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With one plate (fig. 1)

Summary

1. Activity of the specific alkaline phosphatase, 5-nucleotidase, is intense in the epithelium and secretion of the rattlesnake venom gland. Non-specific alkaline phosphatase activity is lacking.

2. Thyroid epithelium, the smooth muscle of great vessels, and (inconstantly) smooth muscle of abdominal hollow viscera show greater 5-nucleotidase than non-specific activity.

3. These findings confirm the specificity of 5-nucleotidase.

Introduction

THERE has been controversy concerning the existence of a number of specific alkaline phosphatases. Evidence is accumulating for the identity of a distinct and specific enzyme which liberates phosphate from 5-nucleotides (e.g. adenosine-5-phosphate, muscle adenylic acid, and inosine-5-phosphate, inosinic acid) but not from 3-nucleotides (adenosine-3-phosphate, yeast nucleic acid).

To this enzyme the name 5-nucleotidase was given by Reis (1934) who described its occurrence in extracts of heart muscle. In subsequent papers (1937 a, 1937 b) Reis reviewed the distribution of 5-nucleotidase, finding it in significant quantities in nervous tissues, thyroid, testicle, aorta and other vessels, lung, retina, and choroid. Species differences were noted. The rat contains the enzyme in nearly every tissue while the pigeon has demonstrable 5-nucleotidase activity only in the lungs.

Few histochemical studies of 5-nucleotidase have been reported. Gomori (1949), using muscle adenylic acid as substrate in his alkaline phosphatase technique, reported colouring of certain brain structures in the mouse and rat, spermatogenic elements in man, mouse, and rat, splenic follicles in man, and smooth muscle of visceral and blood-vessel walls. Newman and others (1950) demonstrated 5-nucleotidase activity in the media of the arteries in many organs and in smooth muscle of the walls of viscera. Elsewhere they found
the activity with muscle adenylic acid as substrate was about the same as with other phosphates. Pearse and Reis (1952) recently reported that they were able to demonstrate histochemically 5-nucleotidase activity in those organs in which chemical estimations had previously indicated substantial activity of the enzyme. McManus, Lupton, and Harden (1952) presented a method for the histochemical demonstration of 5-nucleotidase (see below). Its occurrence in a number of human tissues was reported. The presence of 5-nucleotidase and the absence of alkaline phosphatase in obsolescent human renal glomeruli were indicated by McManus and Lupton (1952). McManus, Lupton, and Etheridge (1952) described features of 5-nucleotidase and other phosphatases studied histochemically in the aorta and other blood-vessels of man.

Because of the interesting distribution of this enzyme in the tissues of man and other mammals, and the reports of Gulland and Jackson (1938) and Zeller (1951) that an enzyme with specific 5-nucleotidase activity has been found in all snake-venoms thus far examined chemically, we have attempted its demonstration in the poison and other salivary glands of snakes and in other snake tissues.

**MATERIALS AND METHODS**

The method used in these studies was the McManus, Lupton, and Harden (1952) modification of Gomori's alkaline phosphatase technique. Tissues were fixed in chilled 80 per cent. alcohol and embedded in paraffin. Sections were incubated for 2 to 4 hours or overnight in Gomori's alkaline phosphatase stock solution at pH 8-5 to 8-8, lacking magnesium ion and containing muscle adenylic acid or inosinic acid as substrate. Corresponding sections were treated by the alkaline phosphatase method of Gomori, with glycerophosphate and hexosediphosphate as substrates. Precipitated phosphates were visualized by the formation of black cobalt sulphide. Tissues were obtained from garter snakes (*Thamnophis sirtalis sirtalis*), juvenile pilot black snakes (*Elaphe obsoleta obsoleta*), western diamond-backed rattlesnake (*Crotalus atrox*), and a few tissues from water-snakes (*Natrix sipedon*). Tissues examined included salivary glands, thyroid, adrenal, intestine, pancreas, spleen, liver, trachea, lung, heart, aorta, testis, ovary, kidney, brain, eye, and Harderian gland.

**RESULTS**

Black precipitates, indicative of enzyme activity, are marked in a number of snake tissues incubated with muscle adenylic acid. Nuclei and nucleoli are often well coloured. However, the intensity of coloration is usually proportional to cytoplasmic activity in the vicinity. In view of the findings of Martin and Jacoby (1949) and others, it is our feeling that this may represent adsorption of calcium phosphate and not intrinsic enzyme activity.

Precipitates are formed in cartilage, lung, the luminal borders of oral and intestinal mucosal cells, vas deferens epithelium, and liver and adrenal
5-Nucleotidase in Snake Tissues

sinusoid endothelium. In the kidney, activity in the proximal convoluted tubules is concentrated at the brush borders, while in the distal convoluted tubules it is associated with granules dispersed throughout the cytoplasm (fig. 1, H). However, there is also blackening in these areas with hexosediphosphate and glycerophosphate. Because activity is manifested towards all three phosphate substrates in these regions, the most that can be said is that an enzyme is present which is effective in hydrolysing 5-nucleotide. The evidence does not permit the conclusion that a specific enzyme for 5-nucleotides only is present.

The most striking proof of a specific 5-nucleotidase is obtained with poison glands of the rattlesnake, Crotalus atrox. The active poison gland is made up of branching tubules with wide lumina lined with a simple columnar epithelium. There is intense staining in both secretion and epithelium, with muscle adenylic acid as substrate (fig. 1, A). With glycerophosphate (fig. 1, B) there is almost no coloration. This is consistent with Zeller's finding that the venom of viperine snakes is rich in 5-nucleotidase but lacking in phosphomonoesterases.

In contrast, salivary glands of both poisonous and non-poisonous varieties show no greater activity with 5-nucleotides than with other substrates. In sections of the superior labial gland of the garter snake (fig. 1, G) the serous cells show phosphatase activity with both muscle adenylic acid and glycerophosphate. By contrast only the mucus-cells react positively with the PAS technique. The rattlesnake labial gland differs from that of the garter snake in having a greater preponderance of mucus-cells.

The smooth muscle of the blood-vessels and hollow viscera is another tissue in which there is a distinct difference in activity between the muscle adenylic acid and glycerophosphate preparations. The media of the large arteries near the heart is consistently darker with muscle adenylic acid (fig. 1, c) than with glycerophosphate (fig. 1, d), although differences in the degree of coloration are not always marked. As has also been noted in mammalian tissues by Gomori and in our laboratory, the coloration in visceral smooth muscle, although often intense, is inconstant and is frequently lacking. Thyroid epithelium also shows greater activity with 5-nucleotide (fig. 1, e) than with glycerophosphate (fig. 1, f).

Histochemically, our findings in the rattlesnake venom gland and the smooth muscle of blood-vessels and hollow viscera, taken in conjunction with work on mammalian tissues in our own and other laboratories, would seem to establish 5-nucleotidase as an enzyme separate from phosphomonoesterase I and hexosediphosphatase.

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REFERENCES

—, 1937a. Enzymologia, 2, 110.
—, 1937b. Ibid., 185.

FIG. 1 (plate), A, C, E, and G (on the left), and H (on the right), are alkaline phosphatase preparations with muscle adenylic acid (MAA) as substrate according to the method of McManus and others; B, D, and F (on the right), glycerophosphate was used as substrate.

A and B, Crotalus atrox. Collapsed tubules of venom gland. 5-nucleotidase activity is intense in epithelium and venom coagulum. Non-specific phosphatase activity is absent. (× 67.)

C and D, Thamnophis sirtalis sirtalis. Heart (above) and arterial trunk (below). Smooth muscle of the media of the arterial trunk shows greater activity towards 5-nucleotide than toward glycerophosphate. (× 67.)

E and F, Crotalus atrox. Thyroid gland. Epithelial cells show greater 5-nucleotidase than non-specific phosphatase activity. (× 67.)

G, Thamnophis sirtalis sirtalis. Section through upper lip. Phosphatase activity (substrate: MAA) is apparent in buccal mucosa (lower right) and epithelium of superior labial salivary gland (left) and its duct; absent in epidermis (upper right). (× 60.)

H, Elaphe obsoleta obsoleta. Kidney. Alkaline phosphatase activity is intense in the brush borders of proximal convoluted tubule cells. Precipitate in distal convoluted tubules (e.g. to lower right of glomerulus) is associated with coarse granules throughout the cytoplasm. (× 233.)
FIG. 1

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