# Further Studies on the Histochemical Specificity of Phosphatases.* (17464)

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In continuation of previous studies,[1] 10 more substrates were tried in histochemical experiments on phosphatases. The new substrates were the following: catechol- and hydroquinone monophosphates; glucose-6-phosphate, fructose-6-phosphate, phosphoglyceric acid; yeast adenylic acid (3-nucleotide), muscle adenylic acid (5-nucleotide); dimethyl- and diphenyl acid pyrophosphates, and casein. The first two were prepared by a modification of the King-Nicholson[2] procedure; instead of the Ba salts the Ca salts were prepared; the crude products were purified by extraction with water at an alkaline reaction and precipitation by 1 to 2 volumes of alcohol. Glucose-6-phosphate was the gift of Dr. B. Vennesland of the Dept. of Biochemistry, University of Chicago; fructose-6-phosphate, phosphoglyceric acid and yeast adenylic acid were obtained from the Schwarz Laboratories, New York; 5-nucleotide was purchased from the Sigma Chemical Co., St. Louis; dimethyl acid pyrophosphate was received from the Victor Chemical Works, Chicago; diphenyl acid pyrophosphate was synthesized according to the method of Neuberg and Wagner.[3]

*Experimental.* The technic was the same as reported previously. The substrates were used at two pH values only, at pH 5 and 9.2. Slides incubated with glycerophosphate were used as standards for comparison at both pH values. No reaction was obtained with casein in any of the tissues either at alkaline or at acid reaction.

In the alkaline range, intense reactions were observed in 2 hours with all other substrates, except the pyrophosphates which required prolonged incubation (up to 24 hours). The picture of distribution of enzymatic activity was essentially identical with all substrates, except 5-nucleotide. Minor and mostly inconstant differences were noted with the pyrophosphates, the nuclei being stained relatively more intensely than with the use of the other substrates.

Pictures obtained with the use of 5-nucleotide were different in several respects from those observed with any of the other substrates. Although in some of the organs (kidney, adrenal, intestinal mucosa, embryonic skeleton, placenta, etc.) the pattern of enzymatic distribution was indistinguishable from that seen with the use of, *e.g.*, glycerophosphate, in other organs patterns entirely different and specific for 5-nucleotide were found. Some of the main differences were as follows:

1. In the brain of the rat and mouse, nonspecific reaction is found mainly in the grey cortex where the reaction is rather diffuse, and in the walls of blood vessels (Fig. 1). With 5-nucleotide, the cortex is only slightly stained, and the reaction is mainly in the axons. Intense staining is obtained at some sites where the nonspecific reaction is practically negative such as the caudate nucleus (Fig. 2), the radiation of the corpus callosum and the

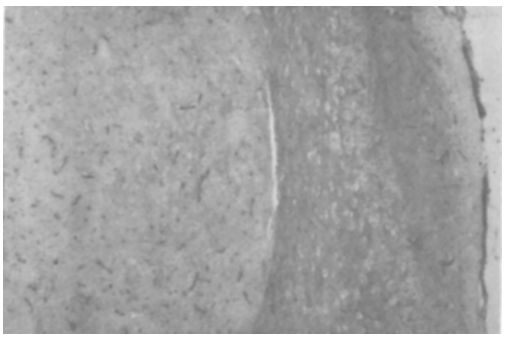

FIG. 1.
Brain of the mouse. Substrate: glycerophosphate. Grey cortex and blood vessels stained.

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1 Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 7.

2 King, E. J., and Nicholson, T. F., *Biochem. J.*, 1939, **33**, 1182.

3 Neuberg, C., and Wagner, J., *Biochem. Z.*, 1926, **171**, 485.

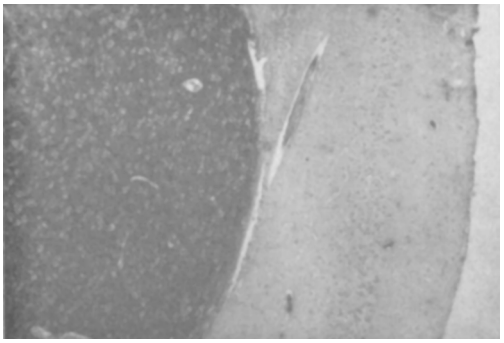

FIG. 2.
Another section of the same block. Substrate: 5-nucleotide. Caudate nucleus intensely stained.

