not involve the intermediate formation of inorganic phosphate in the transmission of the phosphoryl group to the acceptor [1,60, 91]:

$$RO - PO_3H_2 + R'OH \longrightarrow ROH + R'O\,PO_3H_2$$

Which of these two reactions the enzyme catalyzes depends upon the competition between water and other hydroxyl-containing compounds for sites at the surface of the enzyme donor complex [62]. Skillen and Harrison [91] reported significant transphosphorylation at pH 9.5 as measured by the difference in the apparent p-nitrophenyl phosphate activity in bicarbonate and methyl aminopropanol-HCl buffers. At pH 10.5, the degree of transphosphorylation was dependent on substrate concentration. Amador [1] reported that for a given buffer, alkaline phosphatase activation and transphosphorylation paralleled buffer molarity. In testing 23 buffer systems, McComb and Bowers [60] observed that transphosphorylation was demonstrated in the two buffers in which the enzyme was most active. They also observed that for significant transphosphorylation to occur the phosphate acceptor

must contain a hydroxyl group, and either a second hydroxyl group or an amino group.

Activation of alkaline phosphatase

Kay [50] observed that Mg^{++} ions are a powerful stimulant to alkaline phosphatase activity. He also observed that Ca^{++} ions act as a mild inhibitor. Mg^{++} ions are known to activate many preparations of alkaline phosphatase but this effect differs with the enzyme source [28]. Moss and King [63], as well as many others, have used Mg^{++} ions as a stimulant in the measurement of alkaline phosphatase activity. Amador [1] observed that aminated alcohols will stimulate alkaline phosphatase. However, this activation is due to transphosphorylation of alcohol by alkaline phosphatase [1, 60].

Electrophoresis of alkaline phosphatase

Of the many hundreds of intracellular enzymes which have been identified, only a handful can be detected in the blood plasma of normal subjects, and fewer still are in appreciable concentration [41]. Little is known of the process by which enzymes escape from tissue into the blood *in vivo* and, while the characteristics of enzymes demonstrated in tissue extracts are in general retained by the same enzymes after their passage into the serum, some degree of

alteration in properties is nevertheless possible, particularly in those enzymes which are normally attached to intracellular structures [64].

The isoenzymes of alkaline phosphatase which so far have been identified in human zymograms are of liver, bone, intestinal and placental origin. To separate and identify different isoenzymes and tissue sources of alkaline phosphatase, many different electrophoretic techniques as well as colorimetric techniques have been employed, including cellulose acetate [32, 52, 77, 81], agarose thin film [22], polyacrylamide gel [23, 47], paper electrophoresis [5], isoelectric focusing [93], agar gel [74, 98, 114], and starch gel [13, 57, 63, 82, 109, 111]. However, regardless of which technique is being used, other tests must be performed to identify the source of alkaline phosphatase.

Inheritance of alkaline phosphatase

When human serum alkaline phosphatase is examined by starch gel electrophoresis, it is found that some sera show only a single zone of activity, while others show in addition a slower moving zone of variable intensity [53]. The site of origin of the main band which occurs in all subjects is the liver. The site of the second band is the jejunal mucosa [24]. Genetic control of organ-specific alkaline phosphatase has been observed in many organisms: Drosophila,

fowl, sheep and cattle [28]. Ghane [33], in studying alkaline phosphatase isoenzymes of cattle, found no difference in zymograms within 48 monozygous twin pairs, while a pronounced variation existed between pairs. He observed the presence of a particular second band, A, which seemed to be genetically controlled. Those which were of genotype F^0F^0 lacked band A, while those which were of genotype F^AF^A had a dense second band, and genotypes F^AF^0 were intermediate between the F^AF^A and F^0F^0 genotype. Cunningham and Rimer [21], in a population study of alkaline phosphatase, found that 60% of their population contained two alkaline phosphatase bands, a fast moving A and a slow moving B band. Their investigation indicated that the presence of the second band may be genetically controlled. In 1963, Arfors *et al.* [2], in a human twin study, observed that 28 of 89 probably monozygotic twin pairs (alike with respect to sex, A_1A_2BO, MN, Rh (CcDEe) blood groups and Hp and gm serum groups) showed 2 AP bands (Pp^2), in both twins, whereas in the remaining 61 monozygous pairs both twins had only one band (Pp^1). Out of 111 dizygotic twin pairs, 9 were concordant for 2 bands, 63 were concordant for 1 band, and 39 were discordant.

Shreffler [88], in a study of a Caucasian population classified the 2 AP isoenzymes into 5 classes: (0) no detectable phosphatase activity in the position of the slow moving band, (1) very weak or questionable band, (2) definite

but weak band, (3) band of moderate intensity, and (4) with a strong slow band. In all classes band A = liver was present. In some, but not all, band B = intestine was visualized. Both bands A and B were variable in intensity from person to person. Shreffler found that 46% of the samples fell in class 0, having only the liver band.

In 1965, Robson and Harris [80] found that the great majority of samples of placental alkaline phosphatase can be classified into one or another of six distinct phenotypes, namely $P1^f$, $P1^i$, and $P1^s$. To identify all the possible phenotypes they had to have two electrophoretic runs, one at pH 8.6 and another at pH 6.0. By sib and identical twin studies they also demonstrated that the placental alkaline phosphatase phenotype depends on the phenotype of the child.

Relationship of alkaline phosphatase and other blood markers

In 1963, Arfors *et al.* [2] found that the frequency of blood group A was extremely low in individuals who had two alkaline phosphatase bands on their zymogram (Pp^2), and that there is a non-random relationship between the alkaline phosphatase isoenzymes and Lewis blood groups. The individuals who had 2 bands all had Le^{b+} glood group, and not a single individual with 2 bands was found in the Le^{a+b-} group.

Beckman [7] observed that the frequency of Pp^2 was 30.4% in individuals of blood group 0, 30.5% in group B

individuals and 2.3% in group A individuals. There was no significant difference between A_1 and A_2 individuals, and AB individuals were intermediate (15.2%) between groups A and B. Beckman confirmed that there was not a single case of Pp^2 among the Le^{a+b-} blood group individuals. However, he observed that the appearance of 2 alkaline phosphatase bands in the zymogram is related to the ABH secretor status of the individual and not to the Lewis group per se. Out of 271 Pp^2 individuals observed in Beckman's study, not a single one was an ABH non-secretor. These findings have been confirmed by later studies [6, 24, 88].

In 1964, Rendel and Stormont [76] found a strong association between alkaline phosphatase isoenzymes and blood group O in sheep.

In 1966, Langman *et al.* [53] observed that among ABH secretors, individuals of blood groups B and O showed the slow moving alkaline phosphatase band much more frequently than group A individuals, with group AB being intermediate. They classified the genotype of the individuals according to the following scheme: P^{++}, if the slow moving band was relatively intense, P^{+0}, if the band was weak but definitely present, and P^{00}, if the band was present in trace amounts or not present.

In 1967, Robinson *et al.* [79] observed a conspicuously higher frequency of Pp^2 in three Indian tribes, the Montagnais, Naskapi and Sapelo, than in other populations. They

attributed this to the high frequency of the blood group O and secretor genes in Indian populations. This is in accord with the proposal by Langman *et al.* [53] that the occurrence of the slow moving alkaline phosphatase band appears to be determined in part by the secretor locus and the ABO locus, which are separate and unlinked.

Palsson *et al.* [69], in a study of an Irish population consisting of 295 unrelated males age 20-30, found that 72.7% of O individuals were Pp^2, while the frequency of Pp^2 was 45.3% in A individuals and 67.7% in blood group B. In the same year, Walter *et al.* [108] found that the frequency of Pp^2 in group O was 39.5%, in B, 39%, in A, 11.7%, and in AB, 21.5% in a population of 1,145 persons in Hungary.

In 1971, Walker *et al.* [107] studied 6 O secretors, 6 A non-secretors and 6 O non-secretors. All the O secretors showed two alkaline phosphatase bands on their zymograms, and the rest showed only one band. Fritsche and Adams-Park [32] and Sundblad *et al.* [98] also reported that they observed the slow moving alkaline phosphatase band in individuals of blood groups O and B more frequently than in A and AB individuals.

Beckman [7] proposed the following hypotheses:

(1) H substance couples with the alkaline phosphatase enzyme and makes a slow moving band.

(2) H substance complexes with a protein, then the complex binds the enzyme.

He also stated that the presence of blood group A tends to suppress the expression of phosphatase Pp^2. Arfors *et al.* [3] had observed that incubation of alkaline phosphatase of group Pp^2 with anti-Le^b serum would decrease the activity of the slow moving alkaline phosphatase band. Hence, they stated that the slow moving alkaline phosphatase band may represent a complex with which a blood group substance (Le^b or H) is integrated. However, Bamford *et al.* [6] observed that the average level of alkaline phosphatase activity in P^{++} sera was about 30% greater than in P^{00} sera, with the heterozygote intermediate, and that all differences between the three means were highly significant. If the hypothesis of Arfors *et al.* was correct, one would not expect a greater level of serum alkaline phosphatase in those with the slow moving band. Furthermore, when Langman *et al.* [54] extracted alkaline phosphatase of intestinal mucosa, they found that the mucosal alkaline phosphatase tended to be higher in individuals of blood groups O and B than in those of blood group A. When the secretor status was taken into account, the differences between B or O groups and group A became more pronounced and non-secretors had the lowest level of intestinal mucosal alkaline phosphatase. If Beckman [7] was correct in his suggestion that blood group A tends to suppress the expression of Pp^2 phosphatase in the serum, one should not observe the differences in the level of intestinal mucosal alkaline phosphatase in different blood

groups as reported by Langman *et al.* [54]. No relationship has been found between AP and MNSs, Rh, serum Hp [2, 7, 88] or Kell, Duffy, Kidd and P blood groups and Gm, and Gc serum types [88]. Beckman [7] observed that there was no correlation between Lutheran blood group and AP, while he may have found a correlation between AP and Duffy in O secretor females, but not any other group.

Alkaline phosphatase and diet

In 1965, Shreffler [88] observed that the level of alkaline phosphatase Pp^2 varied significantly in samples drawn at different times from the same individual. He suggested that this might be related to diet, seasonal variation, physiological state or diurnal variation. Langman *et al.* [53] observed that after a fatty breakfast total alkaline phosphatase rose most markedly in individuals who were O or B secretors, where the average rise of alkaline phosphatase 7.5 hours after the meal was 24% higher than the average concentration of the fasting sample. A similar rise was also observed in the 5 AB secretors tested. The increase in A secretors was less than half that of B and O secretors, and the increase was still less in the non-secretors.

The electrophoretic findings in these various sera were consistent with the view that all the increase in the enzyme concentration was of intestinal origin and the level

of other phosphatases present in the sera did not change [53, 110]. Warnock [110] believes that the increase of intestinal alkaline phosphatase associated with fat intake points toward a role for alkaline phosphatase in lipid transport across the intestinal mucosal cell.

Lusting [56] observed that intravenous injection of L-homoarginine in rats inhibited bone, intestinal and liver alkaline phosphatases, while the activity of the enzyme increased in kidney and lung tissue. Fishman and Gosh [28] reported that force feeding of amino acids to rats markedly increased the level of intestinal alkaline phosphatase.

The relationship of alkaline phosphatase with sex and age

Phosphatase type 2 (Pp^2) occurs in equal frequency in both sexes, there is no significant difference between parents and offspring, and the equal distribution of frequency between the sexes holds for all blood groups [2, 7, 108]. Roberts [78] stated that some blood constituents undergo significant concentration changes as the subjects age.

In 1963, Gahne [33] observed the influence of age on the alkaline phosphatase enzyme pattern in cattle. Beckman [7] observed a high frequency of Pp^2 group among children below 3 years of age, while observing only slight variations between the other age groups. He also detected an increased

frequency of A and AB blood types among the children with Pp^2 type alkaline phosphatase. In contrast with this, Walter *et al.* [108] found that the frequency of Pp^2 type phenotypes increased with increasing age values.

Shreffler [88] computed a significantly higher level of alkaline phosphatase in individuals below 16 years of age when compared with an older group. However, when he removed B and O secretors from his samples the differences became non-significant.

Warnock [110] observed a second broad band in the ß-globulin area of zymograms in children, in patients with bone diseases, and in extracts of periosteum. Although this band has not been resolved from the liver band thus far, it is readily distinguished from it. According to Fritsche and Adams-Park [32], although the predominant isoenzyme activity in the sera of adults is of hepatic origin, the major enzyme activity of children originates in bone. Yong [114] also observed that the activity of the bone isoenzyme was highest at birth and declined with age.

In 1972 Statland *et al.* [95] studied three age groups of normal individuals, 4 - 12 years, 13 - 17 years, and 18 - 30 years. They observed that the activities of liver and intestinal isoenzymes were independent of the age of the subjects. However, the level of total alkaline phosphatase activity was about 3-fold in the younger groups as compared

to the adults, and the differences among the groups are related mainly to the bone isoenzyme [38, 95].

Sources and levels of alkaline phosphatase

There are many discrepancies as to the sources and levels of different alkaline phosphatase isoenzymes [83]. One reason for these discrepancies is in the variety of experimental techniques and approaches, and another is the fact that such studies involve the effects of activators and inhibitors on phosphatases of different purities.

Schlamowitz [83] observed that about 27% of serum alkaline phosphatase was of intestinal origin. Schlamowitz and Bodanskey [84] reported that approximately 28% to 38% of serum phosphatase of normal individuals was not of bone or intestinal origin. However, Hodson *et al.* [45], using starch gel electrophoresis, concluded that most of the phosphatase enzyme in the serum was probably derived from liver.

Langman *et al.* [53] estimated that among normal individuals the percentage of total alkaline phosphatase activity which can be attributed to the slow moving intestinal component was 24% in P^{++} sera, 16% in P^{+0} sera, and 4% in P^{00} sera. In 1967, Fishman and Gosh [28] concluded that in ABH secretors the intestine must be considered the major source of serum alkaline phosphatase. Several

investigators (Green *et al.* [38], Firtsche and Adams-Park [32] and Skillen *et al.* [90]) have reported that although liver, intestinal and bone alkaline phosphatases are present in the sera of all age groups, in normal children the major source of serum phosphatase is bone, while that of normal adults is the liver.

