Technical Papers

Histochemical Localization of the Mercurial Inhibition of Succinic Dehydrogenase in Rat Kidney

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In a comprehensive review of the actions and uses of diuretics, Pitts and Sartorius (*1*) concluded that the site of the direct tubular action of mercurial diuretics is unknown. The attempts made to localize the site of the tubular reabsorptive depression are based on controversial assumptions supported solely by indirect evidence from toxicologic and excretion studies. We believe that histochemistry provides a more direct line of approach to this problem.

Mercury compounds combine with sulfhydryl groups and inhibit strongly the activity of many essential sulfhydryl groups requiring dehydrogenases, including succinic dehydrogenase (*2*). That the diuretic effect of mercurials is attributable to the sulfhydryl-requiring enzymes seems probable since their renal effects, as well as their systemic toxicity, can be abolished by the administration of BAL, containing two sulfhydryl groups.

In fact, Handley and Lavik (*3*) have found that mercurial diuretics significantly depress the total succinic dehydrogenase activity of the rat kidney. We have made an attempt to localize the site or sites of this inhibition within the renal tubule of the rat kidney using blue tetrazolium (BT)[2] as a hydrogen acceptor for succinic dehydrogenase (*4*).

Novurit "Medica" (mercurphylline, 39.5% Hg) was subcutaneously administered in doses of 10–60 mg Hg/kg of body weight to male albino rats of the Wistar strain, weighing 175–200 g. The rats were killed by decapitation 2–6 hr after the administration of mercurphylline. For determining the toxicity of the subcutaneously administered Novurit "Medica" in this strain some animals were left alive. The doses over 40 mg Hg/kg appeared to be generally lethal within 3 days. The histochemical method of Seligman and Rutenburg (*4*) for the demonstration of succinic dehydrogenase activity was followed in detail, with the only exception that sections of 25 μ were used because, in our hands, such sections were more uniformly stained.

In the kidneys of control animals, the sites of highest enzymatic activity in the form of dark blue intracellular granules of diformazan were consistently localized in the cells of Henle's loops (Fig. 1). A sharp line against the unstained papilla could be seen where the dark blue loops of Henle bent to re-enter the kidney cortex. The convoluted tubules, particularly the proximal ones when bulging from the glomeruli, were also heavily deposited with dark blue granules. The glomeruli were always unstained. Between the cortex and the medulla there was a lighter colored reddish-purple zone through which the heavily stained thick portions of Henle's loops radiated. The collecting tubules were colored with a pale reddish-purple hue, if at all. The reddish color is due to monoformazan, a partial reduction product of BT, indicating lower enzymatic activity. Circulatory endothelium was unstained. These observations are in agreement with the results of Shelton and Schneider (*5*).

FIG. 1. Kidney of a control rat showing heavy precipitation of diformazan, especially in cells of Henle's loops. × 25.

In the kidneys of the test animals doses of 40 mg Hg/kg of weight caused a complete inhibition of succinic dehydrogenase activity within 2–4 hr except in some sections; in these a very pale reddish shade could be seen in the cortex. The doses of 20 and 30 mg Hg/kg inhibited nearly completely, within 2–6 hr, the succinic dehydrogenase activity of Henle's loops in the medullary area (Fig. 2) The kidney cortex of these animals showed a reddish-purple color instead of the dark blue pigmentation of control animals. The staining of the proximal and distal convoluted tubules was diminished roughly in the same proportion. The dose of 10 mg Hg/kg caused within 4 hr a moderate depression of the dehydrogenase activity in the cells of Henle's loops in the medullary area, the activity in the cortex remaining nearly unchanged. The changes after doses of 10–40 mg Hg/kg were reversible within 3 days.

The most characteristic finding in the kidneys of all our test animals was that the succinic dehydrogenase activity disappeared first from the thicker portion of Henle's loop. Duggan and Pitts (*6*) assumed that a

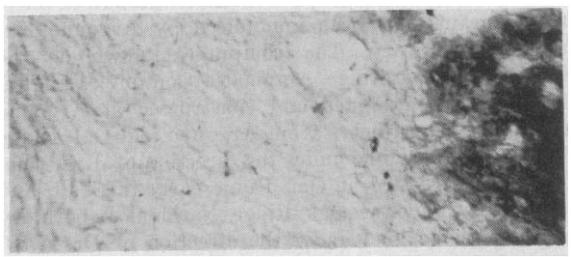

FIG. 2. Kidney of a test animal showing nearly complete inhibition of succinic dehydrogenase activity in the loops of Henle 4 hr after administration of mercurphylline (20 mg Hg/kg of body weight). × 25.

