ether anaesthesia using the dorsolateral approach; the operation was performed within less than 5 min, and the animals recovered from the anaesthesia within 10–15 min. Hydrocortisone and prednisolone (in the form of the water-soluble sodium hemitetrahydrophthalate) were injected intraperitoneally in varying amounts into groups of 10–20 animals per dose at intervals ranging from 7 to 24 h following adrenalectomy. Endotoxin (*Serratia marcescens* lipopolysaccharide[8]) was injected intravenously in an amount (100 µg/kg) which proved lethal in 100% (65 out of 65) of the untreated controls challenged 24 h after removal of the adrenals. Only animals still alive 40 h after administration of the endotoxin were recorded as having been protected from lethal endotoxin shock.

Results. The results are summarized in Figures 1 and 2. It is shown that prednisolone affords protection with as low a dosage as 1 mg/kg, the effect being almost identical regardless of whether the compound is administered 1 or 3 h before the endotoxin. With a dose of 3 mg/kg of prednisolone total or nearly total protection is obtained when the corticosteroid is given either simultaneously with, or 1 h before, the endotoxin; extending the interval to 3 h results in a survival rate of slightly more than 60%; after 17 h, 3 mg/kg prednisolone fails to display any protective activity at all. In order to obtain protection when the interval is 17 h, the dosage of prednisolone has to be increased to 30 mg/kg. Clearly, as the time elapsing between prednisolone treatment and endotoxin injection increases, larger amounts of the corticosteroid are needed to obtain comparable survival rates. Somewhat similar effects were observed when hydrocortisone was used instead of prednisolone, although there were two obvious differences: (a) with hydrocortisone the dose affording roughly 50% protection when administered simultaneously with, or 1 h before, endotoxin is some 5–10 times higher than that of prednisolone, and (b) protective activity subsides at a faster rate than with prednisolone.

Protective effect of prednisolone and hydrocortisone in the adrenalectomized rat.

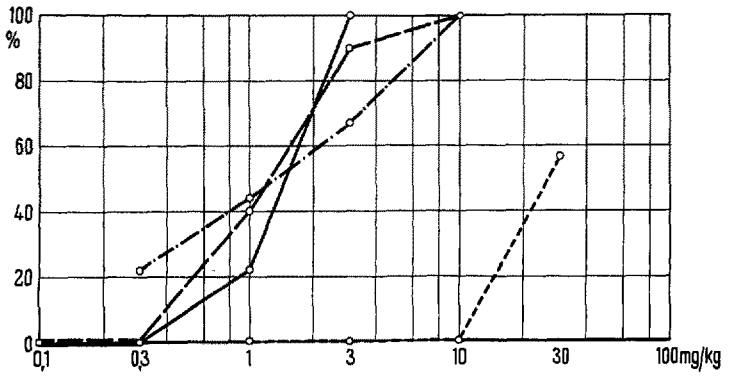

Fig. 1. Prednisolone.

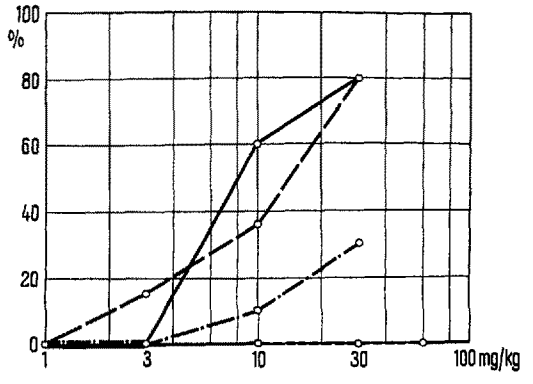

Fig. 2. Hydrocortisone.

Ordinate: Survival (%); each point represents the mean for 10–20 animals. – Abscissa: Dose of steroid (mg/kg, i.p.). – Interval between injection of steroid and challenge with endotoxin: o——o = 0 min, o– – –o = 60 min, o–·–·–o = 180 min, o·····o = 17 h.

Zusammenfassung. An der nebennierenlosen Ratte wurde die Dauer des Schutzeffektes von Prednisolon (P) und Hydrocortison (H) gegenüber letalem Endotoxinschock bestimmt. P war 5–10mal wirksamer als H; ebenso hielt der Schutzeffekt von P 5–10mal länger an als derjenige von H.

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Forschungslaboratorien der CIBA Aktiengesellschaft, Basel (Switzerland), January 17, 1964.

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8 Kindly placed at our disposal by Dr. M. J. SHEAR, National Institutes of Health, Bethesda, Md.
9 Present address: The J. Hillis Miller Health Center, Department of Microbiology, University of Florida, Gainesville (Florida, U.S.A.).

PRO EXPERIMENTIS

Measurement of Glass Microelectrodes

The necessity of using very sharp electrodes to study the electrical properties of the cellular membrane brought about extensive utilization of electrolyte-filled glass microcapillaries during the last twelve years. LING and GERARD[1] and NASTUK and HODGKIN[2] reduced the diameter of the tip of the glass microelectrodes to about 0.5 µ to measure the cellular transmembrane potentials. NASTUK and HODGKIN[2] studying some of the physical properties of the glass microelectrodes, first used electron

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microscopy to measure the external and internal diameters of the tip of their electrodes. Electron microscopy was also utilized by BYZOV and CHERNYSHOV[3] as a guide to design sharper glass electrodes.

