TABLE V. Agglutinins in Sera of Individuals with Sickle Cell Anemia and Sickle Cell Trait.

Erythrocytes of:	Diagnosis*	Saline suspensions	Albumin suspensions	Antiglobulin (Coombs) test
W.B.	SCA	—	—	+
P.R.	"	+	+	+
N.N.	"	++		+
J.W.	"	+		+
A.B.	"	++	+	++
D.W.	"	—	++	+
S.S.	"	++	++++	+
D.G.J.	"	±	+	+
M.S.	"	+	+	+
W.K.	"	±	+	+
W.E.	"	—	++	
C.W.	"	—	++	+
O.W.	"	—	+	+
I.C.G.	T	+	++	+
M.B.	T	+	++	+
W.M.	T	—	—	—
G.E.	T	+	+	±
O.W.	T	—	—	—
A.S.	T	—	—	
C.M.	T	—	—	—
F.T.	T	—	±	±
D.K.	T	—	—	
C.J.	T	—	±	
S.J.	T	—	+	

* SCA indicates sickle cell anemia.
T " " " trait.

the abnormal hemoglobin and about 50% normal hemoglobin. Our results would indicate that there is a *qualitative* difference in the antigenic structure of erythrocytes from individuals with sickle cell trait and those with sickle cell anemia.

The demonstration of agglutinins in the sera of all the thirteen sickle cell anemia patients tested suggests that the hemolytic process in sickle cell anemia may be related to the presence of agglutinin in the patients' sera. Further experiments are in progress to determine the nature of this agglutinin and the significance of its presence in about one-third of our cases of sickle cell trait. Since our rabbit sera have given absolute differentiation between the sickling trait and the anemia, we believe that anti-sickle cell anemia rabbit sera may offer a diagnostic aid in the differentiation of sickle cell anemia from sickle cell trait in those cases in which the latter is complicated by other forms of anemia, and presents a difficult diagnostic problem.

Summary. 1. Erythrocytes from 2 individuals with sickle cell anemia, when injected into rabbits, produced antisera which agglutinated erythrocytes of 19 individuals with sickle cell anemia, but not those of 21 individuals with sickle cell trait nor those of 124 normal individuals.

2. Such sera may be useful in the differential diagnosis between sickle cell anemia and sickle cell trait.

3. The presence of agglutinin antibody has been demonstrated in the sera of all of 13 patients with sickle cell anemia. This finding may explain the hemolysis which is characteristic of this disease.

4. Of 11 patients with sickle cell trait, definite agglutinin antibody was demonstrated in the sera of 4, and doubtful agglutinin antibody in 2.

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Effects of HCl on Alkaline Phosphatase in Kidney and Intestine: Histochemical and Quantitative Study.* (18118)

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Numerous histochemical and biochemical studies have demonstrated the widespread distribution and specific location of alkaline phosphatase in various tissues. However, the

* An abstract covering part of this data appears in *Anat. Rec.*, 1949, v103, 29.

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identity or diversity of the enzymes at these sites remains an unsettled question(1-8). It has been shown(10) that in the mouse, renal phosphatase may be distinguished from intestinal phosphatase by the greater sensitivity of the latter to inhibition by cyanide. Present observations on the effects of HCl provide a further basis for distinguishing between the alkaline phosphatases of these two organs.

Materials and methods. A. Histochemical. Specimens of kidney and intestine, obtained from guinea pigs and albino mice killed by decapitation and from a dog under nembutal anesthesia, were fixed in chilled acetone(9). Sites of alkaline phosphatase activity were demonstrated by the method of Gomori(11).

To study the effect of increasing concentrations of HCl on histochemically demonstrable phosphatase, slides bearing sections of both kidney and intestine were placed for ½ hour at 37°C in solutions of HCl ranging from pH 5.0 to 1.8. After rinsing in distilled water the entire group of slides, each of which had been subjected to a different pH, was then carried simultaneously through the procedure for demonstrating the sites of phosphatase activity.

B. Quantitative. Kidneys, and segments of intestine rinsed with saline, were homogenized in chloroform water, allowed to autolyze at room temperature for 12 to 24 hours, then reground and partially clarified by brief centrifugation. The concentration of the supernatant thus obtained was so adjusted that 0.5 cc of a 1:10 dilution gave a suitable colorimeter reading when incubated for one hour at 37°C in phenolphthalein phosphate substrate(12). Test tubes containing 5.0 cc of HCl solutions ranging from pH 4.5 to 1.8 were warmed to 37°C. To each was added 0.5 cc of homogenate. After ½ hour duplicate 0.5 cc aliquots of each HCl-homogenate mixture were removed for phosphatase determination(12). To ascertain the pH at which the homogenates had been treated, the pH of the remainder of each HCl-homogenate mixture was then determined with a Beckman pH meter. The pH of a 5.0 cc portion of the substrate was lowered only 0.2 pH unit (9.6 to 9.4) by the addition of 0.5 cc of the most acid of these mixtures. The amount of phosphatase activity surviving treatment with the various concentrations of HCl was calculated in arbitrary units.

