

This treatment doubled the number of N-terminal residues in both groups and appears to produce a degraded elastin (possibly by breaking bonds of the type described by Czernawski⁴). The number of N-terminal residues/10⁶ g was found to be greater for group B (mean age 80) than for group A (mean age 70).

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¹ Sanger, F., *Biochem. J.*, **44**, 126 (1945).

² Partridge, S. M., and Davis, H. F., *Biochem. J.*, **61**, 21 (1955).

³ Lansing, A. I., Alex, M., and Rosenthal, T. B., *J. Gerontol.*, **5**, 112 (1950).

⁴ Czernawski, J. W., *Nature*, **194**, 869 (1962).

Isolation of Ergosterol Peroxide from *Trichophyton schönleini*

AN earlier investigation of the free sterols in 14 species of the genera *Epidermophyton*, *Microsporum* and *Trichophyton*, obtained from the hexane extract of the mycelium, revealed the presence of brassicasterol (I) and ergosterol (II)¹.

Exhaustive extraction of 2 kg of the dried milled mycelium of *Trichophyton schönleini* yielded, besides I and II, ergosterol peroxide (III). The total yields, based on dried mycelium, were 0·05, 0·12 and 0·06 per cent respectively. Whereas the hexane extract contained II, the diethyl ether and acetone extracts contained I and III and the methanol extract II and III in amounts sufficient to be isolated in a crystalline state after chromatography.

To our knowledge, III has been isolated only once before as a natural product: from *Aspergillus fumigatus*².

The isolated ergosterol peroxide, m.p. 178°, $[\alpha]_D - 16\cdot2^\circ$ (CHCl_3), was identified by mixed melting point with a synthetic sample³, m.p. 177°, $[\alpha]_D - 18\cdot6^\circ$ (CHCl_3). The infra-red and n.m.r. spectra (quartet at 6·03, 6·18, 6·30, 6·43 p.p.m., 2H, C₆—C₇, olefinic protons; broad signal at 5·17 p.p.m., 2H, side-chain olefinic protons, TMS = 0) and mass spectra were identical and consistent with the accepted structure. The identity was further established by similar comparison of the acetate of III, m.p. 202–3°, $[\alpha]_D - 13\cdot8^\circ$ (CHCl_3), and a synthetic sample.

The mass spectra of the alcohol III showed major peaks at 428, 410, 392 and 376 mass units. The acetate gave major peaks at 470, 452, 436, 410, 392, 376 and 362. All these peaks persisted at approximately the same ratios at lower voltage.

The optical rotations for the peroxide and its acetate were lower than the values reported in the literature^{2,3} (-35° , -29° and -17° , -23° for III and its acetate respectively).

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¹ Blank, F., Shortland, F. E., and Just, G., *J. Invest. Dermatology*, **39**, 91 (1962).

² Wieland, P., and Prelog, V., *Helv. Chim. Acta*, **30**, 1028 (1947).

³ Windaus, A., and Brunkens, J., *Ann.*, **460**, 225 (1928).

PHYSIOLOGY

Saline-filled Micro-electrodes in Relation to Membrane Potential Measurement in Fresh-water Protozoa

THE measurement of membrane potentials in fresh-water protozoa is complicated by the presence of junction potentials existing between the micro-electrode and the medium. These seem to be associated with the fine dimensions of the electrode, coupled with a steep ionic gradient between the contents of the glass electrode and the medium. Adrian¹ was the first to consider in detail the problem of electrode tip potentials and its relationship to membrane potentials. He noted that potentials in frog muscle cells are often increased when the tips of saline-filled micro-electrodes break. Bingley² describes a technique for reducing electrode resistance and tip potential before recording membrane potentials in amoebae. It is necessary to pre-treat micro-electrodes filled with 3·0 M potassium chloride by passing them through agar gel. Potentials were only successfully recorded from amoebae with micro-electrodes which had resistances less than 15·0 M ohm but more than 4·0 M ohm. Above the upper limit, negative tip potentials were too high and no potential was recorded; below, the amoebae sustained damage with a lowering of membrane potential.

Experiments have now been designed to simulate the passage of a micro-electrode from dilute Chalkley's fluid³ into the interior of amoebae in terms of ion changes. Utilizing the technique of flame photometry the internal potassium concentration in *Amoeba proteus* was found to lie within the region of 45 mM potassium/l. (Bingley and Dick, unpublished results.) Changes of electrode resistance and tip potential were monitored using standard electronic and photographic techniques. In Figs. 1–3 electrode resistance is indicated by the height of pulses fed to the micro-electrode through a capacitor at 0·5 sec time-intervals. These are superimposed on d.c. tip potentials. Connexion to the micro-electrode was made by means of standard silver chloride wire. The electrical behaviour of treated and untreated 3·0 M potassium chloride micro-electrodes was examined during reversible exchanges of a solution of 50 mM potassium chloride for one of dilute Chalkley's fluid. The results are shown in Fig. 1. Fig. 1a illustrates a 17·0-mV tip potential of an untreated 20 M ohm 3·0 M potassium chloride filled micro-electrode in dilute Chalkley's fluid; this potential is shown by earthing the silver chloride electrode connexion to the amplifier. The reduction of tip potential and electrode resistance (5·0 M ohm) is shown in Fig. 1b, when the medium is exchanged for a 50 mM potassium chloride solution by dropping it vertically on to the electrode. The change of tip potential when the electrode is in 50 mM potassium chloride is shown in Fig. 1c; d shows the restoration of tip potential (−20 mV) after washing with dilute Chalkley's fluid. The effect of pressure (one atmosphere) applied to the internal contents of the electrode on the tip potential and electrode resistance is shown in e; f shows the reduction in tip potential and electrode resistance by the breakage of the tip. The silver chloride/potassium chloride junction was earthed so that the overall tip potential may be corrected.

Fig. 2a illustrates the zero tip potential produced by a pre-treated 7·0 M ohm 3·0 M potassium chloride micro-electrode in dilute Chalkley's fluid; b, the potential changes during an exchange for 50 mM potassium chloride in the manner described previously; c shows the absence of overall tip potential change and resistance change, while the micro-electrode remains immersed in 50 mM potassium chloride. If the exchange of medium surrounding the micro-electrode is made so that the fluid flow is at right-angles to the axis of the electrode a positive potential excursion (+30 mV) takes place (Bernoulli effect). This is