10^{-5}M and $K_i = 1.0 \times 10^{-5}M \pm 0.1 \times 10^{-5}M$ (SEM) at pH 7.5, compared with $K_i = 0.53 \times 10^{-5}$ reported by Cory and Suhadolnik(6) using Sigma Type I enzyme at pH 7.0. The data were subjected further to more subtle analysis according to Reiner (14). The inhibition was found to be the "complete exclusive E" type, which corresponds to classical competitive. K_3 of Reiner, which is the inhibitor-enzyme dissociation constant, was 0.9×10^{-5}M which agreed well with K_i. Thus, N^6-methyladenosine is a competitive inhibitor of adenosine deaminase.

Because N^6-methyladenosine and MMPA are structurally similar and their K_is are of the same order of magnitude, the kinetic analysis of one may be related to the other by analogy. Thus, the initial conclusion based on the interpretation of Lineweaver-Burk plots that MMPA is a competitive inhibitor of adenosine deaminase appears to be valid.

The relationship, if any, between the inhibition of adenosine deaminase inhibition by MMPA and aminonucleoside nephrosis is not clear at this time.

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Relationship of Alkaline Phosphatase Levels in Intestinal Mucosa to ABO and Secretor Blood Groups.* (31504)

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Since the first discovery of a slowly-migrating serum alkaline phosphatase component on starch gel electrophoresis(1,2), it has been abundantly demonstrated that the ABO and Secretor loci influence the occurrence and strength of this slow-moving (B) phosphatase band(2-7). The band is weak or absent in type A individuals, and is absent almost without exception in ABH non-secretor individuals. In type O or type B individuals who are ABH secretors, the B phosphatase component may or may not be present, and if present may vary in concentration over a wide range; this variation is influenced by genetic as well as non-genetic factors(4,5). Similar blood group-associated serum alkaline phosphatase variation has been reported in cattle(8) and in sheep(9).

There is now a compelling body of evidence to indicate that the serum B phosphatase is the same enzyme as a slowly-migrating component found in extracts of intestinal mucosa. Both enzymes have the same electrophoretic mobility on starch gel, as shown by the results of Hodson *et al*(10), and confirmed in our studies. Both enzymes are similarly in-

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hibited by L-phenylalanine(11,12), and these are the only alkaline phosphatases known to be so inhibited. Aalund *et al*(13) have shown that the blood group-associated serum alkaline phosphatase of sheep is also inhibited by this specific intestinal phosphatase inhibitor. Schlamowitz and Bodansky(14) have shown, with a rabbit antiserum prepared against human intestinal alkaline phosphatase, that human serum contains a cross-reacting phosphatase precipitated by the antiserum, presumably the same enzyme. These authors found the cross-reactive phosphatase to constitute from 13 to 29% of the total serum phosphatase in normal subjects. Fishman *et al*(15) found 20 to 65% by the phenylalanine inhibition method. Robinson and Pierce(12) treated human serum with neuraminidase, and found that only the B enzyme is not altered in mobility by this agent. We have shown (unpublished) that the B phosphatase of intestinal extracts is similarly resistant to neuraminidase. Finally it has recently been observed in our laboratory that an antiserum prepared against extracts of intestinal mucosa, when mixed with intestinal extracts eliminates the intestinal B phosphatase band on starch gel in the manner described by Boyer(16), and also removes the serum B phosphatase band when mixed with serum. This indicates immunochemical similarity, if not identity (Shokeir and Shreffler, unpublished).

Because of the strong influence of the ABO and Secretor loci on the serum B phosphatase expression, and the probable identity of the serum and intestinal B phosphatases, it seemed of considerable interest to determine whether ABO and secretor types have any influence upon the presence or levels of the enzyme of intestinal mucosa. Most specifically, it seemed important to determine whether ABH non-secretors might be unable to synthesize the enzyme. This communication reports the results of such studies, indicating that the level of the intestinal enzyme is not significantly influenced by either ABO type or ABH secretor type.

Materials and methods. Seventeen specimens of duodenum and jejunum were obtained at autopsy, along with a blood sample.† The red blood cells were typed for the ABO and Lewis systems, and the serum was classified for B phosphatase level as previously described(5). The mucosa was scraped from the washed intestine and stored at —80° until analyzed. It was then homogenized in saline in a Waring blender for 3 minutes, spun at 10,000 × *g*, and the supernatant extract saved for analysis. All extracts were adjusted to a volume of 10 ml per g initial dry weight of mucosa. The extracts were analyzed for total phosphatase activity by the method of Grossberg *et al*(17), and electrophoresed on starch gel. The phosphatase pattern was developed by the method previously described (5), and B band intensity was classified on an arbitrary scale from 0 through 6, in a manner similar to that previously described for serum (5). The intestinal extracts were also tested for blood group substances by hemagglutination inhibition to verify the ABH secretor type deduced from the Lewis type of the red blood cells.

