Renal Succinic Dehydrogenase and Mercurial Diuretics

It is generally agreed that the action of mercurial diuretics is predominantly renal and that extra-renal factors are of no or only little importance[1]. There are however, conflicting opinions as to whether the proximal convoluted tubules or the more distal segments of the nephron are acted upon by these drugs[2].

Recently, MUSTAKALLIO and TELKKÄ have reported that Novurit (Medica) (Mercuphylline 39·5%) when given to rats in large doses, depresses histochemically demonstrable dehydrogenase activity, especially in the cells of HENLE's loop, within a few hours[3]. These investigators concluded that mercurial diuretics have a distal tubular action. They found, however, no significant microscopic changes in the kidneys of these rats[4] and no alteration in the staining pattern of histochemically demonstrable sulfhydryl groups[5]. These findings are at variance with observations by WACHSTEIN and MEISEL[6] and by RENNELS and RUSKIN[7]. Using mercuhydrin (Meralluride sodium 39% Hg) as diuretic agent we found within a few hours marked reduction of succinic dehydrogenase activity predominantly in the terminal portions of the proximal convolutions, and none in HENLE's loop. Inactivation of the enzyme coincided with and often somewhat preceded the cellular necrosis which regularly occurs within a few hours after a large dose of mercuhydrin.

In view of these apparent differences in the results obtained and the importance of the proper location of the diuretic action in the mammalian kidney, we wish to report additional experiments with Novurit, the substance used by the Finnish investigators.

Young male albino rats of the Wistar strain weighing 150–220 g were injected intramuscularly with Novurit "Medica" (obtained through the courtesy of K. K. MUSTAKALLIO) in doses of 20 and 40 mg Hg/kg body weight. Fresh frozen sections were cut with a Sartorius microtome at a thickness of 10–15 μ and incubated for 1 or 2 h at 37°C in a mixture of the following composition[8].

0·2% neotetrazolium	10	cm³
0·2 *M* sodium succinate	10	cm³
m/10 phosphate buffer, ph 7·4	10	cm³
0·33 *M* calcium chloride	0·2	cm³
0·6 *M* sodium bicarbonate	2·0	cm³
Distilled water	6·8	cm³

Tissue pieces were also fixed in formalin and prepared for routine hematoxylin-eosin sections.

In the kidneys of animals sacrificed 2–6 h after receiving 20 or 40 mg mercury per kilogram body weight, the changes in routine sections varied considerably in different animals. In some, no evidence of necrosis was noticed. In most instances, however, focal areas of degeneration and necrobiosis occurred in cells within the distal portion of the proximal convolutions. In a few animals the changes were more severe. In kidneys in which no microscopic damage was detected on routine sections, the distribution of histochemically demonstrable succinic dehydrogenase was not different from that observed in the normal rat kidney. Within the inner cortical zone, the ascending thick loops of HENLE contrasted sharply with the less intensely stained, straight, distal portions of the proximal convoluted tubules. Thick limbs of HENLE's loop located in the outer medullary zone presented the characteristic strong staining reaction (Fig. 1). In some of the kidneys, however, in which necrotic changes were seen in routine preparations, distinct diminution of enzymatic activity in portions of the proximal convoluted tubules was noted.

In all kidneys of the animals sacrificed after 24 h extensive necrosis was found in the inner cortical zone located, as in the case with inorganic mercury, in the distal portion of the proximal convoluted tubules and occasionally extending into the more proximal portions. In these necrotic tubules succinic dehydrogenase was markedly reduced and often completely absent. There was no diminution in the staining reaction of HENLE's loops located in the inner cortical zone and in the outer medullary zone (Fig. 2).

One of the apparent differences in the results between the two groups of investigators using mercuhydrin and Novurit respectively is therefore probably due to the fact that mercuhydrin in the large doses used induces renal necrosis regularly at a faster rate as compared to Novurit. However, after 24 h no difference in the action of both drugs can be recognized, although mercuhydrin causes in general more extensive necrosis. Since succinic dehydrogenase activity disappears in necrobiotic cells regardless of the agent which has caused the cellular damage[1], it is not surprising that this phenomenon is observed a few hours regularly with mercuhydrin and not in all animals with Novurit. After 24 h the effect of both drugs was similar. There was, however, no appreciable diminution in enzymatic activity in any of the segments that could be identified as part of HENLE's loop. The difficulty in identifying those tubules which did show reduction in enzymatic activity encountered by the Finnish workers might well be due to their use of comparatively thick sections.