Results. Typical results obtained when tissues from dog, mice, and guinea pig were subjected to acid treatment before staining for phosphatase are shown in Fig. 1. From these histochemical observations it is apparent that in all three species the alkaline phosphatase of the kidney is inactivated by a lower concentration of HCl than is the corresponding enzyme of the intestine.

In agreement with the histochemical observations, the quantitative data (Fig. 2) demonstrate that in crude homogenates renal alkaline phosphatase of the mouse is inactivated at a distinctly higher pH than is intestinal phosphatase. The slight rise in activity of intestinal phosphatase following treatment at pH 3.8 to 2.8 was a constant finding. The first portion of the upper curve in Fig. 2 approximates a summation of the individual curves for kidney and intestine, but beyond pH 3.0 the curve is appreciably elevated above that for the intestine alone. In a preliminary investigation of this phenomenon(13) it was found that acid-inactivated renal homogenate (pH 2.5 for ½ hour, then neutralized) added to intestinal homogenate increased the resistance of the latter to acid inactivation by approximately the amount apparent in Fig. 2. On the other hand, acid-inactivated intestinal homogenate (pH

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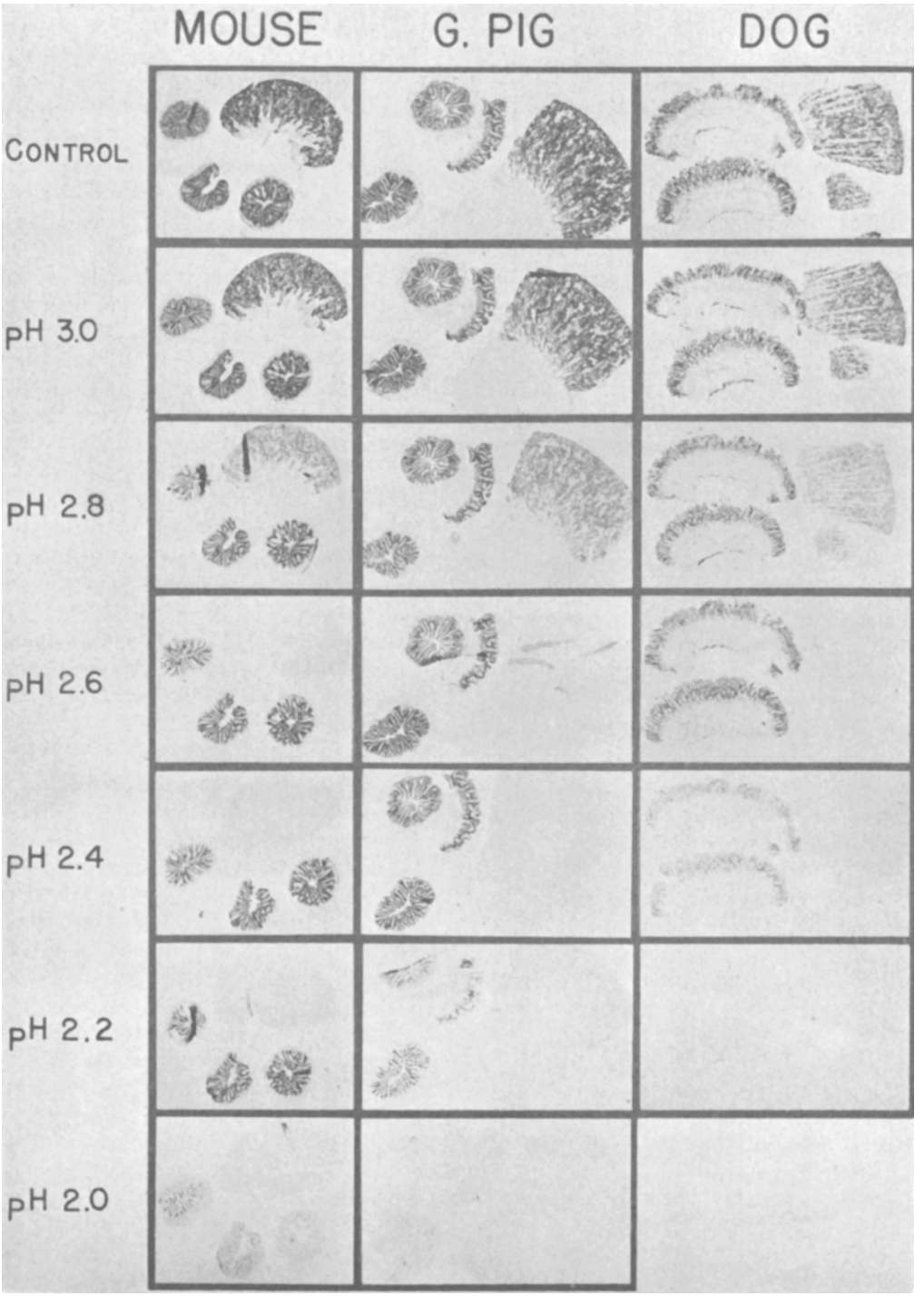