[1] Aided by a grant from the Sigrid Jusélius Foundation.
[2] Obtained from the Dajac Laboratories, Monomer Polymer, Inc., Chicago, Ill.

large dose of a diuretic could completely block reabsorption in that segment of tubule on which it acts. According to them, the proximal segment, in which water and electrolytes are reabsorbed as an isosmotic solution, is responsible for two-thirds or more of the total renal reabsorptive capacity for water and sodium. Their observation that large doses of mercurial diuretics block about 20% of the reabsorptive capacity led them to assume that mercurials act on the distal segment. In the distal segment of the tubule, water must be reabsorbed against an osmotic gradient, a process that requires energy. Succinic dehydrogenase, involved in the citric acid cycle of Krebs, probably participates in formation of this energy.

Our results support the view that mercurial diuretics act on the distal segment of the tubule because the activity of succinic dehydrogenase, after the administration of the mercurial, first disappeared from the region of the distal tubule, in which the concentrative reabsorption of water begins. In the distal convoluted tubules, however, such a complete inhibition could not be observed.

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Manuscript received May 4, 1953.

Observations on the Daily Movements of Fishes[1]

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Earlier work in this laboratory (*1, 2*) described a pre-sundown movement of schools of perch (*Perca flavescens*) from 25–35-ft depths, where they hover in the daylight hours, onto a 18- to 30-ft shelf adjacent to Second Point in Lake Mendota. At sunrise the movement is reversed.

This study was made with an echo-sounding unit in a 40-ft Navy launch that records sound pulses returning from the bottom of the lake and from intervening schools of fish (*3, 4*). An oscilloscope attachment was built into both the transmitting and receiving circuits of a U.S. Navy pattern NJ-9 echo sounder to permit the keying of the sound transmission by the blanking pulse to characterize the echo by a scope-tube trace. The circuit was changed to obtain unsaturated d-c (Fig. 1) and a-c (not illustrated) signals that are proportional to the strength of echo. The oscilloscope permits any part of the trace to be isolated and magnified for more detailed study. In depths less than 10 ft, where the reverberations from the outgoing transmissions tend to blend with and obscure the echo, the amplifier gain can be adjusted so that only the echo will be accepted. Searches, therefore, at depths of 5–10 ft can be made. Details of the circuit will be published elsewhere.

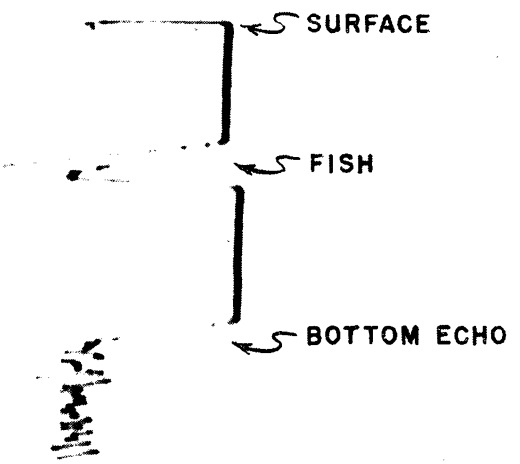

FIG. 1. Echo trace of saturated d-c signal of oscilloscope showing fish midway between surface and bottom.

It was seen on the oscilloscope screen that the schools of perch moved onto the Second Point shelf high over the bottom during their pre-sundown, inshore movement and reached their greatest concentration during the hour before sunset. They settled lower as twilight approached and their echo trace blended finally into the bottom echo. No further evidence of their presence was discernible on the instruments after darkness set in.

A diver, equipped with an aqualung and communicating with the boat via throat microphones, descended to the bottom at intervals during the pre- and post-sundown period. This was repeated on eight different evenings in July and August, 1952. The divers used spotlights for observations after dark.

Pre-sundown observations revealed the perch, 6–11 in. in length, aggregated in tight schools with 8–10 in. between individuals. As long as there was sufficient light for the diver to see without artificial light, the fish moved in schools off the bottom. Figure 1 shows the oscilloscope echo from the fish.

After sundown the divers, aided by spotlights,

[1] Supported in part by a grant from the U. S. Office of Naval Research and in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Conservation Department and the Wisconsin Alumni Research Foundation.

[2] Acknowledgment is made to L. V. Whitney and R. C. Dugdale for aid in designing and assembling the electronic auxiliary units to the NJ-9 echo sounder; to R. A. Ragotkie, W. J. Tudor, J. J. Tibbles, R. J. Loeffler, W. J. Wisby, and J. R. Nursall for aid in servicing the divers, lights, and telephones; to O. von Frisch, J. R. Nursall, and J. T. Durkin, who denied themselves the comforts of terrestrial life to serve as diving observers.