It is admittedly not easy to identify the various portions of the nephron, particularly in those in which cellular details are obscured by deposits of granular dye. The most satisfactory technique for the localization of functional processes in certain parts of the renal tubules consists in the isolation of single renal units[2]. However, the application of this technique to tissue segments stained for unspecific dehydrogenase was unsuccessful due to technical difficulties[3]. Nevertheless, the correct identification of most renal tubules is possible, provided thin sections are studied. With the technique used in this communication, one can easily prepare sections 10–15 μ in thickness and even occasionally sections not thicker than 5μ.

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[1] H. KLUPP, Wiener Z. inn. Med. *34*, 238 (1953).

[2] R. F. PITTS and O. W. SARTORIUS, Pharmacol. Rev. *2*, 161 (1950).

[3] K. K. MUSTAKALLIO and A. TELKKÄ, Science *118*, 320 (1953); Amer. Med. Exper. Biol. Fenn. *32*, 1 (1954). – A. TELKKÄ and K. K. MUSTAKALLIO, Exper. *10*, 216 (1954).

[4] K. K. MUSTAKALLIO and A. TELKKÄ, Amer. Med. Exper. Biol. Fenn. *32*, 1 (1954).

[5] K. K. MUSTAKALLIO and A. TELKKÄ, Amer. Med. Exper. Biol. Fenn. *32*, 1 (1954). – A. TELKKÄ and K. K. MUSTAKALLIO, Exper. *10*, 216 (1954).

[6] M. WACHSTEIN and E. MEISEL, Amer. J. Pathol. *30*, 147 (1954); Science *119*, 100 (1954).

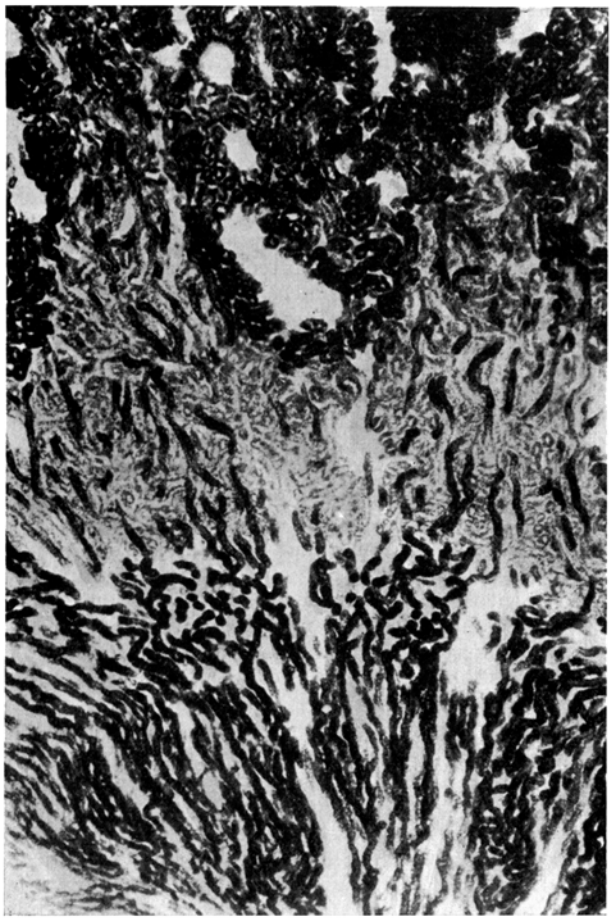
[7] E. G. RENNELS and A. RUSKIN, Proc. Exp. Biol. Med. *85*, 309 (1954).