Results. Table I shows the results of the analysis of extracts and sera, with the specimens grouped by blood type. Three specimens (Nos. 5, 16 and 17), although probably from secretors, could not be conclusively classified for ABH secretor type due to ambiguities or discrepancies between the Lewis type and the inhibition tests of the intestinal extracts. They are, therefore, listed as questionable. No blood sample was available with specimen No. 1, but the blood type was deduced from inhibition studies on the mucosal extract. Two specimens (Nos. 15 and 16), although included, may be abnormal, because of the unusually high levels of serum B phosphatase in these type A_1 individuals.

Fig. 1 shows the starch gel results on the extracts and also demonstrates the similarity in mobility of the serum and intestinal B phosphatase bands. If the mucosal phosphatase is influenced by ABO and secretor type in a way similar to the serum phosphatase, it would be expected that ABH nonsecretors would completely lack the intestinal B phosphatase, and that type A individuals

† I wish to thank Dr. R. Hendrix for supplying these specimens.

would have weak levels or no B phosphatase in the intestine. In fact, the level of intestinal B phosphatase is comparable in all blood group classes, and the enzyme is unquestionably present in ABH non-secretors. The total phosphatase activity of the intestinal extracts is even higher in type A than in type O individuals, and there is no essential difference between type O secretors and non-secretors. There is no significant

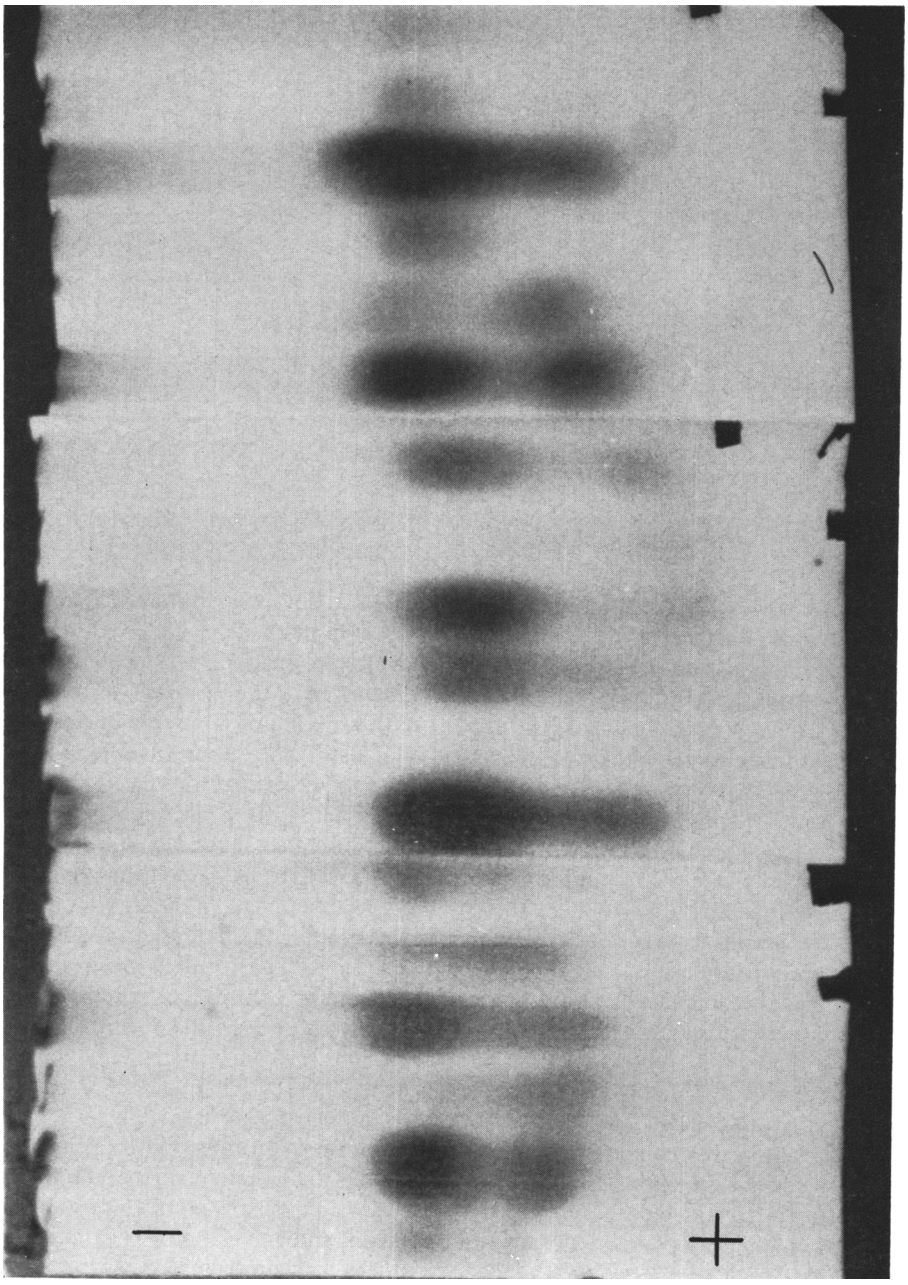

FIG. 1. Results of separation of mucosal extracts by starch gel electrophoresis. The order of samples from top to bottom is the same as the order in Table I. The last sample is a serum having a high level of B phosphatase, showing the similarity in mobility of serum and intestinal B phosphatases.

