bearing somewhat the same relation to the latter as the hydrous micas bear to muscovite. Thus, in spite of the essential homogeneity of the original acidic volcanic rocks or pumiceous tuffs, the alteration products differ because of differences in environment that affect the alteration process. Where the rocks do not undergo leaching, montmorillonite may be produced. In this case, iron and magnesium are retained in the crystal lattice of the clay mineral; when iron is unusually abundant, the peculiar green montmorillonite may be produced. Leaching conditions, on the other hand, yield kaolin or allophane. The leached condition may be caused by surface water or the environment accompanying some coally matter. In these cases, the iron is not retained in the crystal lattice but is converted into limonite. The conditions that favor alteration to glauconite-celadonite are obscure, but it is inferred that the alteration may take place under sea water.

One point deserves special notice; that is, in spite of the acidic character of the original pumiceous rocks, an appreciable amount of iron is included in some of the alteration products. It is probable that the iron was not present in the original rock but was introduced or concentrated in the alteration process. It should also be noted that, in many cases, the alteration products do not consist of one type of clay mineral but mixtures of the several minerals. In such cases identification of the individual minerals is very difficult and can best be accomplished by differential thermal analysis. The complex mineralogical properties of Schichinoe clay offer an example and, in the experimental studies, the differential thermal analysis was very helpful.

The Effect of Magnesium Sulfate on Acid Inactivation of Renal and Intestinal Alkaline Phosphatase

Victor M. Emmel

Department of Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, New York

In studying the effects of various enzymatic digestions on histochemically demonstrable alkaline phosphatase, it became necessary to study independently the effect of HCl and $MgSO_4$ on alkaline phosphatase in histological sections and fresh tissue homogenates of kidney and intestine. It has been reported elsewhere (*1*) that renal phosphatase is distinctly more sensitive to inactivation by HCl than is intestinal phosphatase. The present data demonstrate that in the presence of $MgSO_4$, a well-known activator of alkaline phosphatase, the sensitivity of both renal and intestinal phosphatase to acid inactivation is increased.

Paraffin sections of mouse kidney and intestine fixed in cold acetone were prepared by the usual methods (*2*). Sites of alkaline phosphatase activity were demonstrated by the Gomori technique (*3*). In the manner previously described (*1*), sections were exposed to various concentrations of HCl with and without the addition of $M/100$ $MgSO_4$ at 37° C for ½ hr prior to staining for phosphatase. The observations recorded in Table 1 indicate that when exposed to HCl alone

TABLE 1

EFFECT OF $MgSO_4$ ON ACID INACTIVATION OF HISTOCHEMICALLY DEMONSTRABLE ALKALINE PHOSPHATASE IN MOUSE KIDNEY AND INTESTINE

Treatment	HCl only		HCl + $M/100$ $MgSO_4$	
pH	Kidney	Intestine	Kidney	Intestine
H_2O	++++	++++	++++	++++
5.0	++++	++++	++++	++++
4.5	++++	++++	+++	++++
4.0	++++	++++	++	++++
3.5	++++	++++	0	+++
3.0	++++	++++	0	+
2.5	0	+++	0	0
2.0	0	+	0	0
1.8	0	0	0	0

Figures on left indicate pH at which sections were treated with HCl or with HCl plus $MgSO_4$ at 37° C for ½ hr prior to staining for phosphatase. Amount of activity surviving treatment is recorded as the degree of blackening of the sections.

renal phosphatase is inactivated between pH 3.0 and 2.5, and intestinal phosphatase inactivated at about pH 2.0. When $M/100$ $MgSO_4$ is added to the HCl, the ranges over which inactivation of renal and intestinal phosphatases occurs are shifted to about pH 4 and pH 3, respectively.

In comparable quantitative experiments fresh tissue homogenates (*4*) were treated with HCl or with HCl plus $MgSO_4$, followed by determination of residual phosphatase activity (*5*). Fig. 1 illustrates the results obtained with mouse intestine. Comparison of curves I and II shows the activating effect of $M/100$ $MgSO_4$ on alkaline phosphatase. The gradual merging of these two curves suggests that the enzyme that has survived treatment with HCl may be less subject to activation

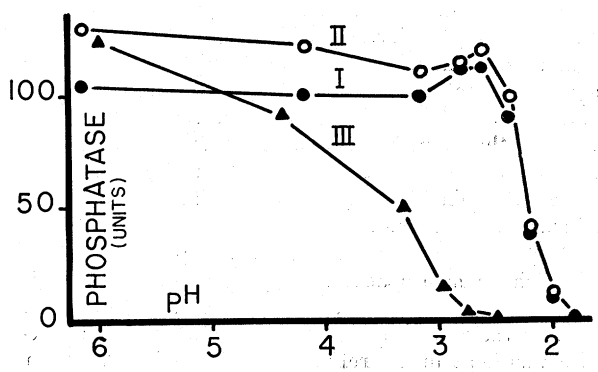

FIG. 1. Effect of $MgSO_4$ on inactivation of intestinal alkaline phosphatase by HCl. Abscissa indicates pH at which fresh tissue homogenates were treated at 37° C for ½ hr prior to phosphatase determinations. Phosphatase units are arbitrary. Curve I, homogenate treated with HCl only, no $MgSO_4$ in substrate; curve II, homogenate treated with HCl only, $M/100$ $MgSO_4$ in substrate; curve III, homogenate treated with HCl plus $M/100$ $MgSO_4$, $M/100$ $MgSO_4$ in substrate.