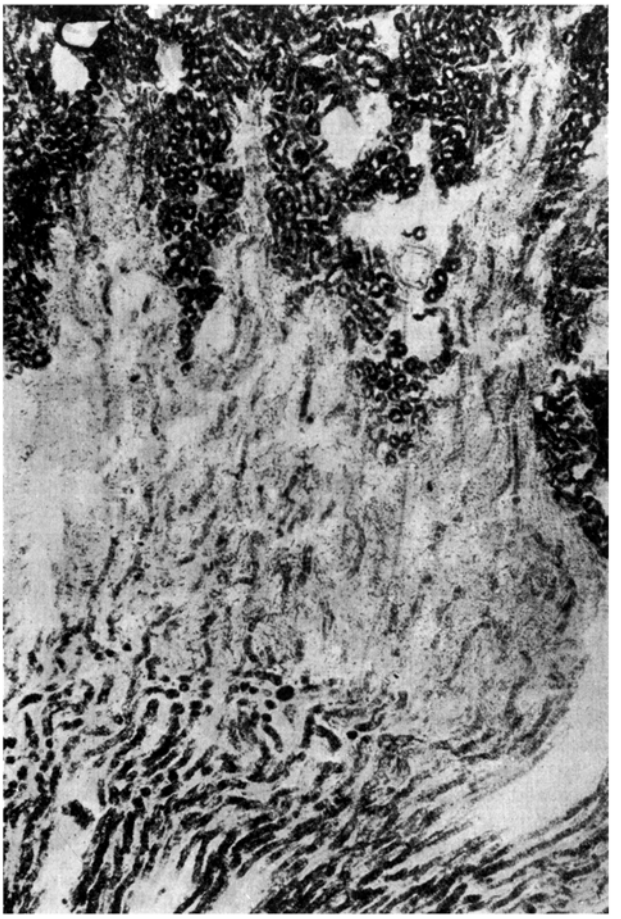
[8] M. WACHSTEIN and E. MEISEL, Amer. J. Pathol. *30*, 147 (1954).

[1] M. WACHSTEIN and E. MEISEL, Amer. J. Pathol. *30*, 147 (1954).

[2] J. OLIVER, Harvey Lect. *40*, 102 (1944–45).

[3] M. WACHSTEIN and E. MEISEL, Amer. J. Pathol. *30*, 147 (1954).





Both photomicrographs are from fresh frozen sections cut at 10–15 μ and prepared for the demonstration of succinic dehydrogenase activity without counter-staining. Both depict corresponding portions of the kidney. On top may be seen a portion of the outer cortex. The center shows the inner cortical zone and at the bottom is seen a portion of the outer medullary zone.

Fig. 1.–Normal Rat: Note the intensely stained proximal portions of the proximal convoluted tubules in the outer cortical zone and the unstained glomeruli. In the inner cortical zone ascending limbs of HENLE contrast sharply with the less intensely stained straight, terminal portions of the proximal convoluted tubules. The outer medullary zone shows predominantly ascending limbs of HENLE with intense activity (× 45).

Fig. 2.–Kidney from a rat sacrificed 24 h after the intramuscular injection of 40 mg Hg/kg body weight of Novurit. Note the extensive inactivation of enzymatic activity located clearly in the terminal portion of the proximal convoluted tubules. Thick limbs of HENLE in the inner cortical and outer medullary zone retain their staining reaction (× 45).

Zusammenfassung

Der genaue Angriffspunkt der Quecksilberpräparate an der Niere ist nicht geklärt. Widersprechende Befunde über den Effekt dieser Diuretica auf mikroskopische Veränderungen und histochemisch nachweisbare bernsteinsaure Dehydrogenase können in folgender Weise geklärt werden:

Mercuhydrin in grossen Dosen führt schon innerhalb weniger Stunden zu einer schweren toxischen Nephrose in der Rattenniere, während Novurit anfangs oft langsamer wirkt. Nichtsdestoweniger ergeben beide Mittel nach 24 h ein ähnliches mikroskopisches Bild. Bernsteinsaure Dehydrogenase ist vermindert und verschwindet schliesslich vollkommen in den absterbenden Zellen. Daher ist schon in den ersten Stunden nach Injektion von Mercuhydrin eine sehr ausgedehnte Inaktivierung in den Zellen der geraden Anteile der Hauptstücke zu sehen. Dieser Effekt wird nur gelegentlich in den ersten Stunden nach Novurit beobachtet. Nach 24 h ist er aber regelmässig vorhanden.

Mit beiden Präparaten konnte keine Verminderung der Enzymaktivität in HENLESsche Schleifen beobachtet werden. Es wird auf die Notwendigkeit hingewiesen, dünne Schnitte zu studieren, weil die verschiedenen Anteile des Nephrons sonst kaum zu unterscheiden sind.

Das Ergebnis dieser Versuche spricht eindeutig für eine Wirkung der Quecksilberdiuretica auf die Hauptstücke und nicht auf HENLESsche Schleifen.

Actions d'un inhibiteur sélectif des acétylcholinestérases, le 3318 CT, sur la transmission neuromusculaire du chat

Il a été montré dans des notes précédentes[1] que le diiodométhylate de la bis (pipéridino-méthylcoumara-

[1] A. FUNKE, J. JACOB et K. VON DÄNIKEN, C. r. Acad. Sci. *236*, 149 (1953). – J. JACOB, Exper. *10*, 53 (1954).