

mycobacterial polysaccharides. Although this report does not describe in detail the structure of arabinogalactan because of the difficulty in preparing pure arabinogalactan from the phenol-treated cell walls, its structure may be similar to that of highly branched arabinogalactan, having arabinose at a nonreducing terminal, isolated from cell wall³, wax D⁶ and whole cell⁷ by other investigators. The existence of arabinose at a nonreducing terminal of polysaccharides also supports the possible linkage between arabinose and mycolic acid.

It is concluded that mycolic acid links to arabinofuranoside of arabinogalactan in the mycolic acid–arabinogalactan–mucopolysaccharide complex of mycobacterial cell wall.

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- 1 F. KANETSUNA, *Biochim. Biophys. Acta*, 158 (1968) 130.
- 2 I. AZUMA AND Y. YAMAMURA, *J. Biochem. Tokyo*, 53 (1963) 275.
- 3 A. MISAKI AND S. YUKAWA, *J. Biochem. Tokyo*, 59 (1966) 511.
- 4 N. P. V. ACHARYA, M. SENN AND E. LEDERER, *Compt. Rend.*, 264 (1967) 2173.
- 5 I. AZUMA, H. KIMURA AND Y. YAMAMURA, *J. Biochem. Tokyo*, 57 (1965) 571.
- 6 E. VILKAS, J.-M. DELAUMENY AND C. NACASCH, *Biochim. Biophys. Acta*, 158 (1968) 147.
- 7 N. HAWORTH, P. W. KENT AND M. STACEY, *J. Chem. Soc.*, (1948) 1220.

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Secretory response to the stimulation of amphibian skin glands

There is a diverse series of reports in the literature on the time course of secretion from the amphibian skin glands in response to stimulation. SHARE AND USSING¹ referred to unpublished observations which demonstrated that the secretion from the skin glands of *Rana temporaria* had a transient character with a duration of about 20 min. SELDIN AND HOSHIKO² reported that, when adrenaline was applied to the inner (corium) surface of isolated skin of *Rana pipiens*, small volumes of fluid were extruded from the glandular pores within 30 sec and this secretory process was apparently completed within 2 min; LINDLEY³ found a similar time course of secretion during cutaneous nerve stimulation.

There have been no published accounts of attempts to record simultaneously the time course of glandular secretion and the electrical response^{4–6} of the isolated amphibian skin under these conditions. I have obtained volumetric records of the secretion from the glands of the isolated skin of the toad, *Xenopus laevis*, and the contemporaneous changes in the potential difference and resistance of the skin.

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Animals were killed by cutting the spine and pithing; abdominal skin was excised, cleaned of adherences and washed in a volume of Ringer solution which had the following composition: 100 mM NaCl, 2.5 mM KCl, 1 mM CaCl_2 and buffered with Tris at pH 7.6. The isolated piece of skin was mounted between the glass half-chambers as shown in Fig. 1. Initially the chamber was clamped in the horizontal position and filled carefully to exclude air bubbles. Subsequently the chamber was rotated into the vertical position so that the hydrostatic pressure (about 15 cm H_2O) held the outer (epithelial) surface of the skin against the perforated nylon disc. The experimental configuration ensured that the skin was constrained to occupy a well-defined position throughout the experiment. From theoretical and experimental standpoints it was concluded that the constraining hydrostatic pressure was too small to generate significant water movement across the skin. The exposed areas of the outer and inner surfaces of the skin were 1.63 and 5.10 cm^2 , respectively, and the skin was bathed on both sides by identical Ringer solutions. During the experimental period, which followed an equilibration period of about 30 min, the volume of the outer solution was monitored by observing the meniscus in the precision bore capillary (internal diameter, 0.5 mm). Because the experimental period was relatively short it was found unnecessary to control the environmental temperature in the volumetric experiments. The potential difference across the skin (inner surface positive) was monitored by Ag-AgCl electrodes connected to a Vibron electrometer (33B-2) coupled to a recording oscillograph (type 5-124, Consolidated Electrodynamics Corp., Calif.).

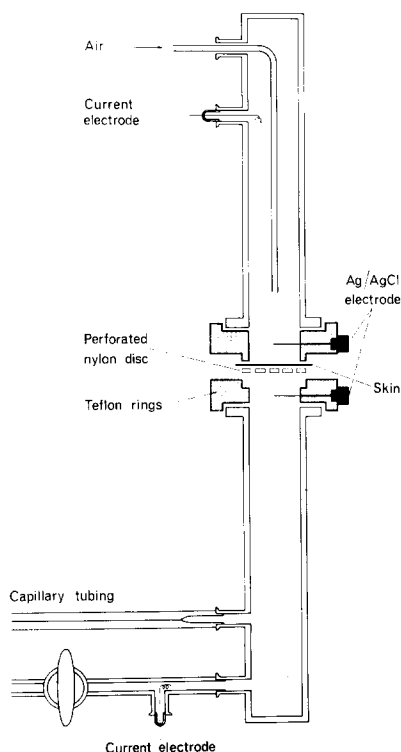


Fig. 1. Diagram of volumetric apparatus.

Constant depolarising or hyperpolarising currents were passed across the skin *via* platinum electrodes in series with a large variable resistance network and a 45-V battery.