TABLE I. Results of Analysis of Serum and Intestinal Extracts.

Specimen	Blood group*		Serum B phosphatase†		Intestinal B phosphatase†		Total intestinal phosphatase activity‡	
1	O	S	–		1		18	
2	O	S	2		2		26	
3	O	S	3	1.7§	6	2.7	219	72
4	O	S	0		2		23	
5	O	?	2		1		192	
6	O	NS	0		5		168	
7	O	NS	0	0.0	3	3.0	45	84
8	O	NS	0		1		38	
9	B	S	1	0.5	5	4.0	61	157
10	B	S	0		3		253	
11	A_2	S	1		0		18	
12	A_1	S	0		6		486	
13	A_1	S	0	1.0	2	3.3	141	296
14	A_1	S	1		1		222	
15	A_1	S	3		4		333	
16	A_1	?	3		2		59	
17	A_1	?	0		5		182	

* S = ABH secretor, NS = ABH non-secretor.

† Numbers represent semi-quantitative classes determined as previously described(5), where 0 indicates absence of the phosphatase after starch gel electrophoresis and 1 through 6 indicate successively greater activities of the enzyme.

‡ Activity = mg P liberated from beta-glycerolphosphate/hr/100 ml extract.

§ Mean of bracketed values.

correlation between level of B phosphatase in the serum and level either of intestinal B phosphatase or of total alkaline phosphatase in the mucosal extracts. Thus the ABO and secretor effects on serum B phosphatase are not observed with the intestinal extracts. The difference between type O and type A individuals in total alkaline phosphatase activity in the mucosal extracts is statistically significant ($t = 2.29$, $P<0.05$), and may be a real effect. However, due to the small number of observations, further data should be collected before it is accepted as valid. The apparent discrepancy in some instances between the total activity of an extract (Table I) and the apparent activity revealed by staining intensity on the starch gel (Fig. 1) is due to differing amounts of phosphatase in the faster-migrating intestinal component, and to activity remaining at the origin of the starch gel in some cases, which is not clearly revealed by the photograph.

Discussion. These results establish that the type A and the ABH non-secretor individuals, who lack the B phosphatase of serum, do have B phosphatase in their intestinal mucosa at levels no different than type O or type B individuals, who usually have the B phosphatase in their serum. This observation indicates that the absence of B phosphatase from the serum of non-secretor and type A individuals, is not due to inability to synthesize it, or to synthesis at a reduced rate relative to type O and type B secretor individuals. The serum difference must, therefore, be due either to a reduced rate at which the phosphatase enters the serum, or to a differential inhibition or destruction of the phosphatase which gets into the serum. The precise role which the ABO and Secretor loci or their products may play in such a process is, of course, a point of great interest which might have an important bearing upon mechanisms of gene action by the blood group genes.

Summary. Autopsy specimens of human intestine were examined to determine whether the ABO and secretor blood group loci exert the same influence upon levels of intestinal alkaline phosphatase in the mucosa as they do upon levels of the intestinal enzyme in the serum. It was shown that these blood group genes do not significantly affect mucosal al-

kaline phosphatase, indicating that they do not control capacity to synthesize the enzyme, but rather must play a role in determining rate of secretion into the serum, or in destroying or inhibiting the activity of the enzyme after it is secreted into the serum.

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The Phenylalanine-Hydroxylating System of Avian Liver. Pattern of Appearance During Embryonic Development and Limiting Component.* (31505)

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The inability of livers in phenylketonuric individuals to convert phenylalanine to tyrosine(1-3) has prompted attempts in several laboratories(4-7) to characterize the phenylalanine-hydroxylating system of mammalian liver. The overall process catalyzed by the system involves two separate reactions, which may be formulated as follows:

(1) Phenylalanine + tetrahydropteridine + O_2 → tyrosine + dihydropteridine + H_2O

(2) Dihydropteridine + NADPH‡ + H^+ → tetrahydropteridine + $NADP^+$

(3) Sum: Phenylalanine + O_2 + NADPH + H^+ → tyrosine + $NADP^+$ + H_2O

While the specific phenylalanine hydroxylase catalyzing Reaction 1 appears to be localized in liver, the enzyme system catalyzing Reaction 2, reduction of the pteridine cofactor, has been prepared from several mammalian tissues. The cofactor, which is closely related to dihydrobiopterin(8), may be replaced by certain synthetic pteridines in *in vitro* assay systems(7). It has been demonstrated that in the genetic disease phenylketonuria, the pteridine cofactor and the cofactor reductase are present(9) but the phenylalanine hydroxylase is missing(3,9,10).

The initial studies on development of the phenylalanine-hydroxylating system in rats indicated that the activity of the overall system is negligible in the livers of fetal rats or rats less than 24 hours old, and that the activity approaches the adult liver level several days after birth(11-13). It was further

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‡ Abbrevations: NADP and NADPH, oxidized and reduced forms, respectively, of nicotinamide adenine dinucleotide phosphate; NADH, reduced nicotinamide adenine dinucleotide; DMTP, 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine.