proteins. After removal of the $CuSO_4$-$Ca(OH)_2$ precipitate by centrifugation, the sample containing the lactic acid is neutralized. One cc. (containing 200 γ or less) is incubated with 1 cc. of yeast suspension in phosphate buffer and 1 cc. of potassium ferricyanide for $\frac{1}{2}$ hour at 30° in open test tubes. Air does not interfere with the determination. At the end of the incubation period, the mixture is centrifuged. To 2 cc. of the supernatant fluid ferric sulfate solution is added, the mixture is diluted, and the amount of resulting Prussian blue determined using a photoelectric colorimeter with a yellow-green filter. In our experiments the Cenco-Sheard-Sanford photelometer was used. For this instrument we found it best to use two standard curves—one for samples containing less than 75 γ of l-lactic acid/cc., the other for samples containing 75–150 γ. If the sample contains less than 75 γ/cc., 2 cc. of the supernatant fluid from the enzyme mixture are placed directly in a cuvette, 1 cc. of ferric salt solution and 2 cc. of water are added, and the reading made immediately. For samples containing 75–150 γ, 2 cc. of the supernatant fluid from the enzyme mixture are placed in a 10-cc. volumetric flask, 2 cc. of Fe^{+++} salt added, the mixture diluted to volume, and the Prussian blue determined.

A practically straight-line relationship is obtained when per cent transmission is plotted against concentration. The curve is completely reproducible with the same yeast preparation and when incubated for the same length of time. We found it best, however, to make a standard curve with each set of analyses by setting up one blank determination and one with a known amount of lactic acid, in this way correcting for any variation in the yeast suspension or in the procedure.

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Histochemical Demonstration of Alkaline Phosphatase in Decalcified Dental and Osseous Tissues[1]

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Histochemical studies on alkaline phosphatase in bones and teeth have been restricted to the incipient stage of mineral deposition, due to the fact that it very soon becomes impossible to section these structures without first decalcifying them. Employment of acids to remove the mineral has resulted in destruction of this enzyme. In rats, therefore, it has not been possible to follow the alkaline phosphatase in odontogenesis later than 4 days past birth (*1*).

It is obviously desirable to learn the localization and relative activity of this enzyme in bones and teeth throughout their developmental history. It would be expected that such information might have functional significance. The dental tissues are of extraordinary interest in this regard, since they are highly specialized for mineral deposition.

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In the development of a method for the successful demineralization of the hard structures without at the same time destroying the alkaline phosphatase, we have been guided by a study of the activity of this enzyme *in vitro* under a variety of environmental and chemical factors (*2*).

The heads of 28-day-old rats were split in the midsagittal plane, fixed in cold, 80 per cent alcohol, and placed in the following solutions of specified pH:

I. (a) equal parts of 20 per cent sodium citrate and 2 per cent formic acid ..... pH 4.9
   (b) equal parts of 20 per cent sodium citrate and 5 per cent formic acid ..... pH 4.2
II. (a) 5 per cent aqueous ammonium citrate ..... pH 4.8
   (b) 10 parts 5 per cent ammonium citrate and 1 part 15 per cent citric acid ..... pH 4.2

Completion of decalcification, as shown by the needle test, required 1–5 days. During dehydration of the tissue blocks all alcoholic solutions were buffered to pH 9.3 ± 0.1, a step important in preserving the maximum phosphatase activity. In line with this objective it was found necessary to use a minimum of heat to expand the paraffin ribbon; we allowed 10–15 seconds at 45°–50°C. when using paraffin with m.p. 56°–58°C.

The upper incisors and molars with bony encasements in each case were sectioned longitudinally. The sections were uniformly incubated for 3 hours at 37°C., using sodium glycerophosphate as a substrate. Visualization of the enzymatic action was brought about by the generally accepted method of Gomori (*3*), which results in the formation of black cobalt sulfide at the site of enzyme activity.

The range in hydrogen-ion concentration over which decalcification could be accomplished without destroying alkaline phosphatase was found to be very narrow, *i.e.* pH 4.8–5.0. When this range was exceeded on the alkaline side, the rate of decalcification became unduly slow; with a slight increase in acidity the alkaline phosphatase activity was irreversibly lost. This is illustrated by the observation that little or no phosphatase activity was demonstrable in the tissues decalcified in solutions Ib and IIb at pH 4.2. The phosphatase activity was well preserved, however, in comparable tissue blocks decalcified at pH 4.8 and 4.9 (solutions Ia and IIa, respectively). The purpose in buffering all the alcoholic solutions used subsequent to decalcification was to provide immediately an optimum condition for the phosphatase to retain and perhaps to regain activity. That this may be an important factor was further demonstrated by allowing sections to stand 24 hours in an aqueous medium buffered at pH 9.3 ± 0.1 prior to incubation; there was a definite increase in phosphatase activity in comparison with adjacent sections not so treated and which contained demonstrable phosphatase activity. The reaction was also accentuated by the addition of magnesium chloride to the substrate. In fact, when sections were pretreated in the manner just described and subsequently incubated with magnesium ions in the substrate, an amount of precipitate was deposited in the odontogenic and osteogenic tissues which was considered to represent a maximum alkaline phosphatase reaction.

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