The inhibition of alkaline phosphatase

It has been proven inadequate to have a classification of alkaline phosphatase isoenzymes based solely on starch-gel electrophoresis data. Additional biochemical studies of L-phenylalanine sensitivity, heat sensitivity, ABH blood types and secretor status can help in classifying the sources of different alkaline phosphatase isoenzymes [28].

In 1937, Bodansky [12] used bile acids to inhibit bone and kidney alkaline phosphatases. Bile acid proved to be ineffective in inhibiting intestinal alkaline phosphatase.

Schlamowitz [83] prepared antisera for bone and intestinal alkaline phosphatases. Individually or in mixtures intestinal and bone antisera selectively precipitated their respective phosphatases. Under conditions where the enzymes are present in low concentrations, such as in serum, cross reaction of bone antisera may occur with intestinal

phosphatases. However, the cross reaction of intestinal antisera with bone phosphatase did not seem to occur. Schlamowitz and Bodansky [84] observed that anti-bone antibodies precipitated 40 - 59 percent of the alkaline phosphatase in the serum of normal fasting individuals.

In 1962, Hodson *et al.* [45] indicated that human liver phosphatase can be precipitated by anti-human bone phosphatase serum. This, of course, could lead to errors in identification.

Boyer [13] also used immunologic means to delineate the inter- and intra-organ relationships of alkaline phosphatase isoenzymes.

Fishman *et al.* [27] reported that human alkaline phosphatase of intestinal origin, but not of bone, kidney and spleen, was 78% inhibited by L-phenylalanine. D-phenylalanine did not so affect alkaline phosphatase.

Gosh and Fishman [35] reported that intestinal alkaline phosphatase activity in the presence of L-phenylalanine shifts its optimum pH toward the alkaline range and the inhibition is pH dependent. The extent of inhibition of the enzyme by L-phenylalanine is likewise greatly dependent on substrate concentration, and the energy of activation in the presence of the inhibitor is nearly three times greater than the corresponding value in its absence. The inhibition of the rat's intestinal alkaline phosphatase by L-phenylalanine is of the non-competitive type.

Horne *et al.* [46] tested the effect of L-phenylalanine on liver, bone and intestinal alkaline phosphatases. They found that 5×10^{-3} M L-phenylalanine inhibits liver, bone and intestinal alkaline phosphatases to the extent of 37%, 30%, and 83%, respectively. The L-phenylalanine sensitive isoenzyme has been attributed to the intestinal isoenzyme [15, 77, 95]. Fishman and Sie [31] used L-homoarginine, an inhibitor of bone and liver alkaline phosphatase, in conjunction with L-phenylalanine to measure the different alkaline phosphatase isoenzymes. A 10% non-specific inhibition for both of these inhibitors has been reported [31, 38].

Many authors [27, 28, 38, 46] have reported a double specificity of L-phenylalanine. If this observation is correct, then the exclusive inhibition of intestinal alkaline phosphatase by L-phenylalanine and not D-phenylalanine suggests a unique difference in the catalytic site of the intestinal enzyme from that of the enzyme prepared from other sources [27]. Presant *et al.* [73] reported that, unlike others, their findings confirmed a significant D-phenylalanine inhibition of enzyme activity at all concentrations of D-phenylalanine employed.

Many researchers (Posen *et al.* [71], Horne *et al.* [46], Cadeau and Malkin [14], Green *et al.* [38] and many others) have used heat treatment at 56°C or urea to denature alkaline phosphatase.

In serum the heat sensitivity of each enzyme source remains characteristic and independent of the influence of the others in the mixture. The resultant heat inactivation is an additive function of the heat sensitivities of members of the mixture [46].

Some bone phosphatase activity remains after urea treatment [4, 46, 95]. Urea also inactivates part of the liver, intestinal and placental phosphatase isoenzymes [4, 46, 51].

Urea inhibits bone phosphatase, but also introduces other artifacts that are distinct from those produced by heat. Heat denaturation usually causes limited inhibition of all other isoenzymes except that from placenta [47]. Bahr and Wilkinson [4] and Fennelly *et al.* [26] stated that urea at low concentrations might cause uncompetitive deformation of the enzyme molecule without interfering with its catalytic activity. At higher concentrations, however, more complete unfolding of the enzyme molecule would progressively destroy its enzymatic activity.

Moss *et al.* [64] observed that the differences in protein or urea content between different individuals are unlikely to be great enough to lead to significant variations in alkaline phosphatase heat stability.

Storage of alkaline phosphatase

Green *et al.* [38] froze aliquots of a serum pool and assayed them over a 10-month period. For 42 samples, the mean and standard deviation of the heat inactivation was 74.1 ± 4.0% with a coefficient of variation of 5.5%.

Massion and Frankenfeld [59] observed that storage increases the activity of alkaline phosphatase, and that the lower the initial activity of a given lot, the greater was the rate of increase. The difference in rate of increase among materials from different sources varied widely. Refrigeration greatly decreased, but room temperature restored the rate of change. Ten freshly drawn sera were tested. The activity of these sera increased by an average of 0.9%, 2.7%, and 6.1% in 6, 24, and 96 hours; that of pooled serum, frozen and thawed, increased about 1% per hour. Cold storage for several months did not significantly change the level of alkaline phosphatase [71, 98].

Alkaline phosphatase polymerization

In 1962, Moss and King [63] stated that if each organ does contain only one alkaline phosphatase, the main phosphatase zone probably corresponds to the free enzyme protein. The subsidiary zone seen on starch gel electrophoresis may then represent proportions of the enzyme for which the electrophoretic mobility has been modified in some way.

Factors which may affect electrophoretic mobility include aggregation or disaggregation, modification of the enzyme by environmental factors (i.e., removal of charged molecules), or attachment of the enzyme protein to indifferent protein fractions [58].

A very slow-moving band of alkaline phosphatase close to the origin has been observed in some samples. Its position coincides with β-lipoproteins [63, 88]. Markert [52] stated that under suitable conditions of electrophoresis a single LDH isoenzyme may be represented by two, three, or more closely spaced bands. The multiple bands cannot represent distinct isoenzymes in terms of protein composition but may be produced by minor changes in molecular migration, perhaps due to physical-chemical environment in the cell at the site of protein synthesis.

The heavier form of the enzyme can be converted to the lighter form. These two forms of enzyme have been reported to be kinetically indistinguishable from each other [36, 37, 63].

Beckman [8] states that the serum intestinal alkaline phosphatase, which is resistant to neuraminidase, has lost its sialic acid in the intestinal form and is combined with lipids, therefore moving slower on electrophoresis. The native form of this enzyme does not occur in serum, but is always found in extracts of fetal jejunal mucosa and sometimes in biopsy specimens from the mucosa of adults.

Smith and Fogg [92] reported that after reconstitution a high alkaline phosphatase component predominated; during subsequent spontaneous activation this component decreased, and there was a concomitant increase in a low-molecular weight alkaline phosphatase component. The result of butanol extraction suggested that the observed change may be attributed to the breakdown of a complex between alkaline phosphatase and lipoproteins.

Moss [65] observed that the antigenic determinants of the major fast electrophoretic zones are also present in both the larger and smaller slow components, and that polymerization of the enzyme or combination with non-enzymic molecules does not mask the antigenically reactive groups.

The relationship of alkaline phosphatase and different diseases

Alkaline phosphatase has been observed to be elevated in hepatobilary diseases and bone disorders [16, 19, 41, 49, 52, 67, 71, 84, 114]. Gutman [41] stated that cell injury or aging may result in abnormal leakage of intracellular enzymes into extracellular fluid at the expense of the tissue enzyme content, which doubtless accounts for the increased number and quantity of circulatory enzymes encountered in a variety of diseases.

In 1959 Schlamowitz and Bodansky [84] reported that, while the ratio of bone to intestinal phosphatase is 1.4 -

4.6 to 1 in normal individuals, it was 6 - 190 to 1 for cancer patients. Schwartz [85] also reported high levels of alkaline phosphatase activity in cases of cancer tumors including bone or liver.

Chiandussi *et al.* [19] found that Paget's disease, intrahepatic obstructive jaundice, extrahepatic obstructive jaundice, and hepatic cancer were associated with high alkaline phosphatase.

Korner [52] reported that in the bone disorders with high alkaline phosphatase a large and sometimes predominant part of the increase is due to beta globulin phosphatase activity, and that alpha 2 activity is also raised. Alpha 1 activity, though usually low, may be increased. In hepatobiliary disorders with a high phosphatase concentration, the increase is due mainly to raised alpha 2 phosphatase activity, and there is also an increase in alpha 1 phosphatase.

Gutman [41] proposed two main hypotheses to explain the elevation of alkaline phosphatase in disease conditions:

1. Retention or impaired excretion, which assures the source of plasma alkaline phosphatase to be bone, and the liver has only an excretory function by the way of bile passage. If these passages are blocked, then the bone enzyme is retained.

2. Hepatogenic theory: most serum alkaline phosphatase is of hepatic origin, and that the hepatobiliary system,

when disordered, contributes wholly or in large part to the increase in plasma alkaline phosphatase.

In contrast, Yong [114] found that electrophoresis of bile reveals an isoenzyme having the same electrophoretic mobility as liver type alkaline phosphatase but not bone type. Furthermore, Kaplan and Righetti [49] observed that in rats the rise in the serum alkaline phosphatase in obstructive jaundice was intimately related to de novo synthesis of this enzyme by liver.

Griffith *et al.* [40] observed a generally high degree of correlation between abnormalities of serum alkaline phosphatase and 5'-nucleotidase in hepatobiliary diseases. Phelan *et al.* [70] state that it is now apparent that the increase in serum alkaline phosphatase in liver disease arises largely from a de novo synthesis of enzyme protein by liver cells, and while there is as yet no evidence that a similar mechanism accounts for the elevation of serum 5'-nucleotidase, changes in the relative levels of the two phosphatases at different stages of disease may be due to alterations in the extent to which a single type of cell capable of producing both enzymes is stimulated to synthesize one rather than the other.

Betro [11] observed that 23% of cases who had both high alkaline phosphatase and lactic dehydrogenase also had congestive heart failure.

Skillen *et al.* [90] observed that increases in the serum intestinal alkaline phosphatase are likely to be an indication of a disorder of the hepatobiliary system or chronic renal failure.

Alkaline phosphatase, Km and the effect of pH

In 1934 Linweaver and Burk [55] proposed the theory of the dissociation constant, $Ks = (E)(S)/_{(ES)}$. On the basis of this theory, the rate of the observed reaction is directly proportional to the concentration of the enzyme substrate complex (ES) at all values of the concentration of the substrate (S); Ks is proportional to (S) only at low values of substrate.

In 1962, Moss and King [63], using β-naphtylphosphate as the substrate, observed that alkaline phosphatase extract of different tissues had different affinities, while different bands of the same tissue had the same affinities for the substrate. The bands of bone phosphatase had Km values of 0.110 mM and 0.118 mM, that of liver and 0.067, 0.067, and 0.070; kidney phosphatase had Km values of 0.105, 0.103, and 0.096 for different bands and intestinal phosphatase had Km values of 0.090 and 0.098. These findings clearly point out the existence of a single alkaline phosphatase in each tissue, with properties characteristic of the tissue of origin.

Gosh and Fishman [37] purified two interconvertible placental phosphatases. The lighter form had a molecular weight of 70,000 and the heavier form had a molecular weight above 200,000 by sucrose density gradient centrifugation. The Km of the two variants were identical (18 mM) at pH 10.6. However, when pH was raised to 10.7, Km raised to 72 mM of phenylphosphate.

Skillen and Harrison [91] observed that the optimum substrate concentration in a bicarbonate buffer, with p-nitrophenyl phosphate as substrate, was 55 mM for liver, 50 mM for bone and 14 mM for intestinal phosphatase. Although this observation does not clearly distinguish between bone and liver phosphatases, it clearly separates these two from intestinal phosphatase.

Regan isoenzyme

Regan isoenzyme is named after the cancer patient in whose serum the isoenzyme was first discovered. Fishman *et al.* [30] observed that the Regan phosphatase in cancer tumors resembled placental phosphatase and not those of the tissue of origin. They also observed that the isoenzyme appeared in cancer cells, as well as in the serum of patients with bronchogenic cancer.

Fishman and coworkers [29, 66, 96] observed that Regan isoenzyme was indistinguishable from placental alkaline phosphatase on immunological as well as on biochemical

grounds. It was suggested that these findings could be an indication of derepression of the genome by the tumor and that this may be general for tumor proteins. There is a high incidence of Regan isoenzyme in carcinoma of the ovary followed by pancreatic, gastric and lung carcinoma. The lowest incidence was observed in bronchogenic carcinoma and breast cancer. Blood grouping of those patients with Regan isoenzyme showed a sharp decrease in group A and a sharp increase in group O.

In 1972, Higashino *et al.* [44] observed the Regan isoenzyme in patients with hepatocellular carcinoma. Their biochemistry also resembled that of placental alkaline phosphatase, although the Km values were 1.1 mM and 1.6 mM of substrate for the variant and placental alkaline phosphatase, respectively.

Epilepsy, genetics and enzymes

There is an elevation of glutamate oxalacetate transaminase (GOT) in cerebrospinal fluid of epileptics [43, 61]. Hain and Nutter [43] observed that an age factor was also involved which should be taken into consideration. In 1969 Niebroj-Dobosz and Hetnarska [68] found elevated lactic dehydrogenase in idiopathic epileptics. Other enzymes were normal (creatine phosphokinase, phosphohexosisomerase, aminotransferase, aspartate transaminase, malate dehydrogenase and lactate dehydrogenase). Wright and Pollitt [113],

in an epileptic patient, observed high levels of plasma ornithine and 240 µg/100 ml. of ammonia (upper limits of normal are 60 µg/100 ml.). Ornithine carbamoyl transferase was just higher than normal. There was no significant urinary excretion of ornithine, but large amounts of homocitrulline. Plasma ornithine levels in parents and siblings were just twice that of normal.