by magnesium. In control experiments it was found that serial dilution of the homogenate, thereby reducing its phosphatase activity, did not in itself significantly alter the activating effect of magnesium. Comparison of curves II and III demonstrates the distinctly increased sensitivity of intestinal phosphatase to acid inactivation in the presence of magnesium. With kidney homogenates magnesium similarly caused increased sensitivity to inactivation by HCl.

Further observations will be necessary to provide a basis for explaining the manner whereby magnesium increases the sensitivity of alkaline phosphatase to inactivation by HCl. It is pertinent to note, however, that Bodansky (*4*) has found that the activating effects of magnesium and cobalt on purified preparations of rat bone and intestinal phosphatase were markedly altered in the presence of certain amino acids. It is possible that in the present experiments the magnesium-phosphatase complex was more labile and hence more subject to acid denaturation than the noncomplexed form of the enzyme.

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Growth Effects on Chick Fibroblast Cultures of Fractions of Adult and Embryonic Tissue Extracts Following Differential Centrifugation[1]

R. S. Hoffman, J. A. Dingwall, and W. DeW. Andrus

Department of Surgery, New York Hospital and Cornell University Medical College, New York City

In a previous paper from this laboratory (*1*) some of the physicochemical properties of the growth-promoting substances contained in a Tyrode's extract of adult sheep heart have been described. In the present communication, attempts to determine roughly the distribution of the active factors between the macro- and micromolecular fractions of the crude extract, as well as that obtained from chick embryos, are reported. Tennant, Liebow, and Stern (*2*) have previously described high-speed differential centrifugation of embryonic extract.

Preparation of extract of adult sheep heart: Sterile adult sheep hearts denuded of pericardium and epicardial fat were cut up and minced in a blender with 6 times their volume of Tyrode's solution. This mixture was kept in the refrigerator for 24 hr and then centrifuged at 2,500 rpm for 20 min. The decanted supernatant extract could be stored in the refrigerator for several months without losing its activity.

The method of preparation of chick embryonic extract was as follows, being similar to that described by Claude (*3*). Eight-day-old chick embryos were collected under sterile conditions and after washing with Tyrode's solution and freeing of all extraneous material were finely ground with sterile sand and extracted with 6 volumes of Tyrode's solution. The resulting mixture was allowed to stand overnight and then centrifuged for 20 min at 2,500 rpm. The decanted supernatant fluid was used for the experiments.

TABLE 1

SUMMARY SHOWING COMPARISON OF GROWTH EFFECT OF ULTRACENTRIFUGED FRACTIONS OF ADULT AND EMBRYONIC EXTRACTS WITH WHOLE EXTRACTS AND TYRODE'S SOLUTION CONTROLS

No. cultures	Fluid phase added	Average size of cultures at 7 days (mm^2)
10	Adult sheep heart extract, *supernatant*	68.5
10	Adult sheep heart extract, *total*	56.5
15	Adult sheep heart extract, *residue*	22.0
5	Tyrode's solution	12.0
10	Embryonic chick heart extract, *supernatant*	33.5
10	Embryonic chick heart extract, *total*	31.0
15	Embryonic chick heart extract, *residue*	17.0
5	Tyrode's solution	12.0

The extracts of adult and embryonic tissues were each centrifuged in sterile lusteroid tubes in the concentration rotor of an air-driven high-speed centrifuge for periods of 1–3 hr at 30,000 rpm. The resulting closely packed pellets, representing the macromolecular fractions in each case, were resuspended in Tyrode's solution in amounts corresponding to the original volume of extract, and the growth-promoting activity of both the redissolved macromolecular and the supernatant fractions was tested on chick fibroblast cultures growing *in vitro,* by the following technique.

Third passage hanging-drop fibroblast cultures from 7-day-old embryonic chick hearts were divided into halves. Each sister half was placed in a Carrel flask (3.5) containing 0.5 ml chicken plasma, 1 ml Tyrode's solution, and 1 drop of chick embryonic extract in order to facilitate formation of a coagulum. The cultures were allowed to grow in this medium for 48 hr, and the increase of surface area during this period was estimated according to the method of Ebeling (*5*). The 2 fractions of the heart extract were then added to the flasks so that their effects were compared on sister halves of the same cultures, or the activity of each fraction was evaluated by comparison with sister halves of cultures containing only Tyrode's solution in the supernatant phase. Table 1 depicts the results of a series of such experiments.

A survey of the table indicates that the supernatant

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