Comments and Communications

Method for Obtaining Venous Blood from the Orbital Sinus of the Rat or Mouse

BLEEDING by cardiac puncture has been a frustrating problem to those working with rats in experiments requiring considerable quantities of blood. Death of the animal from shock is too frequent, and investigators must be prepared for decimation of their animal population over any extended period of time.

This note is prompted by the enthusiastic reception given our demonstration of a simple method, originated by B. N. Halpern of the Hôpital Broussais in Paris, France. This permits frequent bleeding without serious effects on the animal. Over 1 ml of venous blood can consistently be obtained from the orbital sinus of the rat.

A glass tubing, 6–8 in. in length and 4–6 mm in diameter, drawn out to pipet form is again drawn to provide a capillary puncturing end about 1 in. long. The bore of the puncture end should easily permit the free flow of blood. The animal is held immobile on its side against a table top by a firm grip, permitting the head to be pressed down with the thumb and forefinger. The capillary end of the glass tubing is introduced into the orbit of the eye at the anterior angle formed by the lids and the nictitating membrane. A short thrust past the eyeball will enter the slightly resistant thorny membrane of the sinus. The eyeball itself remains uninjured. As soon as the sinus is punctured, blood enters the tubing by its own pressure.

The tube can be sealed and the blood centrifuged without transfer. Heparinization of the tubes permits the taking of larger samples. Stress on the animal can be further reduced by 2 drops of 2% cocaine administered topically before bleeding. We have used this method, applicable also to the mouse, upon hundreds of rats without fatality.

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

IN a recent communication, Mustakallio and Telkka (*1*) stated that after the administration of mercurial diuretics to the rat, succinic dehydrogenase first disappeared from the ascending portions of Henle's loops. Since this observation, if confirmed, would represent the first direct microscopic proof for an action of mercurial diuretics on distal segments of the tubular system, we wish to communicate our own findings.

In a report dealing with the influence of experimental kidney damage on histochemically demonstrable succinic dehydrogenase activity in the rat, we also studied the influence of a mercurial diuretic (*2*). We injected Meralluride Sodium[1] (mercuhydrin sodium) in amounts of 10 mg Hg/kg of weight. This amount will produce regularly renal necrosis (*3*) which apparently is localized, as with mercury bichloride, within the terminal portions of the proximal convolutions (*4*). We found, as with other kidney damaging agents, e.g., *dl*-serine, reduction of the histochemically demonstrable succinic dehydrogenase 45–60 min after the subcutaneous injection of the drug within the damaged cells of the proximal convoluted tubules. The thick loops of Henle which can be easily recognized by their intense staining reaction, however, retained their enzymatic activity. The same results were obtained when even larger doses of up to 40 mg Hg/kg of weight were used, as suggested by Mustakallio and Telkka. With these large doses necrosis of the proximal convolutions extended obviously beyond the terminal portions into almost all segments of the proximal convolutions. Inactivation of enzymatic activity in these necrotic tubules was complete after a few hours. In contrast, the ascending limbs of Henle's loops both located within the inner cortical zone and the outer portions of the medulla retained their full dehydrogenase activity. Superior preparations were obtained with thin frozen sections (about 10 μ) incubated in a buffered solution of neotetrazolium which contained, besides the sodium succinate, calcium chloride and sodium bicarbonate as activators. The use of blue tetrazolium with or without activators gave less satisfactory preparations. The results of our experiments would support our previously expressed view that the microscopically demonstrable effect of mercurial diuretics, even if given in very large amounts, is restricted to the proximal convoluted tubules (*3*).

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[1] Lakeside Laboratories, Inc., Milwaukee, Wis.

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