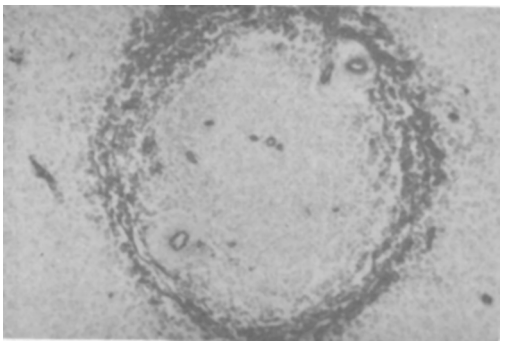

FIG. 3.
Human spleen. Substrate: glycerophosphate. Positive reaction at the periphery of a Malpighian body.

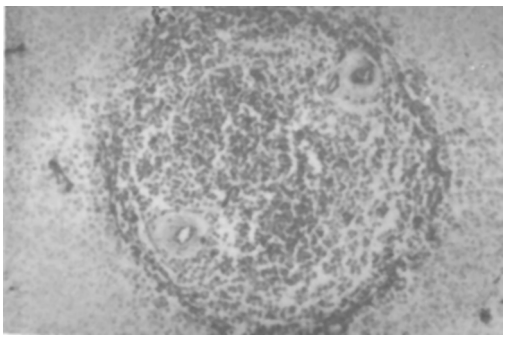

FIG. 4.
Consecutive serial section to the previous one. Substrate: 5-nucleotide. Reaction throughout the Malpighian body.

structures of the cornu Ammonis. The blood vessels are less intensely stained.

2. In the human spleen, only the periphery of the Malpighian bodies shows a nonspecific activity (Fig. 3); with 5-nucleotide, the centers are also intensely stained (Fig. 4).

3. In the testis of man, the mouse and the rat, 5-nucleotidase activity is quite intense in the nuclei of spermatogenic elements while nonspecific activity is much weaker.

4. Smooth muscle of vessel walls and of some viscera (urinary bladder, stomach, etc.) stains intensely although neither uniformly nor consistently when the substrate is 5-nucleotide, while the nonspecific reaction is negligible in the same locations. On the other hand, longitudinal muscle of the uterus of the mouse stains equally well with all substrates.

These findings seem to confirm previous contentions[4,5,6] that 5-nucleotidase is a specific enzyme, different from nonspecific alkaline phosphatase. Whether the multiple overlapping of patterns of distribution is genuine or in part due to impurities of substrate nature in the muscle adenylic acid used in these experiments will require further investigation.

In the acid range, good results were obtained with catechol monophosphate and with glucose- and fructose-6-phosphates; with the other substrates the reactions were much weaker and variable. No significant differences in localization of activity were seen with different substrates, except that phenylpyrophosphate tended to show a predominantly nuclear pattern of activity. The findings of Reis[7] according to which prostatic acid phosphatase is also primarily a 5-nucleotidase could not be confirmed histochemically since the intensity of the reaction was far more marked either with glycerophosphate or with 3-nucleotide than with 5-nucleotide as a substrate.

*Summary.* The pattern of distribution of alkaline phosphatase activity in tissues is, when 5-nucleotide is used as a substrate, different from patterns obtained with any other substrate. The specificity of 5-nucleotidase appears to be an established fact.

[4] Reis, J., *Bull. soc. chim. biol.*, 1934, **16**, 385.
[5] Reis, J., *Enzymologia*, 1937-38, **2**, 110.
[6] Gulland, J. M., and Jackson, E. M., *Biochem. J.*, 1938, **32**, 590, 597.
[7] Reis, J., *Enzymologia*, 1938, **5**, 251.

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