The measure of the exact dimensions of the glass microelectrodes is very important because the electrical charges, or Zeta potentials, at the surface of the glass walls introduce large errors in the measurements made by the electrode if the diameter at the tip is reduced under a certain value[4,5]. Moreover, it is possible to design cation-sensitive microelectrodes of a diameter small enough to permit the Zeta potentials at the tip to become the main parameter by these electrodes[6,7].

In this communication we propose a method to study the tip of the glass microelectrodes. Microcapillaries were drawn from Corning No. 0150 glass and Pyrex capillaries (both having an OD of 1 mm and an ID of 0.5 mm) and compared. After cursory observation with the light microscope, the selected microcapillaries, 2 to 3 cm long, were affixed to the top surface of the adjustable specimen holder of the RCA EMU-3F electron microscope. A beam current of 12 μa or less was necessary to avoid deformation of the Corning glass tips. All observations were made at 50 KV except the high magnification of the Pyrex tip at 100 KV. A Baird grating replica was positioned at the specimen plane for calibration purposes. Uptake of 3 *M* KCl was used as a test for usability of the tips before and after entry into the electron microscope.

Microcapillaries drawn from Corning glass capillaries ranged from 0.1 μ to 0.3 μ in diameter at the tip. Figure 1(a) shows a Corning tip measuring 0.16 μ in diameter with a diameter of 1 μ at 8 μ back from the tip. One of the Pyrex microcapillaries is illustrated in Figure 1(b). These range from 0.09 μ to 0.14 μ at the tip. A 1 μ diameter is seen 12 μ from the tip. Higher magnification of a Pyrex tip, Figure 2(a), reveals an inside diameter of about 700 Å and a tip diameter of about 1300 Å. The walls measure 300 to 330 Å at the tip. Figure 2(b) shows the taper of the Pyrex tip. The inside diameter is only slightly increased whereas the capillary walls have doubled in thickness in 1 μ. The ratio of OD to ID is about 2 to 1 at the tip and about 3 to 1 back 1 μ from the tip. The tips were found to be open before and after observation with the electron microscope.

The results shown above indicate the possibility of accurate measurements of the external and internal diameter of the glass microelectrodes near the tip, since at this level the thickness of the glass wall is small enough to permit the glass wall to be transparent to the electron beam. Such information is essential in view of the recent developments of the glass microelectrodes. An exact appreciation of the damage done to the tip by different filling methods is possible by electron microscopy. Moreover, a more quantitative approach to the problem of the influence of the Zeta potentials at the tip of the electrodes is also available if one knows with precision the diameters and the taper of the glass microelectrodes at their tips.

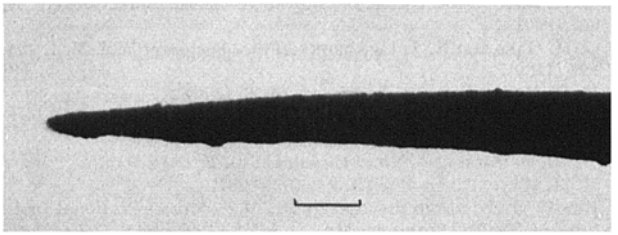

Fig. 1(a)

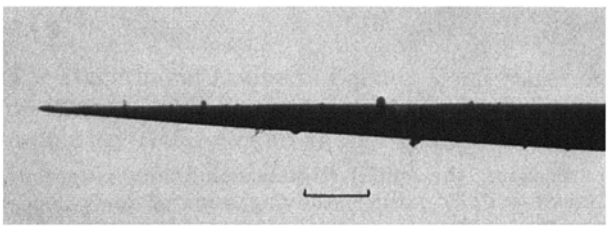

Fig. 1(b)

Fig. 1. Electron micrographs of glass microelectrodes drawn from (a) Corning No. 0150 glass capillary; (b) Pyrex capillary. (Bar indicates 1.0 μ.)

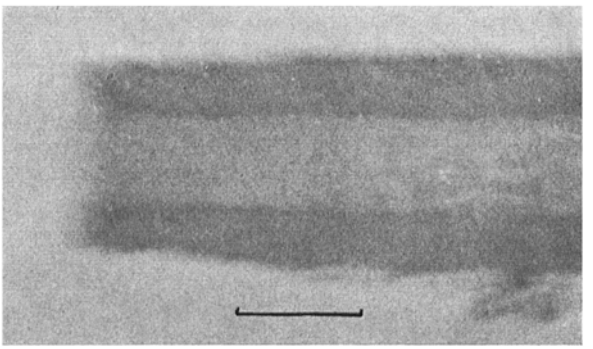

Fig. 2(a)

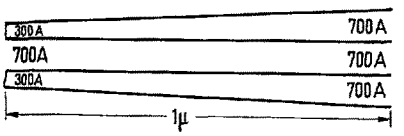

Fig. 2(b)

Fig. 2. Pyrex microelectrode: (a) high magnification EMG; (b) diagram. (Bar indicates 0.1 μ.)

Zusammenfassung. Zur Untersuchung der elektrischen Zellmembraneigenschaften sind bekanntlich besonders scharfe Glaselektroden Voraussetzung. Mit Hilfe eines modifizierten Elektronenmikroskops kann sowohl Wandbreite, wie auch innerer Durchmesser der Mikroelektrodenspitze gemessen werden.

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