FIG. 1.

Acid inactivation of alkaline phosphatase in kidney and intestine of mouse, guinea pig, and dog. Figures on left indicate pH of HCl to which specimens were subjected at 37°C for ½ hour prior to treatment with Gomori technic. Degree of blackening is related to amount of phosphatase activity. Not counterstained. Dark streaks in guinea pig kidney at pH 2.6 are due to folds in the section.

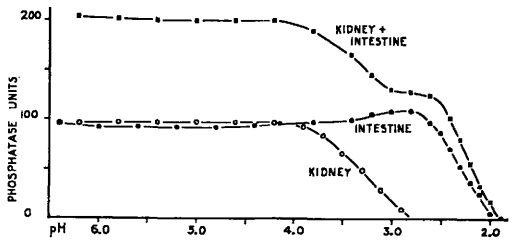

FIG. 2.

Acid inactivation of alkaline phosphatase in homogenates of mouse kidney and intestine and in a homogenate containing equivalent amounts of each. Abscissa indicates pH at which homogenates were treated at 37°C for ½ hour prior to determination of phosphatase activity. Enzyme units are arbitrary. Each curve represents an average of 3 experiments.

1.8 for ½ hour, then neutralized) slightly decreased the resistance of renal homogenate to acid inactivation.

Discussion. A problem which arises in the interpretation of histochemical studies of enzyme inhibitors(5,7) is that local differences in concentration of a single enzyme may be misinterpreted as indicating differences in sensitivity or resistance to a given agent. For example, in the action of cyanide on mouse kidney and intestine(10), it might be supposed(5) that the apparently greater resistance of renal phosphatase is due merely to a higher enzyme concentration in this organ. If such a quantitative difference were the only factor, then with any agent capable of suppressing phosphatase activity the kidney should always be found more resistant than the intestine. The fact that renal phosphatase is actually less resistant to acid inactivation renders such a simple interpretation untenable; since on the basis of the cyanide effects it would be necessary to assume a higher enzyme concentration in the kidney, and on the basis of the HCl effects it would be necessary to assume a higher concentration in the intestine. It thus becomes justifiable to conclude that in addition to whatever quantitative differences there may be in the phosphatase content of these two organs, there must also be qualitative differences.

It should be pointed out that the degree of difference between kidney and intestine may vary with species. The difference in HCl sensitivity between renal and intestinal phosphatase is greater in the mouse than in the dog (Fig. 1). Species variation also occurs with respect to cyanide sensitivity(10): in mouse the intestine is more sensitive than the kidney, while in dog(13) both organs are about equally sensitive. Gomori(5) has implied that in some species (not identified) the kidney may be more sensitive to cyanide than is the intestine.

The chemical basis for the differences between the alkaline phosphatases of these two organs is not understood. In crude homogenates the presence of substances acting as inhibitors, activators, or protective agents might contribute to the observed differences in HCl sensitivity. However, the curve obtained with the mixed homogenates indicates that whatever effects such substances may have, the renal and intestinal phosphatases retain their individuality in the presence of one another. The fact that the HCl sensitivity of the alkaline phosphatase in fresh tissue homogenates closely approximates that demonstrable histochemically in specimens which have been fixed, washed and subjected to fat solvents would seem to minimize the importance of dializable substances in the homogenates and suggests that the differences may lie in the enzymes themselves, or be due to something which remains firmly associated with the enzyme even during the rigors of histological preparation.

Summary. The inactivation of renal and intestinal alkaline phosphatases by HCl was studied by histochemical and by quantitative methods. In the mouse, guinea pig and dog the renal enzyme is inactivated at a distinctly higher pH than is the intestinal enzyme. These observations support the view that renal and intestinal alkaline phosphatases are not identical.

13. Emmel, V. M., unpublished data.

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