In 1970 Mabry *et al.* [57] reported a syndrome of very high levels of alkaline phosphatase in three siblings and a first cousin, all of whom had epilepsy and mental retardation. All these individuals were from consanguineous marriages. They also reported normal levels of serum alkaline phosphatase in 129 severely retarded individuals, many of whom had seizures.

Casey *et al.* [16] found 15 males and 6 females with elevated alkaline phosphatase among 18 male and 12 female epileptics. However, the number of epileptics compared to the total number of 17,431 individuals was not large enough for statistical studies.

Tsairis *et al.* [106] observed epilepsy in two daughters, a son and their mother. One daughter and their father did not have epilepsy. The mother and two daughters had elevated blood and cerebrospinal fluid pyruvate and lactate levels in a basal state, and abnormally high blood levels after glucose loading.

In 1972, Faed *et al.* [25] found a ring chromosome in many cells of a girl who was epileptic since the age of four. She had no previous head injury.

Causes of abnormalities in blood metabolites

It is well known that an increased rate of enzyme synthesis or a decreased rate of enzyme degradation can induce an increase in enzyme concentration. Certain substances can stabilize pre-existing enzyme proteins and delay their degradation. A third mode of induction is an increase in the activity of individual enzyme molecules. Griffin and Cox [39] observed that prednisolone mediates a configurational change in alkaline phosphatase during its synthesis that leads to an increase in the number of catalytic sites or a lowering of energy level of the enzyme substrate transition state. Using radioactive leucine, these workers observed that prednisolone did not produce an increased enzyme protein level.

In 1972, Singh *et al.* [89] used aniline derivatives, salicylic acid derivatives, hydrazines, catechol amines, and purine derivatives to observe their effect on blood metabolites. These drugs did not influence the levels of inorganic phosphate, blood urea nitrogen, cholestrol, total protein, albumin and alkaline phosphatase.

Effects of high blood ammonia

In 1954, Sherlock *et al.* [86], by using ammonium chloride and high protein diets, were able to cause an altered mental state, characteristic tremor, and electroencephalographic changes indistinguishable from impending hepatic coma.

Bessman and coworkers [9, 10] observed a significant arterial-venous difference of free ammonia, suggesting that the free ammonia is converted by brain and muscle to a bound form. They advanced the following hypothesis: of the many chemical reactions utilizing ammonia in the body, two are of significance in the brain, glutamine synthesis, and the reversal of glutamate oxidation, namely, reductive amination of α-ketoglutarate. They suggested that the synthesis of glutamic acid from the α-ketoglutarate generated by the Krebs cycle in the brain was the mechanism of hepatic coma when the arterial ammonia concentration rises. It was also suggested that transamination of aspartic acid to replace the α-ketoglutarate necessary for the Krebs cycle cannot be accepted, since due to impermeability of the brain to organic anions the brain cannot obtain an exogenous source of aspartic acid. Summerskill, Wolfe and Davidson [97] measured different arterial/venous blood ammonia levels in hepatic coma patients. However, they did not observe a good correlation with clinical status.

In 1963, Stahl [94] observed that elevated blood ammonia level, especially arterial, may precede coma by many days. Subsequently, the patients lapsed into coma with increasing arterial and venous ammonia, increasing arteriovenous difference, and later, a progressively diminishing arteriovenous difference. A favorable clinical outcome was usually preceded by a decline in blood ammonia concentration with an increasing arteriovenous difference. However, some patients remained comatose even after a normal level of blood ammonia had been reached. Stahl suggested that this finding strongly favored Bessman's hypothesis of a long active depletion by ammonia of the Krebs cycle in the brain.

In 1965, Warren *et al.* [112] observed very little change in electroencephalograph readings before and during an ammonia tolerance test. However, after five days of high protein diet and ammonium chloride intake, the tracing was significantly lower in one patient, and marked changes consistent with those seen in hepatic coma were observed in four out of ten patients.

Cohn and Castell [20] observed electroencephalographic changes in only 1 of 19 persons when they loaded them with ammonium acetate. This observation caused them to suggest that the ammonia which is immediately available to the brain from the blood is not the major determinant of gross changes in the electroencephalography seen in patients with hepatic encephalopathy.

Glasgow *et al.* [34] observed that the highest blood ammonia levels were seen in the most deeply comatose patients in Reyes syndrome. Shih *et al.* [87] found a similar condition of ammonia intoxication of the central nervous system in patients with enzyme defects of the urea cycle.

Carter *et al.* [18] indicated that most ammonia in the blood is in the ionized ammonium (NH_4^+) form and that an increase in blood pH would increase the amount that could readily penetrate the blood brain barrier.

Blood urea nitrogen

A high incidence of significantly low urea nitrogen occurs in various mental disorders, namely paranoid states and personality disorders in males, and neurosis, schizophrenia and psychoses in males and females. No relationship was observed between low urea nitrogen and elevated leucine aminopeptidase, alkaline phosphatase, glutamic oxalacetic transaminase or lactic dehydrogenase [16, 17].

In 1973, Redman *et al.* [75] observed that the birth weight of neonates was, on average, inversely proportional to the maternal urea. This trend appeared at 28 weeks gestation, but was most striking at 32 weeks when a urea level above 20 mg/100 ml was associated with significantly smaller babies.

MATERIALS AND METHODS

Blood was collected by venopuncture from a group of mentally retarded individuals in institution A in 1971 (IA1) and from another group in 1972 (IA2), from employees of institution A as a normal control (IAC), and from residents of institution B (IB1). (Over 95% of the residents in institution B are epileptics.) Blood was also collected from the institutionalized sib pairs in 1971 (Sib Pairs 1971), and in 1973 (Sib Pairs 1973). In 1973 blood was collected from all the known epileptics in institution A (IAE). A control was derived by selecting the data for that sib in each pair whose first name was alphabetically last (IAS). When it was desired to have a population which was age 20 and older, persons in the sib pair sample who were less than 20 years old were, where possible, replaced in the sample by sibs of over 20. After clotting, the blood was centrifuged at a speed of 2200 RPM and the serum was capped and stored in a refrigerator for analyses.

A Technicon two channel AutoAnalyzer was used to measure the level of the following metabolites: glucose (Glu), blood urea nitrogen (BUN), serum glutamate oxaloacetate transaminase (SGOT), uric acid (UA), calcium (Ca),

inorganic phosphate (Pi), total protein (TP), albumin (Alb), and alkaline phosphatase (AP).

Glucose

Glucose was measured by the inverse colorimetric technique, measuring the decrease in color of potassium ferricyanide as it is being reduced by reducing sugars. The procedure, adapted to automation by Technicon from the method of Hoffman [J. Biol. Chem. 120: 51, 1937], involves the dilution of the sample in saline followed by dialysis into air-segmented alkaline potassium ferricyanide. The dialisate is then heated to 95° in a heating bath and the color change is measured at 520 mμ wave length (Technicon Pamphlet N-16b).

BUN

The AutoAnalyzer urea nitrogen method is a slightly modified version of the procedure described by Marsh *et al.* [Clinical Chemistry 11: 624, 1965, as described in Technicon Pamphlet N-16b]. The BUN procedure is a modification of the carbamido-diacetyl reaction as applied to the determination of urea nitrogen. It is based on the direct reaction of urea and diacetyl monoxime under acid conditions. The presence of thiosemicarbazide intensified the color of the reaction product and enables the determination to be run without the need of concentrated acid reagents (Technicon

Pamphlet N-16b). Saline-diluted serum is dialized into an air-segmented stream of diacetyl monoxime-thiosemicarbazide mixture. The mixture is acidified by introducing into it a mixture of ferric chloride-phosphoric acid and sulfuric acid. The acidified solution is heated to 95° in a heating bath where urea reacts with diacetyl. The color product is then measured at 520 mμ in a 15 mm tubular flowcell.

SGOT

SGOT is measured by the procedure described in Technicon Pamphlet N-25b and it is based on the procedure of Morgenstern *et al.* [Clin. Chemistry 12: 95-111, 1966]. Serum samples incubated with SGOT substrate are brought to 37° in a heating bath coil and the resultant oxalacetate produced by the action of serum enzyme is dialyzed into a reagent stream of citrate buffer. The dialysate is then reacted with the diazonium salt of N-butyl-4-methoxymetanilamide (Azone Fast Red. PDC) which couples with the oxalacetate during passage through the second 37°C heating bath coil. The colored product is read at 460 mμ in the colorimeter in a 15 mm. flow-cell. Blank interferences are eliminated by the use of dialysis and the specificity of the color reaction.

Uric acid

Uric acid is measured by the procedure described in Technicon Pamphlet N-13b. The automated procedure is adapted

from the manual method described in *Practical Physiological Chemistry* by Hawk, Oser and Summerson [13th ed., p. 564]. The sample is diluted with physiological saline and then dialyzed. To the dialysate is added a mixture of sodium cyanide - urea followed by the addition of phosphotungstic acid reagent. The quantitative measurement of uric acid involved the reduction of phosphotungstate complex to a phosphotungstite complex. The presence of cyanide intensifies the color and prevents turbidity. After mixing, the blue color of the reaction product is measured at 660 mμ in a 15 mm flowcell.

Calcium

Calcium was measured by the procedure described in Technicon Pamphlet 26a. The method is based on the procedures of Gitelman [Anal. Biochem. 18: 521, 1967], who incorporates the use of 8-hydroxyquinoline with the method of Kessler and Wolfman [Clin. Chem. 10: 686-702, 1964] to virtually eliminate the interference of magnesium. The serum is diluted with 0.25 N HCl to release the protein-bound calcium, and the mixture is dialyzed. Cresolphthalein complexone, containing 8-hydroxyquinoline and diethylamine potassium cyanide base, are then added to the dialysate. A colored calcium dye complex is formed in the presence of diethylamine. The developed color was measured in a 15 mm flowcell at 580 mμ.

Inorganic phosphate

The automated inorganic phosphate procedure was adapted to the AutoAnalyzer by Kraml [Clin. Chim. Acta. 13: 442, 1966] from the procedure reported by Hurst [Can. Jour. of Biochem. 42: 287, 1964] (Technicon Pamphlet N-26a). The serum sample is diluted with 0.25 N HCl and dialyzed. An acidic solution of ammonium molybdate is added to the dialysate. Phosphomolybdic acid is formed and this is immediately reduced by stannous chloride-hydrazine sulfate which is introduced into the air-segmented stream of dialysate-ammonium molybdate. The absorption of the blue product is measured at 660 mμ in a 15 mm flowcell.

Total protein

The procedure for determination of total protein is described in Technicon Pamphlet (N-14b). The procedure was adapted to the AutoAnalyzer by D. L. Stevens and is a modification of the biuret reaction, proposed by Weichselbaum [Amer. J. Clin. Path. 7: 40, 1946].

The sample stream is diluted with an air-segmented stream of biuret reagent. The biuret reaction depends upon the formation of a purple colored complex of copper in an alkaline solution, with two or more carbamyl groups (-CO-NH-) which are joined directly together or through a single atom of nitrogen or carbon. The developed color is measured at 550 mμ using a 15 mm flowcell.

Albumin

The AutoAnalyzer procedure for albumin (Technicon Pamphlet N-15c) is based on the work of Ness *et al.* [Clin. Chim. Acta. 12: 532, 1965]. It is based on the quantitative binding of the anionic dye, 2-(4-hydroxyazobenzene) benzoic acid (HABA), specifically to serum albumin. Nishi and Rhodes [*Automation in Analytical Chemistry*, edited by Skeggs, 1966, pp. 321-23], adopted the method of Ness *et al.* to the AutoAnalyzer. A serum sample is introduced into an air-segmented HABA dye diluted in a phosphate buffer. The developed color is measured at 505 mμ in a 15 mm flowcell.

Alkaline phosphatase

The automated alkaline phosphatase procedure is based on the method of Morgenstern *et al.* [Clin. Chem. 11: 876, 1965], who modified the manual process of Bessey *et al.* [J. Biol. Chem. 164: 321, 1946]. The serum is diluted in an air-segmented stream of the substrate p-nitrophenyl phosphate dissolved in 2-Amino-2-methyl-1-propanol buffer at pH 10.25±0.05. The mixture of serum-substrate is incubated at 37°C for 4 minutes. The enzyme breaks p-nitrophenyl phosphate into p-nitrophenol and phosphate. The mixture is then dialyzed into an air-segmented stream of 2-amino-2-methyl-1-propanol buffer. The dialyzed p-nitrophenol is highly colored under alkaline conditions and thereby provides its own chromagen. Dialysis eliminates the interference of

bilirubin and the need for blank correction (Technicon Pamphlet N-6b). The absorbance of p-nitrophenol is measured at 410 mμ in a 15 mm flowcell.

The blood from the sib pairs in institution A (Sib Pairs 1973) and from the epileptic patients in institution A (IAE) were studied for the level of ammonia, the levels of bone, liver and intestinal alkaline phosphatase, alkaline phosphatase substrate specificity, ABO and Lewis blood groups and their secretor status. A number of other institutionalized individuals with high alkaline phosphatase were also tested for the level of their ammonia.

For determination of liver, bone and intestinal alkaline phosphatase the automated method of Morgenstern *et al.* described above was adapted to the two channels of the AutoAnalyzer (Figure 1). The total alkaline phosphatase was first determined as described previously. Then a 1 cc aliquot of serum was heated in a bath for 15 minutes at 56.0± 5°C (Horne *et al.*[46]) and immediately transferred to an ice water bath for 3 minutes. The serum samples were then brought to room temperature and assayed on the AutoAnalyzer. Bone alkaline phosphatase is more sensitive to heat than liver or intestinal alkaline phosphatase (Horne *et al.* [46], Warnock [109, 110] and many others). In one channel p-nitrophenyl phosphate was used. This would measure the heat-stable alkaline phosphatases. In the other channel p-nitrophenyl phosphate + .005 M L-phenylalanine was used

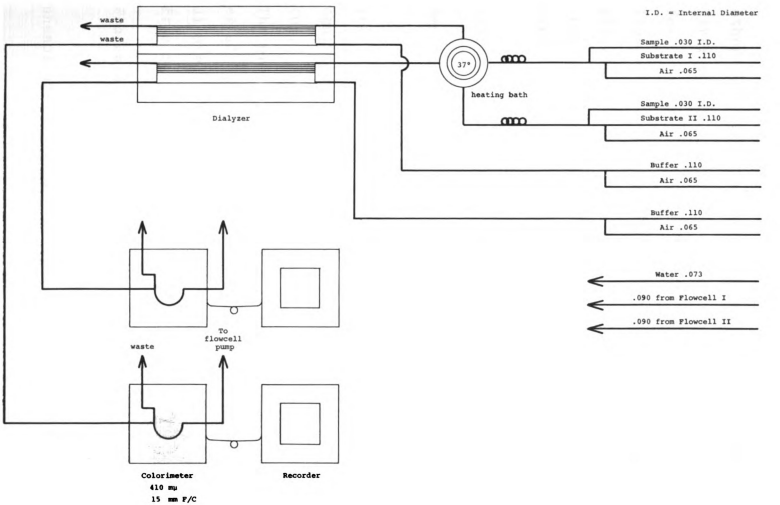

Figure 1. Adaptation of the method of Morgenstern *et al.* for measurement of alkaline phosphatase to both channels of AutoAnalyzer.

as substrate. L-phenylalanine inhibits intestinal alkaline phosphatase (Horne *et al.* [46], Warnock [109, 110], and many others). The remaining alkaline phosphatase is mostly of liver origin. Horne *et al.* [46] reported that after heating for 15 minutes at 50°C, 20.8% of the activity of liver alkaline phosphatase and 89.8% of the activity of intestinal alkaline phosphatase were recovered. L-phenylalanine inhibited the liver alkaline phosphatase 37% and intestinal alkaline phosphatase was 83% inhibited.

There is no satisfactory way to determine bone alkaline phosphatase.

Ammonia

For determination of ammonia, fresh blood was drawn from each patient by venopuncture and after clotting, centrifuged at 2200 RPM for 10 minutes without removing the top. The serum was then immediately used for the assay. The procedure is a modification of the procedure of Kaplan *et al.* [48]. The reagents in this determination were not diluted. The water used in this procedure was made ammonia-free by passing it through an acidic Dowex resin.

Reagents

1. Phenyl-nitroprusside: A solution of 2.5×10^{-2} phenol and 2.5×10^{-4} of sodium nitroprusside (listed by

many American chemical companies as Sodium nitroferricyanide), Na_2 Fe $(CN)_5NO,2H_2O$, was made in ammonia-free water.

2. Alkaline hypochlorite solution: A solution of 25 gr NaOH per 1000 ml containing 40 ml of commercial hypochlorite with the strength of at least 5% NaOCl by weight, was made with ammonia-free water.

3. Standard:

(a) stock standard: a solution of 0.472 gr of pure $(NH_4)_2SO_4$ was made in one liter of ammonia-free water.

(b) working standard: 500 µg%. 5 ml of stock standard was diluted to 100 ml with ammonia-free water.

Procedure: To 1 ml of fresh blood serum, working standard, or ammonia-free water, was added in succession 1 ml of phenol-nitroprusside and 1 ml of alkaline hypochlorite. The test tube was corked and shaken for about 5 seconds.

The tubes were then transferred to a 37°C bath and incubated for 20 minutes. After incubation and development of color the solutions were transferred to cuvets and read against the standard at 560 mµ. The upper limit of normal is 100 µg/100 ml.

Blood typing

The red blood cells of the patients were washed with physiological saline 3 times and then tested for ABO and

Lewis blood groups by the standard test tube technique using anti-A, B, Le^a and Le^b antisera. A drop of red blood cell suspension was mixed with 2 drops of antiserum and incubated for 15 minutes at room temperature. After incubation the tubes were serofuged for 15 seconds and read. For anti-A serum blood was collected from a B-blood type individual and for anti-B serum from an A individual. The donors had no previous transfusion. The anti-Lewis serums were kindly provided by Dr. Emanuel Hackel.

Secretor status

One gr of ulex seeds were soaked in 20 ml physiological saline overnight. The mixture was then ground with a mortar and pestle, followed by centrifugation at 2200 RPM. The supernatant was then used as Anti-H. To 2 drops of saliva 2 drops of anti-A, B or H were added and mixed. The mixture was serofuged for 15 seconds and allowed to incubate at room temperature for 15 minutes. To the tube with anti-A serum, type A red cells, to the tube with Anti-B serum type B red cells, and to the tubes with Anti-H, O type red cells were added. The tube contents were mixed for 20 minutes. After incubation the tubes were serofuged for 15 seconds and read macroscopically.

Substrate specificity

To determine substrate specificity five patients were selected randomly and Km was calculated for them and the values were plotted on a graph. The values for v (velocity) were graphed on the ordinate and the values for substrate concentration plotted on the abcissa. The reaction curve was plotted and the Km value was found to be about 5×10^{-5} of substrate.

To determine the substrate specificity for all the patients, in one channel of the AutoAnalyzer (substrate I) a substrate concentration of 2×10^{-2} M was used to get V_{max} and in the other channel (substrate II), the substrate concentration was 5×10^{-5} M which is equal to Km and should give $v = \frac{1}{2} V_{max}$. After the values were read, V_{max} was divided by v_{Km} and the values recorded. The result of $V_{max}/v_{Km} = 2$ was arbitrarily taken as normal affinity. If the result was less than 2 it indicated a high affinity of alkaline phosphatase for the substrate, and if it was more than 2 it indicated a low affinity.

RESULTS

The populations of two institutions, one for non-specific mentally retarded individuals and the other for the epileptic mentally retarded, were screened for metabolites: glucose (Glu), blood urea nitrogen (BUN), uric acid (UA), serum glutamate-oxalacetate transaminase (SGOT), calcium (Ca), inorganic phosphate (Pi), total protein (TP), albumin (Alb), and alkaline phosphatase (AP). The employees of the first institution were tested for the same metabolites and used as a normal control.

All together there are four populations in this part of the study. The control population (IAC), some non-specific mentally retarded residents of institution A who were tested in 1971 (IA1), other such residents of institution A who were tested in 1972 (IA2), and residents of institution B, of which over 95 per cent are mentally retarded epileptics (IB1).

Table 1 indicates for each metabolite the means and standard deviations for the control group and the normal range as defined by two standard deviations above and below these means.

The mean results of analyses performed on different days were often strikingly dissimilar. This indicates that "run variation" was present, that is, that the standards were

Table 1. The means and standard deviation for the control group and the normal ranges.

Metabolite	Mean	S. D.	Range	Units
BUN	14.92	3.87	7.2 - 22.7	mg%
SGOT	28.27	7.13	14 - 42.5	Karmen unit
Uric Acid	5.73	1.47	2.8 - 8.7	mg%
Ca	10.79	0.51	9.8 - 11.8	mg%
Pi	4.17	0.51	3.2 - 5.2	mg%
Total Protein	7.57	0.50	6.6 - 8.6	gr%
Albumin	4.43	0.36	3.7 - 5.2	gr%
Alkaline Phosphatase	72.80	24.52	24.0 -122.0	mIU/ml

sufficiently variable from run to run to cause wide variation in the mean results. Such run variation was observed for glucose, SGOT, Ca, Pi, total protein (TP), and albumin (Alb). In addition, the BUN values for 1972 differed from those of 1971 because of a change in standards, although there does not appear to be run variation for BUN within a given year.

Run variation makes it impractical to compare population means by simple statistical tests. However, the means will be similar in all runs so that the frequency of outlying values should be comparable. The standard deviations may be compared because the variability within a sample should remain constant while the mean varies slightly.

The various biochemical tests were statistically analyzed and are presented in Tables 2-10. Where appropriate, each sample of institutionalized persons was compared with the control group to determine whether the mean or standard deviations were significantly different by t or F test, respectively. Similar comparisons were made between IA1 and IA2 to check for the existence of variation in methodology over the interval of a year, and between IA2 and IB1 to check for a difference due to epilepsy.

Values outside of the normal ranges are termed "outliers" and their numbers are presented in Tables 11 and 12. The frequency of individuals with an unusual amount of each metabolite was analyzed by a standard 2 x 2 chi-square test which tested two populations for the number of high

Table 2. Analysis of the test for serum glucose.

Population	N	mean mg%	standard deviation
IAC	230	97.48	21.36
IAC less 2 outliers	228	95.97	12.96
IA1	668	80.20	14.89
IA2	726	89.55	12.78
IB1	364	72.62	11.79

Table 3. The analysis of the test for blood urea nitrogen (BUN).

Population	N	Mean mg%	Standard deviation
IAC	230	14.94	3.87
IA1	668	17.03	4.33
IA2	726	15.59	4.33
IB1	364	13.61	4.55

Populations compared	F test	t test
IAC and IA1	1.25*	6.85**
IAC and IA2	1.25*	2.15**
IAC and IB1	1.38*	3.81**
IA1 and IA2	1.00	6.20**
IA2 and IB1	1.10	7.07**

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 4. The analysis of the test for SGOT.

Population	N	Mean Karmen units	Standard deviation
IAC	230	28.27	7.13
IA1	668	22.89	8.22
IA2	726	22.32	11.12
IA2[#] less outliers	663	22.38	7.72
IB1	364	35.51	13.78
IB1[@] less high AP and SGOT individuals	297	31.09	7.56

Populations compared	F test	t test
IAC and IA1	1.32*	9.48**
IAC and IA2[#]	1.17*	10.56**
IAC and IB1[@]	1.12	4.35**
IA1 and IA2[#]	1.13	1.16
IA2[#] and IB1[@]	1.04	16.25**

* Significant at 0.05 level.

** Significant at 0.01 level.

#,@ Populations used in comparisons.

Table 5. The analysis of the test for uric acid.

Population	N	Mean mg%	Standard deviation
IAC	230	5.73	1.47
IA1	668	5.59	1.36
IA2	726	5.19	1.35
IB1	364	5.16	1.30

Populations compared	F test	t test
IAC and IA1	1.16*	1.27
IAC and IA2	1.18*	4.95**
IAC and IB1	1.27*	4.81**
IA1 and IA2	1.00	5.49**
IA2 and IB1	1.07	0.35

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 6. The analysis of the test for serum calcium.

Population	N	Mean mg%	Standard deviation
IAC	230	10.79	0.51
IA1	668	11.18	0.93
IA2	726	10.60	0.69
IB1	364	10.01	0.58

Table 7. The analysis of the test for serum phosphate.

Population	N	Mean mg%	Standard deviation
IAC	230	4.17	0.51
IA1	668	3.88	0.71
IA2	726	4.13	0.61
IB1	364	3.78	0.64

Table 8. The analysis of the test for serum total protein.

Population	N	Mean gr%	Standard deviation
IAC	230	7.57	0.50
IA1	668	7.71	0.88
IA2	726	7.54	0.58
IB1	364	7.50	0.54

Table 9. The analysis of the test for serum albumin.

Population	N	Mean gr%	Standard deviation
IAC	230	4.43	0.36
IA1	668	4.20	0.43
IA2	726	4.10	0.56
IB1	364	4.16	0.42

Table 10. The analysis of the test for serum alkaline phosphatase.

Population	N	Mean mIU/ml	Standard deviation
IAC	230	72.80	24.52
IA1	668	91.75	41.85
IA2	726	91.70	46.43
IA2 less high AP & SGOT#	624	90.84	43.56
IB1	364	158.55	71.17
IB1 less high AP & SGOT@	297	147.30	69.59

Populations compared	F test	t test
IAC and IA1	2.91**	8.28**
IAC and IA2#	3.15**	7.58**
IAC and IB1@	8.05**	17.13**
IA1 and IA2#	1.08	0.21
IA1 and IA2	1.23**	0.02
IA2# and IB1@	2.55**	12.84**

#,@ Populations used in comparison.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 11. The distribution of high outliers for the different tests performed.

	BUN		SGOT		Uric Acid		Ca		Pi		Tp		Alb		Ap	
	out-liers	others	out-liers	others	out-liers	others	out-liers	others	out liers	others	out-liers	others	out-liers	others	out-liers	others
IAC	5	225	5	225	5	225	4	226	6	224	6	224	0	230	9	221
IA1	69	599	17	651	19	649	30	638	-	-	21	562	1	667	90	578
IA2	39	687	23	703	20	706	17	646	19	645	32	594	-	-	103	623
IB1	11	353	14	283	8	356	0	364	2	362	7	357	0	364	165	132

Table 12. The distribution of low outliers for the different tests performed.

	BUN		SGOT		Uric Acid		Ca		Pi		Tp		Alb		Ap	
	out-liers	others	out-liers	others	out-liers	others	out-liers	others	out-liers	others	out-liers	others	out-liers	others	out-liers	others
IAC	1	229	0	230	0	230	3	227	2	228	0	230	2	228	0	230
IA1	3	665	23	645	3	665	16	652	-	-	1	582	43	625	0	668
IA2	7	719	33	693	5	721	38	625	25	639	10	716	-	-	0	726
IB1	14	350	1	364	3	361	114	250	65	299	1	363	31	333	0	364

(or low) outliers in each population compared with all other individuals in the populations (Table 13). If there is a correlation between a moderately frequent form of mental retardation or of epilepsy and unusual values for a given metabolite, significant frequencies of outliers may be recorded.

Glucose

The results of the glucose determinations are presented in Table 2. The mean and standard deviation for the IAC population are 97.48 and 21.36, respectively. However, if two advanced diabetics with fasting blood sugars of 244 and 304 are taken out of the population, the mean drops to 95.97, while the standard deviation becomes 12.96, or about two-thirds of the original standard deviation.

A comparison of the IA1 and IA2 populations shows about 11 per cent deviation between the means. This is partly due to run variation.

The mean and standard deviation for the IB1 population are 72.62 and 11.79, respectively. The drastic drop in the sugar values could be the result of incomplete separation of the red blood cells from the serum before transportation and other chance factors contributing to run variability.

Table 13. The chi-square test for high and low outliers in the different populations.

χ^2 Between	BUN		SGOT		Uric Acid		Ca
	high out-liers	low out-liers	high out-liers	low out-liers	high out-liers	low out-liers	high out-liers
IAC & IA1	15.15**	0.00	.10	8.13**	.30	1.04	3.56
IAC & IA2	4.07*	.59	.61	10.83**	.23	1.59	.51
IAC & IB1	0.37	6.66**	2.41	0.0	.00	1.91	6.37*
IA1 & IA2	11.96**	1.30	.48	1.10	.01	.35	3.63
IA2 & IB1	3.06	10.65**	1.44	17.06**	.30	.06	9.49**

χ^2 0.01 = 6.63

χ^2 0.05 = 3.84

* Significant χ^2 0.05

** Significant χ^2 0.01

Pi				TP		Alb		AP	
low out-liers	high out-liers	low out-liers	high out-liers	low out-liers	high out-liers	low out-liers	high out-liers	low out-liers	
.98	-	-	.51	.40	.34	11.14**	15.94**	0.0	
7.64**	.04	4.89*	2.48	3.20	-	-	17.83**	0.0	
80.27**	4.50*	40.64**	.31	.63	0.00	15.71**	156.30**	0.0	
9.52**	-	-	1.64	5.64*	-	-	.15	0.0	
22.00**	6.28*	58.45**	6.18*	2.95	-	-	186.56**	0.0	

BUN

The results of BUN analysis are presented in Table 3. The mean and standard deviation for the IAC population are 14.94 and 3.87, respectively. The mean for IA1, IA2 and IB1 are 17.03, 15.59 and 13.61, respectively, and the standard deviations are 4.33, 4.33, and 4.55, respectively. The F test indicates that the three retarded populations do not have significantly different variations and, at the same time, are significantly higher than the normal population.

The mean BUN values of the IA1 and IA2, although differing from each other, are both higher than the normal population, while the BUN mean for the epileptic population is significantly lower than any of the other populations.

The results presented in Tables 11 and 13 indicate that the IA1 and IA2 populations have significantly more high outliers for BUN than the IAC population, while the IAC and IB1 groups are not significantly different. The IA1 and IA2 populations also differ significantly from each other at the 0.001 level in the number of high outliers, 10.33% and 5.23% for the two populations, respectively. At least part of the discrepancy between the means of the tests in the IA1 and IA2 populations could be attributed to the number of high outliers in IA1.

The results presented in Tables 12 and 13 indicate that IB1, the epileptic population, had significantly more

low outliers when compared to the IA2 or IAC. Comparison of the IA1, IA2 and IAC did not demonstrate a significant difference between the number of low outliers in these three populations.

SGOT

Table 4 presents the results of SGOT studies. The mean and standard deviation for IAC are 28.27 and 7.13, with the normal range being 14 - 42.5. The means for the IA1 and IA2 are 22.89 and 22.32 and their standard deviations are 8.22 and 11.12. The F test indicates that these populations have different variances. If three outliers with values of 113, 127 and 176, and one of the buildings in which most of the residents had unusually low values are taken out of the analysis, the mean for IA2 becomes 22.38 or an increase of 0.06 units, and the standard deviation becomes 7.72, a decrease of over 30 per cent. The F test becomes non-significant, indicating that the IA1 and IA2 populations have the same variance.

In either case the means for the IA1 and IA2 populations are not significantly different and variation between the years is not observed for SGOT.

The mean and standard deviation for the IB1 population are 35.51 and 13.78, respectively. If those individuals who are high in both AP and SGOT are taken out of the population, the mean and standard deviation become 31.09 and

7.56, respectively. These individuals are taken out of the population because they are assumed to have liver damage, which greatly increases SGOT levels. The F test indicates that there is no significant difference in this case between the distribution of the IB1 from either the IAC or IA2 populations.

Tables 11 and 13 present the number of high outliers in the four populations. IAC, IA1, and IA2 show no significant difference in this respect among themselves. However, IB1 is significantly different from the IAC and IA2, the two populations it is compared with. When the individuals with high AP and SGOT are taken out of the IB1, the differences between this population and the IAC and IA2 populations lose significance.

The differences between the number of low outliers for these populations are presented in Tables 12 and 13. The IA1 and IA2 populations are significantly different from the IAC and IB1 in this respect. However, the IA1 and IA2 populations do not differ from each other. Furthermore, there is no significant difference between the IAC and IB1 populations. The point should be made here that although in this study the lower limit of SGOT is considered to be 14, the literature consistently reports the normal lower limit of SGOT to be zero.

Uric acid

The mean and standard deviation for uric acid studies are presented in Table 5. The means for the IAC, IA1, IA2, and IB1 are 5.73, 5.59, 5.19 and 5.16, and the standard deviations are 1.47, 1.36, 135, and 1.30 for the four populations, respectively.

The mean difference between the IAC and IA1 populations is not significant, nor is the mean difference of IA2 and IB1.

However, there is a significant difference between IA1 and IA2 which could be attributed to the different standards used from year to year. F tests for the four populations indicate that there is a significant difference between the variances of the normal and the retarded populations. However, there are no significant differences among the variance of the retarded populations.

Tables 11, 12 and 13 show that there are no significant differences between the high or low outliers in these four populations.

Tables 6, 7, 8 and 9 present the results for the Ca, Pi, total protein and albumin studies. The day to day run variation makes it impossible to make valid comparisons between these four populations.

Alkaline phosphatase

Table 10 presents the means and standard deviations for alkaline phosphatase (AP) in the four populations IAC, IA1, IA2 and IB1. The means for these populations are 72.80, 91.75, 91.70 and 158.55 mIU (Milli-International Units) per ml and the standard deviations are 24.52, 41.85, 46.43 and 71.17 for the four populations, respectively. The normal range for AP is 24-122 (Table 1).

The F tests indicate that the variances of all four populations are significantly different from one another. However, there are two individual outliers with AP values of over 400 in the IA2 populations, and these could be the primary cause of the difference between IA1 and IA2. When these two individuals are removed from the IA2 population, the mean and standard deviations become 90.84 and 43.56, respectively, and the F test for IA1 is 1.08, which is not significant. The means of the IA1 and IA2 populations are almost identical. However, the mean values for retarded populations are significantly higher than for the normal population, and the mean of the epileptic population is significantly higher than the mean of each of the other three populations. This phenomenon holds true even when the individuals who are high both for AP and SGOT are taken out of IB1. Tables 11 and 12 show the number of high and low outliers for the four populations. None of the populations has any low outliers in the AP test.

All three mentally retarded populations have significantly more high outliers than the normal population (Table 11). IA1 and IA2 are not significantly different in the number of high outliers, but the IB1 population has a significantly higher number of high outliers than the IA2 population.

Table 14 presents the cumulative percentages of AP values for individuals in the IA2 and IB1 populations. The median for the IA2 populations is about 80 mIU/ml and the mean for this population is 91.70. The median of the IB1 population, primarily an epileptic group, is between 125 and 130 mIU with a mean of 147.30 mIU. These findings clearly demonstrate an enormous upward shift in the AP levels in the epileptic population. This shift in the AP levels of the epileptic population IB1 is further demonstrated in Figure 2, where the cumulative percentages of IB1 and IA2 from Table 14 are graphically demonstrated.

Sib studies

In 1971 and 1973 serum was collected from all of the sib pairs in institution A. There were 64 sib pairs in 1971 and 33 pairs in 1973. The samples had an overlap in that 17 pairs of these sibs were present in both samples. To prevent run variability from affecting the results, all sib pairs were analyzed on the same day. The metabolites tested in 1971 were glucose, BUN, SGOT, uric acid, Ca, Pi, total

Table 14. Cumulative percentage of individuals for different levels of AP in IA2 and IB1 populations.

	Populations					
	IA2		IB1		IB1 less high AP & SGOT individuals	
mIU of AP	Cumulation	Percentage of Total	Cumulation	Percentage of Total	Cumulation	Percentage of Total
35	2	0.3	-	-	-	-
40	16	2.2	2	0.6	2	0.7
45	36	5.0	-	-	-	-
50	69	9.5	3	0.8	3	1.0
55	86	11.8	5	1.4	5	1.7
60	143	19.7	6	1.7	6	2.0
65	191	26.3	11	3.0	11	3.7
70	242	33.3	15	4.1	15	5.1
75	292	40.2	22	6.0	22	7.5
80	369	50.8	34	9.7	34	11.5
85	403	55.4	43	11.8	43	14.6
90	455	62.6	57	15.7	57	19.3
95	487	67.0	68	18.7	68	23.1
100	544	74.9	80	22.0	80	27.1
105	564	77.6	85	23.4	85	28.8
110	588	80.9	94	25.9	94	31.9
115	600	82.5	105	28.9	105	35.6
120	623	85.7	132	36.7	132	44.8
125	627	86.2	143	39.4	142	48.1
130	634	87.2	155	42.7	151	51.2
135	640	88.0	164	45.2	158	53.6
140	651	89.5	179	49.3	171	58.0
145	654	90.0	189	52.1	179	60.7

Table 14. Continued

Populations						
	IA2		IB1		IB1 less high AP & SGOT individuals	
mIU of AP	Cumulation	Percentage of Total	Cumulation	Percentage of Total	Cumulation	Percentage of Total
150	660	90.8	197	54.3	186	63.1
155	664	91.3	203	55.9	189	64.1
160	671	92.3	216	59.5	198	67.1
165	672	92.4	221	60.9	202	68.5
170	675	92.8	226	62.3	206	69.8
175	687	93.3	239	65.8	217	73.6
180	682	93.8	250	68.9	225	76.3
185	687	94.5	257	70.8	230	78.0
190	691	95.0	263	72.5	234	79.3
195	693	95.3	277	76.3	244	82.7
200	701	96.4	285	78.5	249	84.4
205	703	96.7	289	79.6	251	85.1
210	707	97.2	293	80.7	254	86.1
215	-	-	296	81.5	255	86.4
220	708	97.4	303	83.5	261	88.5
225	710	97.7	304	83.7	-	-
230	712	97.9	306	84.3	263	89.2
235	713	98.1	308	84.8	264	89.5
240	714	98.2	313	86.2	266	90.2
245	715	98.3	317	87.3	269	91.2
250	-	-	322	88.7	272	92.2
255	-	-	324	98.3	274	92.9
260	718	98.8	327	90.0	275	93.2
265	-	-	330	90.9	276	93.6
270	719	98.8	337	92.8	277	93.9

Table 14. Continued

	Populations					
	IA2		IB1		IB1 less high AP & SGOT individuals	
mIU of AP	Cumulation	Percentage of Total	Cumulation	Percentage of Total	Cumulation	Percentage of Total
275	-	-	-	-	-	-
280	-	-	-	-	-	-
285	-	-	341	93.9	281	95.3
290	-	-	345	95.0	283	95.9
295	-	-	-	-	-	-
300	-	-	350	96.4	286	97.0
305	720	99.0	-	-	-	-
310	721	99.2	351	96.7	-	-
315	722	99.3	352	97.0	287	97.3
320	-	-	-	-	-	-
325	-	-	353	97.2	-	-
330	-	-	-	-	-	-
335	-	-	354	97.5	-	-
340	723	99.4	355	97.8	288	97.6
345	-	-	-	-	-	-
350	-	-	-	-	-	-
355	-	-	-	-	-	-
360	-	-	-	-	-	-
365	-	-	-	-	-	-
370	-	-	-	-	-	-
375	724	99.6	-	-	-	-
380	-	-	356	98.0	289	98.0
385	725	99.7	-	-	-	-
390	-	-	357	98.3	290	98.3
395	-	-	-	-	-	-
400	727	100.0	363	100.0	295	100.0

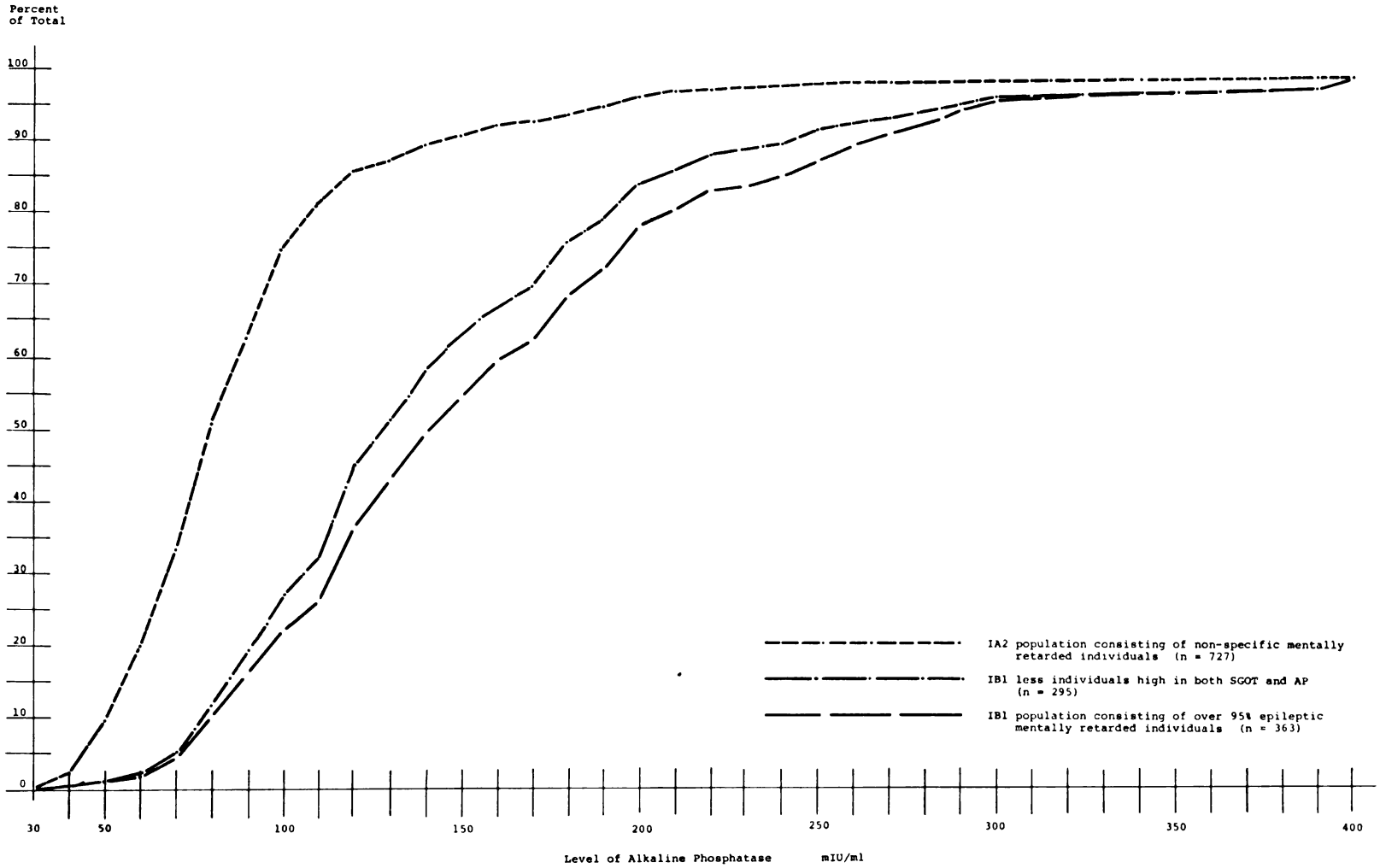

Figure 2. Cumulative curve of AP values in IA2 and IB2 populations.

protein, albumin, and AP. In 1973 three additional metabolites: ammonia, heat and L-phenylalanine resistant AP (APL) and heat resistant, L-phenylalanine sensitive AP, (API) were also tested

The results of intra- and inter-family difference analyses for 1971 and 1973 are presented in Table 15.

The intra- and inter-family mean differences were not significant for either 1971 or 1973, for metabolites glucose, SGOT, uric acid, Ca and albumin. However, except for glucose, the mean for inter-family differences exceeded the mean for intra-family differences, as expected. In 1971, the mean inter-family difference in metabolites BUN, Pi and AP were significantly higher than the mean for intra-family differences, while there were no significant differences for these metabolites in 1973. Although in these cases there was a change from significant to non-significant or the reverse from one year to the next, the differences were always in the same direction.

For the three additional metabolites, ammonia, APL and API tested in 1973, the mean inter-family difference was 33.6, 27.8 and 66 per cent above the intra-family difference, respectively. The differences were not significant for APL and ammonia, while that of API was significant at the 0.05 level.

Table 15. Analyses of intra- and inter-family differences of institutionalized sib pairs observed in 1971 and 1973.

1971		Glu mg%	BUN mg%	SGOT Karmen Units	UA mg%	Ca mg%	Pi mg%	TP gr%	Alb gr%	NH_3 µg%	AP mIU/ml	APL# mIU/ml	API@ mIU/ml
Mean	W	10.63	3.14	8.98	1.19	0.56	0.51	0.45	0.27	---	49.56	---	---
	B	11.78	4.34	12.83	1.26	0.73	0.94	0.56	0.36	---	90.36	---	---
S.D.	W	10.81	2.37	14.75	1.02	0.52	0.47	0.41	0.28	---	66.56	---	---
	B	11.12	2.87	17.30	1.26	0.62	0.68	0.50	0.28	---	99.30	---	---
S.E.	W	1.35	0.30	1.84	0.13	0.06	0.06	0.05	0.04	---	8.98	---	---
	B	1.39	0.36	2.16	0.16	0.08	0.09	0.06	0.04	---	13.39	---	---
N.		64	64	64	64	64	64	64	64	---	55	---	---
F		1.06	1.47	1.38	1.54*	1.46	2.16**	1.53*	1.00	---	2.23*	---	---
t		0.50	2.80**	1.36	1.97	1.67	4.20**	1.28	1.78	---	2.53*	---	---

Table 15. Continued

1973		Glu mg%	BUN mg%	SGOT Karmen Units	UA mg%	Ca mg%	Pi mg%	TP gr%	Alb gr%	NH_3 µg%	AP mIU/ml	APL# mIU/ml	API@ mIU/ml
Mean	W	19.81	3.30	6.54	1.29	0.48	0.53	0.54	0.34	33.87	57.42	9.81	1.00
	B	18.82	3.85	8.54	1.41	0.58	0.56	0.89	0.49	45.27	71.96	12.54	1.66
S.D.	W	22.19	2.05	7.11	0.76	0.35	0.47	0.49	0.26	28.60	66.92	9.77	1.08
	B	24.92	3.32	7.65	1.09	0.62	0.45	0.79	0.39	36.72	72.66	12.98	1.45
S.E.	W	3.86	0.36	1.23	0.13	0.06	0.08	0.08	0.04	4.97	11.65	1.70	0.18
	B	4.33	0.58	1.33	0.19	0.10	0.07	0.13	0.06	6.36	12.64	2.25	0.25
N.		33	33	33	33	33	33	33	33	33	33	33	33
F		1.26	2.61**	1.16	2.03*	3.27**	1.09	2.62**	2.31*	1.63	1.18	1.76	1.77
T		0.17	0.81	1.10	0.52	0.80	0.26	2.15*	1.79	1.41	0.85	0.97	2.09*

* Significant at 0.05 level.

** Significant at 0.01 level.

APL# Heat and L-phenylalanine resistant alkaline phosphatase.

API@ Heat resistant L-phenylalanine sensitive alkaline phosphatase.

Epileptic and retarded studies in 1973

In 1973 all the known retarded individuals with constitutional epilepsy in institution A were tested for the metabolites: ammonia, glucose, BUN, SGOT, uric acid, Ca, Pi, total protein, albumin, AP, APL, and API. In addition, they were also typed for their ABO and Lewis blood group systems and secretor status. Throughout the text this population will be referred to as IAE.

This study was performed on the same day as that of the sib pairs, with the two groups going through the Auto-Analyzer in random order.

One member of each sib pair was selected as a control. The control individual was that sib whose given name was alphabetically the last in the pair. Where a test eliminated persons less than 20 years old, the other sib replaced the selected sib, if possible. This group will be referred to as IAS.

The correlations between the various metabolites and age are presented in Table 16. The age correlations for the various metabolites: ammonia, glucose, BUN, uric acid, Ca, total protein, APL and API are not significant in either the IAE or IAS populations.

In the IAE population age 10 years and older, there is a significant correlation between age and Pi, V_{max}/v_{Km} and AP determinations. However, when individuals in the age

Table 16. The correlation between age and the various metabolites tested in the IAE and IAS populations.

Population	Metabolites													
	NH_3	Glu	BUN	SGOT	UA	Ca	Pi	TP	Alb	AP	APL	API	V/K	N
IAE over 10 years old	.17	.10	.19	-.28	-.17	.04	-.34*	-.18	-.20	-.49**	-.16	-.15	-.35*	61
IAE over 20 years old	.04	.30	-.09	-.02	-.10	-.17	.03	-.03	.02	.06	.08	-.17	.19	44
IAS over 10 years old	.30	-.13	.08	-.52**	-.05	.17	-.28	-.03	-.48*	-.42*	-.14	.04	-.27	33
IAS over 20 years old	.32	-.13	.18	-.23	.04	.10	.07	.08	-.22	.31	-.12	.04	.34	24

* Significant at 0.05 level.

** Significant at 0.01 level.

group 10-19 years old are taken out of the population, the correlation coefficient for all the metabolites tested is not significant.

In the IAS population there is a significant correlation between age and metabolites: albumin, AP and SGOT. However, these correlations also are not significant after removal of individuals 10-19 years old.

Tables 17-24 present the results of analyses of variance for the metabolites ammonia, glucose, SGOT, Ca, Pi, total protein, albumin and for age. The mean difference analyses for these metabolites show no significant differences between the IAE and IAS populations, whether the ages are 10 and older or 20 and older. Figure 3 shows an upward shift in the level of ammonia in epileptics, when compared to the non-epileptic mentally retarded individuals. However, both populations have large standard deviations and the mean differences are not significant.

When the many high outliers (ammonia levels over 100) in the two populations are compared, there are 8 of 33 in the IAS and 20 out of 61 in the IAE population. The chi-square value for the comparison is 0.75 which is not significant at 0.05 level.

Table 25 presents the results of the BUN analysis. The mean and standard deviation are 13.12 mg/100 ml and 4.24 for the IAE population age 10 and older, and 15.12 mg/100 ml and 3.73 for the IAS population age 10 and older,

Table 17. The analysis of the test for serum ammonia.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	89.97	40.95	1.02	1.31
IAS population age 10 and older	33	78.45	40.53		
IAE population age 20 and older	44	95.11	44.82	1.56	1.03
IAS population age 20 and older	24	84.17	35.87		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 18. The analysis of the test for serum glucose.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	98.20	22.34	1.70	.23
IAS population age 10 and older	33	97.18	17.13		
IAE population age 20 and older	44	97.30	18.67	1.27	.21
IAS population age 20 and older	24	96.33	16.54		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 19. The analysis of the test for serum protein.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	7.55	0.68	1.06	.34
IAS population age 10 and older	33	7.50	0.70		
IAE population age 20 and older	44	7.46	0.67	1.36	.22
IAS population age 20 and older	24	7.50	0.78		

F or t test performed between IAE and IAS over 10 years, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 20. The analysis of the test for serum albumin.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	4.58	0.40	1.38	0.36
IAS population age 10 and older	33	4.61	0.34		
IAE population age 20 and older	44	4.51	0.33	1.21	0.12
IAS population age 20 and older	24	4.50	0.30		

F or t test performed between IAE and IAS over 10 years, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 21. The analysis of the test for serum calcium.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	10.24	0.57	1.04	1.28
IAS population age 10 and older	33	10.40	0.58		
IAE population age 20 and older	44	10.26	0.50	1.69	1.34
IAS population age 20 and older	24	10.45	0.65		

F or t test performed between IAE and IAS over 10 years, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 22. The analysis of the test for serum phosphate.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	3.54	0.57	1.04	.64
IAS population age 10 and older	33	3.62	0.58		
IAE population age 20 and older	44	3.37	0.56	1.04	.49
IAS population age 20 and older	24	3.44	0.57		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 23. The analysis of the test for serum SGOT.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	22.49	9.31	1.72*	1.26
IAS population age 10 and older	33	20.33	7.10		
IAE population age 20 and older	44	20.34	6.87	1.04	1.69
IAS population age 20 and older	24	17.54	5.80		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 24. The analysis of the test for the age of the individuals.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	2.26	1.08	2.03*	1.06
IAS population age 10 and older	33	2.58	1.54		
IAE population age 20 and older	44	2.75	0.87	2.20*	1.70
IAS population age 20 and older	24	3.25	1.29		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

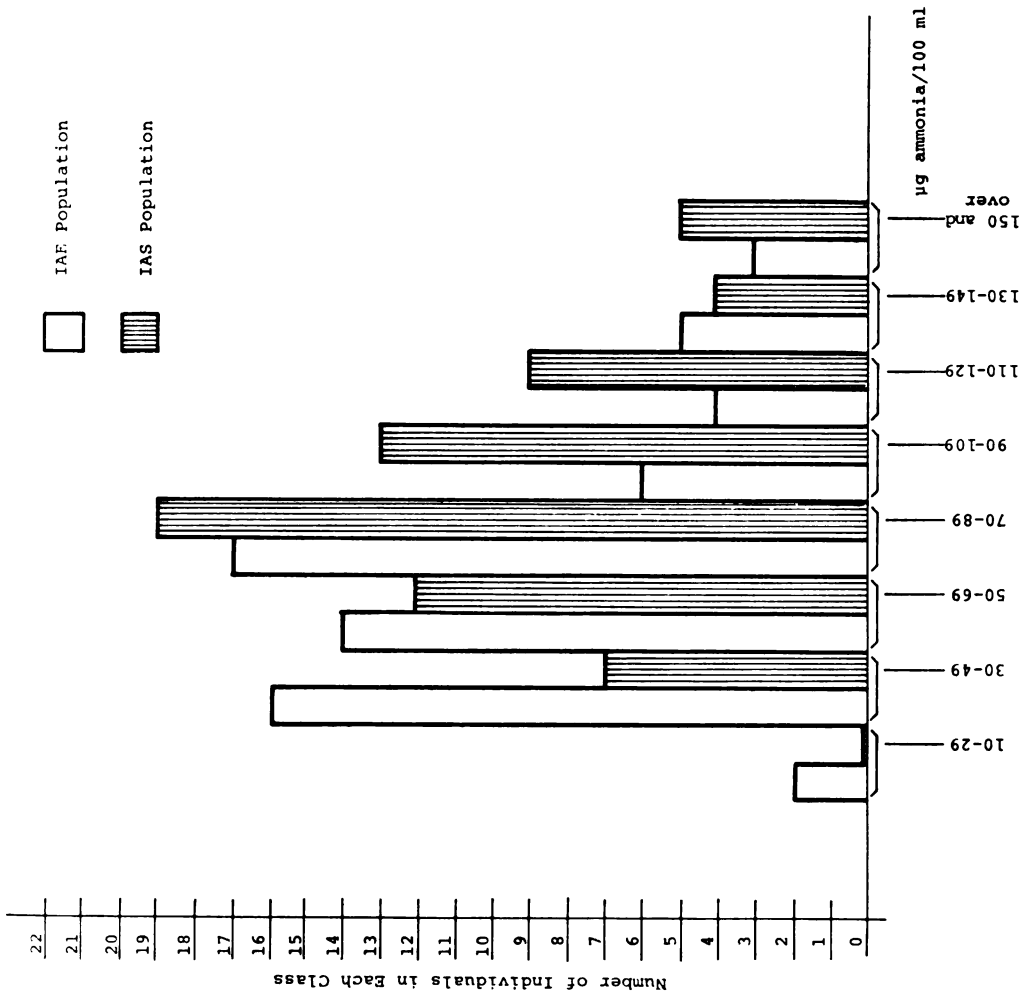

Figure 3. Distribution of ammonia in the IAE and IAS populations.

Table 25. The analysis of the test for blood urea nitrogen.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	13.12	4.24	1.29	2.05*
IAS population age 10 and older	33	15.12	3.73		
IAE population age 20 and older	44	13.98	4.00	1.24	1.02
IAS population age 20 and older	24	14.98	3.59		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

respectively. The F value for the populations is 1.29, which is not significant. The mean difference analysis with t = 2.05 is significant at the 0.05 level, indicating a lower value for the epileptic population. There is no significant difference in the BUN values without persons 10 - 19 years old.

Table 26 presents the results of the uric acid determinations. The means are 5.41 and 5.97 mg/100 ml, and the standard deviations are 1.15 and 1.43 for the IAE and IAS populations age 10 and older, respectively. The F test is not significant, but t = 2.06 for the mean difference analysis is significant at the 0.05 level. The means are not significantly different when only individuals 20 years or older are compared, although F = 1.79 is significant indicating different variances in the two populations. Apparently the level of uric acid is lower in the epileptic compared with the non-epileptic mentally retarded populations.

Table 27 presents the results of serum alkaline phosphatase determinations. The means are 137.00 and 88.16 mIU and the standard deviations are 80.20 and 35.74 for the IAE and the IAS populations age 10 and older, respectively. F = 5.04 is significant at the 0.01 level indicating that the two populations have different variances. The mean difference analysis with t = 4.07, which is significant at the 0.01 level, indicates a higher level of AP in the IAE

Table 26. The analysis of the test for serum uric acid.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	5.41	1.15	1.55	2.06*
IAS population age 10 and older	33	5.97	1.43		
IAE population age 20 and older	44	5.30	1.12	1.79*	1.54
IAS population age 20 and older	24	5.84	1.50		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 27. The analysis of the test for serum alkaline phosphatase.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	137.00	80.20	5.04**	4.07**
IAS population age 10 and older	33	88.15	35.74		
IAE population age 20 and older	44	103.01	36.19	3.67**	4.99**
IAS population age 20 and older	24	69.75	18.85		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

population. Comparable differences are found if individuals under age 20 are excluded from the samples. Figure 4 shows the distribution of AP in different age groups in the IAE and IAS populations. The higher level of AP is clearly observed in all age groups of the IAE population.

The results of API are presented in Table 28. The means are 2.34 and 1.61 mIU/ml and the standard deviations are 1.53 and 1.17 for the IAE and IAS populations age 10 and older. The mean difference analysis with t = 2.39 is significant at the 0.05 level.

The means for the IAE and IAS populations are 2.25 and 1.63 mIU/ml and the standard deviations are 1.12 and 1.21 for the two populations, respectively, for age 20 and older. The mean difference analysis with t = 2.12 is significant at the 0.05 level, indicating the higher values of API in all age groups of the epileptic sample.

For APL, the means are 11.84 and 7.68 mIU/ml and the standard deviations are 8.24 and 7.32 for the IAE and IAS populations age 10 and older, respectively (Table 29). The mean difference analysis, t = 2.43, is significant at the 0.05 level. When persons age 10 - 19 are excluded from the samples, the means are 10.45 and 7.13 mIU/ml and the standard deviations 6.62 and 7.98. The mean difference analysis, t = 1.84, is not significant.

Table 30 presents the $V_{max/v_{Km}}$ values for the two populations. The means are 1.94 and 1.89 and the standard

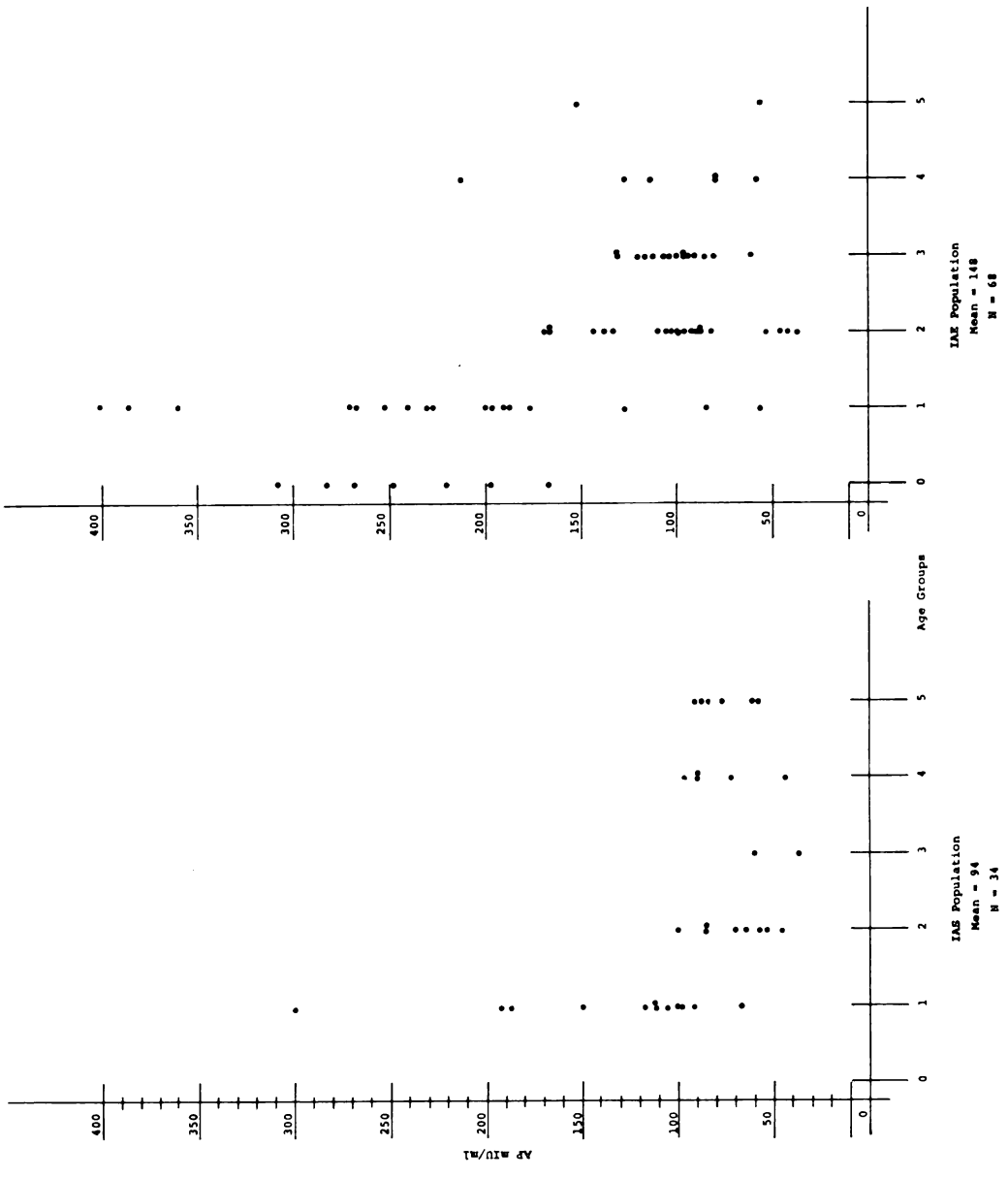

Figure 4. The distribution of alkaline phosphatase in different age groups of the IAS and IAE populations.

Table 28. The analysis of the test for serum intestinal alkaline phosphatase.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	2.34	1.53	1.71	2.39*
IAS population age 10 and older	33	1.61	1.17		
IAE population age 20 and older	44	2.25	1.12	1.17	2.12*
IAS population age 20 and older	24	1.63	1.21		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 29. The analysis of the test for serum liver alkaline phosphatase.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	11.84	8.24	1.27	2.43*
IAS population age 10 and older	33	7.68	7.32		
IAE population age 20 and older	44	10.45	6.62	1.45	1.84
IAS population age 20 and older	24	7.13	7.98		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

Table 30. The analysis of the test $V_{max/v_{Km}}$ for serum alkaline phosphatase.

	N	Mean	Standard deviation	F	t
IAE population age 10 and older	61	1.94	0.15	2.77**	2.04*
IAS population age 10 and older	33	1.89	0.09		
IAE population age 20 and older	44	1.88	0.11	1.89*	1.37
IAS population age 20 and older	24	1.85	0.08		

F or t test performed between IAE and IAS over 10 years old, or IAE and IAS over 20 years old.

* Significant at 0.05 level.

** Significant at 0.01 level.

deviations 0.15 and 0.09 for the IAE and IAS populations age 10 and older, respectively. The mean difference analysis, t = 2.04, is significant at the 0.05 level. Although the mean difference is only 0.05, the small standard errors make this difference significant.

When the 10 - 19 year age group is taken out of the population, the mean difference drops to 0.03, and t = 1.37 which is not significant.

Those individuals who were high in the level of their total protein, AP or both in the original screen were re-tested, and the level of their blood ammonia was also measured. These individuals were also checked to see if they had epilepsy. Eleven out of 49 of these tested individuals had epilepsy (22.5 per cent), and 6 out of 11 epileptics had high ammonia (55.5 per cent) (Table 31). The frequency of epileptics in institution A is stated by the medical personnel to be about 4 per cent.

Table 32 shows the number of individuals observed in each ABO and Lewis blood group phenotype and the number of secretors and non-secretors in each population. The chi-square analyses indicate that there are no significant differences with respect to blood groups and secretor status between the IAE and IAS populations.

In Table 33 the IAE and IAS samples are compared with a large sample of the general population from Southeast Michigan (provided by Dr. E. Hackel) with respect to

Table 31. The distribution of persons with high blood ammonia/epilepsy in groups with high AP or total protein.

	N	High NH_3 not epileptic	Epileptic, normal NH_3	Epileptic with high NH_3
High protein, normal AP	11	3	0	2
High AP, normal protein	17	5	3	1
High protein and AP	21	6	2	3

Table 32. The distribution of ABO, and Lewis blood groups and secretor status in the IAE and IAS populations.

Sub - Table A	O	A	B	AB	Le-	le le	Sec.	sec sec
IAE population age 10 and older	29	26	9	4	58	10	50	18
IAS population age 10 and older	22	7	4	1	31	3	26	8
	χ^2=4.83 d.f.=3				χ^2=0.71	d.f.=1	χ^2=0.10	d.f.=1
Sub - Table B	O	A	B	AB	Le-	le le	Sec.	sec sec
IAE population age 20 and older	17	19	6	2	38	6	33	11
IAS population age 20 and older	15	5	3	1	22	2	19	5
					χ^2=.42	d.f.=1	χ^2=.15	d.f.=1

Table 33. The chi-square analysis of ABO group distributions in the IAE and IAS populations.

IAE population	Observed	Expected	(o-e)	$(o-e)^2/e$
A	26	23.80	2.20	0.20
B	9	8.84	.16	0.00
AB	4	3.40	.60	0.11
O	29	31.96	2.96	0.27
Total	68	68		0.58
	$\chi^2=0.58$	d.f.=3		

IAS population	Observed	Expected	(o-e)	$(o-e)^2/e$
A	7	11.90	4.90	2.02
B	4	4.42	0.42	0.04
AB	1	1.70	0.70	0.29
O	22	15.98	6.02	2.27
Total	34	34		4.62
	$\chi^2=4.62$	d.f.=3		

ABO blood groups. A test of the difference between IAS and the general population (3° of freedom) yields $\chi^2=4.61$, which is not significant. The comparison of IAE and the general population (3° of freedom) gives $\chi^2=0.58$, which also is not significant.

A test of (1) B and O secretors, (2) A and AB secretors and (3) non-secretors of all blood types (Table 34), indicates that these three groups do not differ significantly in frequency between the IAE and IAS populations.

A comparison of the level of API in B and O secretors, A and AB secretors, and the non-secretors of all blood types in the IAE and IAS populations (Table 35), indicates that in all three groups the IAE population has a higher level of API than IAS population. Within each population the B and O secretor group has a significantly higher level of API than the other two groups.

Table 34. The comparison of the distribution of the B and O secretor, A and AB secretors and the non-secretors of all blood phenotypes in the IAE and IAS populations.

Sub - Table A	B and O secretors	A and AB secretors	Non-secretors	Individuals with gene A	Individuals without a gene A
IAE population age 10 and older	31	19	18	30	38
IAS population age 10 and older	19	7	8	8	26
	χ^2=1.03	d.f.=2		χ^2=4.11	d.f.=1
Sub - Table B	**B and O secretors**	**A and AB secretors**	**Non-secretors**	**Individuals with gene A**	**Individuals without a gene A**
IAE population age 20 and older	19	14	11	21	23
IAS population age 20 and older	14	5	5	6	18
	χ^2=1.55	d.f.=2		χ^2=3.35	d.f.=1

Table 35. Mean API in different blood groups and secretor status in IAE and IAS populations.

	O and B secretors		A and AB secretors		Non-secretors	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
IAE population age 10 and older	3.00	1.73	1.73	0.87	1.66	0.97
IAS population age 10 and older	2.26	1.52	0.85	0.89	1.25	0.70
F		1.29		1.06		1.92
Mean diff. analysis t	1.53		2.27*		1.07	
d.f.	48		24		24	

* Significant at 0.05 level.

DISCUSSION

Mental retardation may be caused by some basic defect in the genetics of the individual or may be due to some environmental factor (including maternal-fetal incompatibility). Almost any environmental cause of mental retardation will not have a detectable effect on the physiology of the adult individual. However, some of the genetic causes of mental retardation will be related to permanent shifts in the level of some metabolite.

In general, a mentally retarded population will consist of individuals whose retardation is due to a large variety of conditions. If a distinct group of these individuals has the same condition and it is associated with an abnormal amount (either too much or too little) of a measured metabolite, these individuals will contribute to the mean of that metabolite being too high or too low (though rarely will the difference be statistically significant because of this). The variance of the metabolite may show a statistically different value from a normal population if the change in the level of the metabolite is extreme, and if there are enough of the retarded population with the condition. A more sensitive test for a condition of this type might be for an increased number of high or low

outliers, even though they do not change the mean or variance to a significant degree. For these reasons, the mean, variance, and number of outliers have been examined by appropriate tests.

In the first part of this study the IA1, IA2 and IB1 populations were compared to the IAC population to find out whether the non-specific retarded and the retarded epileptic populations were different from the normal population. Comparison of the IA1 and the IA2 populations, consisting of non-specific mentally retarded individuals in institution A tested in different years, would indicate any yearly variation in the methodology. The IA2 and IB1 populations are compared because they were tested in the same year, and also retarded. Therefore, any difference in the level of their metabolites would primarily be due to the epileptic condition of the IB1 population.

The inter- and intra-family mean differences were calculated and compared for each of the sib groups in 1971 and 1973. If the level of a particular metabolite is controlled largely by one or two genes, as opposed to a combination of many genetic and environmental factors, we would expect to find a difference between the inter- and intra-family mean difference. However, if the level of a given gene is controlled by a combination of many genetic and environmental factors, the inter- and intra-family mean differences are expected to be the same.

Finally, the IAE and IAS populations were retarded individuals living in the same environmental conditions, and only one of them had epilepsy (the IAE population). Comparing these populations might confirm if one of the metabolites is affected in the epileptic group when it is compared to the non-epileptic mentally retarded population.

BUN

In comparison with the normal controls (IAC), serum BUN is higher in mentally retarded individuals (IA1 and IA2) and lower in epileptics (IB1) to a statistically significant degree. Confirming this, the mentally retarded sib pair group (IAS) has a significantly higher BUN than the comparable epileptic group (IAE), and the IB1 epileptic group also has a significantly large number of individuals who are low outliers.

The serum ammonia levels of the sib (IAS) and the epileptic (IAE) populations are highly variable, but the average level is much higher among the epileptics. It can be argued that one might expect higher serum ammonia levels in a population that has low BUN levels. Perhaps many of the epileptic individuals are unable to synthesize appropriate amounts of urea (leading to a low BUN). These individuals would, therefore, have high titers of free serum ammonia. This might lead to physiological changes that are interpreted as epilepsy. For example, Sherlock *et al.* [86]

caused an altered mental state, characteristic tremor, and electroencephalographic changes indistinguishable from impending hepatic coma by using ammonium chloride and high protein diets.

Bessman and Bessman [9] suggested that the excess free ammonia in the brain is converted to bound form by several mechanisms; for example, by glutamine synthesis, and by the reversal of glutamate oxidation. Bessman and Bradely [10] suggested that hepatic coma could be induced by high arterial ammonia levels which cause the synthesis of glutamic acid from the α-ketoglutarate generated by the Krebs cycle in the brain. It was also suggested that transamination of aspartic acid, to replace the α-ketoglutarate, which is necessary for the Krebs cycle, cannot occur because the brain is unable to utilize an exogenous source of aspartic acid.

In 1963 Stahl [94] observed that an elevated blood ammonia level may precede coma by many days. A favorable clinical outcome was usually preceded by a decline in the blood ammonia concentration. Stahl suggested that this finding strongly favored Bessman's [9, 10] hypothesis of a long active depletion by ammonia of the Krebs cycle in the brain.

Carter *et al.* [18] indicated that most ammonia in the blood is in the ionized ammonium NH_4^+ form, and that an increase in blood pH would increase the unionized NH_3 form

in the blood. This would raise the amount that could penetrate the blood-brain barrier.

The results of the present study, combined with the findings by others, suggest the possibility that many institutionalized retarded individuals who are classified as epileptics may, in reality, be suffering from ammonia intoxication. This hypothesis could be further supported by the fact that many of these "epileptics" are being administered different epileptic suppressant drugs, often in extensive combinations, without an effective control of their conditions. Appropriate electroencephalography and analysis of the blood ammonia level may be indicated for every institutionalized "epileptic."

Alkaline phosphatase

The alkaline phosphatase (AP) levels that were determined showed a number of differences, all of which were highly significant. The IA1 and IA2 populations were both higher in mean AP than the IAC control group, while the IB1, largely epileptic group, was much higher than the mentally retarded groups. Some of the difference appears to be due to a general increase in AP levels, particularly for the epileptic group, but the number of persons with very high values, registering as high outliers, is greatly increased in the mentally retarded and especially in the epileptic

group. Similarly, the IAE group had a significantly higher AP level than the IAS group.

Mabry *et al.* [57] found four closely related individuals with what appeared to be a syndrome of mental retardation, epilepsy, and high AP levels. They stated that a sample of 129 retardates, many of whom had epilepsy, had normal AP levels, but their report does not compare these levels with any other population. Casey *et al.* [16] appear to report a high frequency of epileptics with high AP levels, confirming the observations of this study.

It has been stated that high levels of glutamate oxalacetate transaminase (GOT) occur in the cerebrospinal fluid of epileptics, though not in their serum [43, 61]. The IB1 population contained 81 high outliers for serum GOT (SGOT), which is an extremely high frequency. However, high SGOT and high AP levels simultaneously are a sign of liver damage, and 67 of 81 high individuals did combine these two signs. If the group is dropped from the IB1 population, the remaining individuals still have a significantly higher mean AP level than is found in the mentally retarded or control populations. Figure 2 compares the cumulative distribution of AP levels for the IA2 population with that for the IB1 population both with and without the 67 individuals with high AP-high SGOT levels. The results indicate an upward shift in the level of AP in the epileptic population.

Alkaline phosphatase in man as well as in other mammals has been found to have an inverse correlation with age [7, 32, 33, 88, 95, 110, 114]. This inverse correlation was significant in the IAE and IAS populations age 10 and older. However, the significance of age effect disappears if the individuals in age group 10-19 are removed from these populations. The IAE population age 20 or greater still shows a significantly higher level of AP than does the comparable IAS group (Figure 4).

An increased rate of enzyme synthesis or a decreased rate of enzyme degradation can induce an increase in enzyme concentration [39]. A third mode of induction is an increase in activity of an enzyme due to elimination of inhibitors, increase of activators or a configurational change. Griffin and Cos [39] observed that prednisolone mediates a configurational change in alkaline phosphatase during its synthesis. This altered configuration or assembly of the enzyme which occurs during synthesis leads to an increase in the number of catalytic sites or a lowering of the energy level of the enzyme substrate transition state. This third mode of change clearly involves a change in the Km value.

The IAE and IAS populations age 10 and older significantly differed from each other in the mean $V_{max/v_{Km}}$ value. However, there was a significant age correlation in the IAE population for this value.

When the IAE and IAS populations age 20 and older were compared, the age correlation was not significant and the mean $V_{max/v_{Km}}$ of the two populations did not significantly differ from each other. This finding tends to eliminate a configurational change as a cause of elevation of alkaline phosphatase in epileptics.

In 1971 Phelan *et al.* [70] found that the increase of serum alkaline phosphatase in liver diseases was largely due to a *de novo* synthesis of the enzyme by liver cells. Skillen *et al.* [90] observed that the increase of intestinal alkaline phosphatase (API) may be an indication of a defect of the hepatobiliary system or a chronic renal failure. Liver alkaline phosphatase (API) also has been observed to be elevated in hepatobiliary diseases by many other investigators [16, 19, 41, 49, 52, 84, 114].

The APL means in the IAE and IAS populations age 10 and greater were significantly different from each other. When the effect of age was removed, the mean APL difference was not significant, although the IAE still had a greater mean.

The mean API was significantly greater for the IAE than the IAS population in both age 10 and older or 20 and older groups.

From these findings, it can be suggested that AP is elevated in epileptics due to 1) an increase in the enzyme synthesis or 2) a decreased rate of enzyme degradation.

Furthermore, if taken along with the BUN and ammonia results, the elevation of liver and intestinal alkaline phosphatases suggest many cases of hepatobiliary disfunction in the epileptic group, which leads to a high *de novo* synthesis of AP. The increase of intestinal phosphatase in epileptics does not seem to be an indication of chronic renal failure. Otherwise, we would expect to get high and not low BUN values [78].

If we accept the hypothesis of hepatobiliary disfunction, the decreased synthesis of BUN and increased level of free ammonia observed in the epileptic group will be understandable, and the excess ammonia in the system could cause ammonia intoxication of the brain. This would tend to make a thorough re-evaluation of the institutionalized "epileptics" valuable. Many of these persons may be severely misdiagnosed and are being improperly treated.

The second possibility would be that, due to internal changes in the epileptics, either physiological or environmental changes induced by drugs, the half-life of the AP is increased in the epileptic group. Perhaps we are observing elevated AP in epileptics due to a decreased degradation of the enzyme and not because of an increased *de novo* synthesis.

It has been observed that the level of total AP was about three times higher in young individuals as compared to adults (95 and many others). However, it has also been observed that the level of the liver and intestinal

isoenzymes were independent of the age of the subject [95]. The results of this experiment are in complete agreement with the above finding. There was no significant correlation between age and either APL or API. However, the correlation between age and total AP was highly significant.

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A Population Study in Human Biochemical Genetics: The Relationships of Several Metabolites in Epilepsy and Mental Retardation.

presented by

Habibollah Fakhrai

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Zoology

Herman M. Slatis

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Date May 30, 1974

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The relationship of different alkaline phosphatase genotypes with ABO blood groups and secretor status has been observed by Arfors *et al.* [2], Beckman [7], Bamford *et al.* [6], Evance [24], Shreffler [88], Langman *et al.* [53], and many others. They found that intestinal alkaline phosphatase was elevated in the blood serum of blood group O and B secretors when compared to A and AB secretors or the non-secretors of all blood types. In 1968, Langman *et al.* [54] found that the alkaline phosphatase of intestinal mucosal extract was significantly higher in individuals of blood group O and B than in those of blood group A. When the secretor status was taken into account the differences between group B or O compared to group A became more pronounced and non-secretors had the lowest level of intestinal mucosal alkaline phosphatase.

When the level of API was calculated for B and O secretors, A and AB secretors and the non-secretors of all blood groups in both the IAE and IAS populations, the O and B secretors had significantly higher levels of API than A and AB secretors or the non-secretors of all blood groups. However, there was no significant difference between the A

and AB secretor group and the non-secretors in either of the two populations. One possible way to explain this finding when compared to that of Langman *et al.* [54] is that although the A and AB secretors have more AP in their intestinal mucosa compared with the non-secretors, the A and AB secretors are releasing the enzyme to the blood at a lower rate than the non-secretors. The second possibility would be that perhaps the level of AP in the intestinal mucosa of the retarded population is quantitatively different than the normal populations in A and AB secretors, and the non-secretors of all blood groups.

There was no significant difference between the distribution of individuals with ABO blood type of the IAE and IAS populations when compared with the general blood group frequencies of the counties where these individuals originated. The Lewis blood group and the secretor status of the IAE and IAS populations were also compared and no significant difference in these genes were observed between the epileptic and the non-epileptic groups.

Hackel [42] studied the relationship of blood groups and mental deficiency in Michigan. He observed no relationship between these blood markers and mental retardation. The findings of this study are in complete agreement with this finding. He also observed an excessive number of type B and a decreased number of type A individuals in his samples. In this study the number of B individuals is as expected,

and in the IAS population, but not the IAE population, a decreased number of A type individuals is observed. However, this could very well be due to the small sample size.

A number of other tests were also performed; however, the results of these tests are not of significance and, therefore, will not be discussed.

SUMMARY

Blood was collected by venopuncture from populations composed of non-specific mentally retarded individuals in an institution, from the employees of this institution as a normal control, and from the mentally retarded epileptics in a second institution. The total number of individuals in these populations was 1988. Each serum was tested for various metabolites: glucose, blood urea nitrogen, uric acid, serum glutamate oxalacetate transaminase, calcium inorganic phosphate, total protein, albumin, and alkaline phosphatase.

In 1971 blood was collected from all 64 sib pairs in the first institution and subjected to the above tests.

In 1973 blood and saliva were collected from 33 sib pairs and 61 individuals with idiopathic epilepsy, and subjected to the above tests. In addition, tests were performed for various alkaline phosphatase isoenzymes, blood ammonia, ABO and Lewis blood types, and their secretor status.

The tests show that the level of alkaline phosphatase is higher and the level of blood urea nitrogen lower in epileptic than in non-epileptic populations. The elevation in the level of serum alkaline phosphatase held true for all

the isoenzymes. By testing the Km values it was observed that the change in the level of alkaline phosphatase did not involve a change in the nature of the enzyme. Therefore, it was suggested that the change in the alkaline phosphatase activity in epileptics was due either to an increased *de novo* synthesis of the enzyme in this group or because of a decreased rate of enzyme degradation which leads to an increase in the accumulation of the enzyme protein.

The low blood urea nitrogen and the high ammonia levels combined with the results of the alkaline phosphatase studies indicate that in many instances the individuals classified as epileptic may, in reality, have a liver dysfunction and may be suffering from ammonia intoxication. This finding suggests that a more thorough investigation of institutionalized epileptics should be performed. A profile composed of the results of electroencephalography, the levels of ammonia, blood urea nitrogen and different alkaline phosphatase isoenzymes, may be necessary before the individual is treated for epilepsy.

The results of ABO and Lewis blood types and the saliva ABH types indicated that there were no differences between the epileptic and the non-epileptic mentally retarded populations. Furthermore, there was no significant difference between the ABO distribution among the mentally retarded groups and the general population.

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