Fig. 2 shows the results of a typical experiment where noradrenaline was added to the inner medium to give a final concentration of $7\text{ }\mu\text{g/ml}$ (or $4 \cdot 10^{-5}\text{ M}$). Following a latency of about 30 sec an efflux from the skin glands occurred and reached a maximum about 90 sec after stimulation; the time course of the volumetric data in Fig. 2 agree very well with my photographic observations of the secretion from the glands in *Xenopus* skin. The record of the potential difference across the skin shows an initial depolarising phase followed by a hyperpolarising phase; this response is similar to those reported by SCHOFFENIELS AND SALEE⁴ for cutaneous nerve stimulation and application of noradrenaline to the skin's inner surface. The voltage responses to the brief depolarising current pulses ($50\text{ }\mu\text{A}$) are proportional to skin resistance and it is evident that noradrenaline produces a sharp initial drop in skin resistance. The initial resistance of the skin is $1200\text{ }\Omega \cdot \text{cm}^2$ and the resistance reaches a minimum of $620\text{ }\Omega \cdot \text{cm}^2$ about 90 sec after noradrenaline application; subsequently the resistance slowly increases to a value of $670\text{ }\Omega \cdot \text{cm}^2$ at the end of the experimental period. It is possible that the change in skin resistance reflects the activation of the skin glands and that after stimulation the gland cell membranes and the excretory channel of the gland constitute a low resistance pathway across the skin. Thus, the initial depolarising phase of the potential record might result from the emergence of this transient shunt in the skin. There are several alternative explanations of the depolarising phase in terms of alterations in the permeability characteristics of the epithelial cell membranes and these must be thoroughly explored.

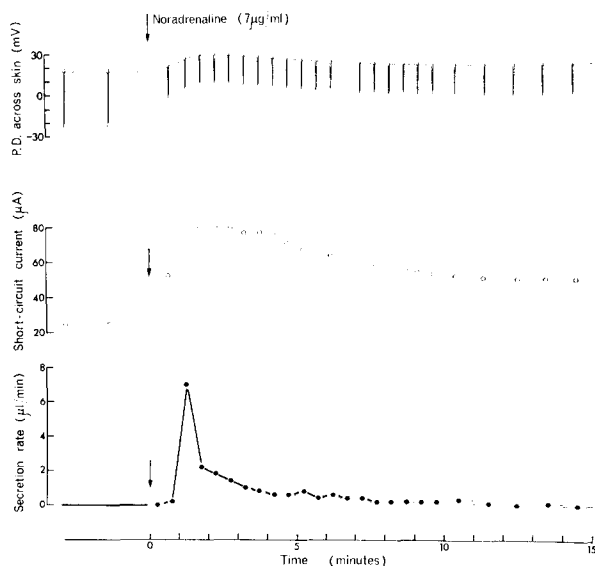


Fig. 2. Time course of the secretory responses to the stimulation of amphibian skin glands by noradrenaline. At the time indicated by the arrow noradrenaline was added to the internal Ringer's fluid to give a final concentration of $7\text{ }\mu\text{g/ml}$. The brief depolarisations on the potential record were produced by constant current pulses of $50\text{ }\mu\text{A}$. P.D., potential difference.

From the magnitudes of the depolarising current pulses and the potential difference, estimates of the short-circuit current were obtained and plotted in Fig. 2. When the skin is bathed by Ringer solution the short-circuit current is an accurate measure of the active influx of Na^+ (ref. 7).

KOEFOED-JOHNSEN, LEVI AND USSING⁸ have reported that the addition of adrenaline to the inner medium produces an increase in short-circuit current which cannot be completely attributed to an increase in active sodium transport and they proposed that under these conditions there is an active Cl^- efflux from the skin glands. My records of the short-circuit current demonstrate that this parameter increases to a peak with approximately the same time course as the secretion rate from the glands. BASTIDE AND JARD⁹ showed that the initial action of $3 \cdot 10^{-5}$ M noradrenaline on the skin of *Rana esculenta* was characterised by a transient increase in short-circuit current which reached a maximum value within 5–10 min after the application of this substance; moreover, these workers claimed that the different time courses of Cl^- influx and efflux under these circumstances were responsible for the transient increase in short-circuit current. Therefore, the proposal that the glands actively secrete Cl^- is compatible with my volumetric records of the secretory rate in *Xenopus* skin.

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- 1 L. SHARE AND H. H. USSING, *Acta Physiol. Scand.*, 64 (1965) 109.
- 2 J. P. SELDIN AND T. HOSHIKO, *J. Exptl. Zool.*, 163 (1966) 111.
- 3 B. D. LINDLEY, *Federation Proc.*, 26 (1967) 767.
- 4 E. SCHOFFENIELS AND M. L. SALEE, *Comp. Biochem. Physiol.*, 14 (1965) 587.
- 5 M. L. SALEE AND M. VIDREQUIN-DELIEGE, *Comp. Biochem. Physiol.*, 23 (1967) 583.
- 6 C. GONZALEZ, J. SANCHEZ AND J. CONCHA, *Biochim. Biophys. Acta*, 120 (1966) 186.
- 7 H. H. USSING AND K. ZERAHN, *Acta Physiol. Scand.*, 23 (1951) 110.
- 8 V. KOEFOED-JOHNSEN, H. LEVI AND H. H. USSING, *Acta Physiol. Scand.*, 25 (1952) 150.
- 9 F. BASTIDE AND S. JARD, *Biochim. Biophys. Acta*, 150 (1968) 113.

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