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SHORT COMMUNICATION

THE EFFECTS OF THERMALLY INDUCED ACTIVITY *IN VIVO* UPON THE LEVELS OF SODIUM, CHLORINE AND POTASSIUM IN THE EPITHELIA OF THE EQUINE SWEAT GLAND

BY STUART M. WILSON[1], HUGH Y. ELDER[1], DAVID McEWAN JENKINSON[2] AND SCOTT A. McWILLIAMS[1]

[1]*The Institute of Physiology, University of Glasgow, Glasgow G12 8QQ* and [2]*Moredun Research Institute, Edinburgh EH17 7JH*

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Evaporation of moisture from the skin surface is an important means of thermoregulatory heat loss in Equidae, which respond to thermal stress with a smooth increase in the rate of sweat output to a plateau level which may be maintained for several hours (Allen & Bligh, 1969; Robertshaw & Taylor, 1969; Montgomery, Jenkinson & Elder, 1982). This pattern is similar to that which occurs when humans are exposed to a warm environment (Montgomery *et al.* 1984). The mode of sweat formation in both the horse and man is similar, involving cell death, fluid transport and the loss of vesicles both by exocytosis and microapocrine secretion (Montgomery *et al.* 1982, 1984). However, the secretions produced by the two glands differ markedly in electrolyte composition. Human sweat is a hypotonic fluid containing sodium chloride as its principal solute (Schulz, 1969; Sato, 1977), whereas equine sweat is hypertonic and contains a high concentration of potassium (Soliman & Nadim, 1967; Kerr & Snow, 1983). This could be a reflection of differences in the underlying mechanism of ionic transport within the gland.

Recent X-ray microanalytical studies of atrichial (eccrine) sweat glands from both the human back (McWilliams *et al.* 1987) and rat footpad (McWilliams *et al.* 1988) have demonstrated changes in intracellular elemental concentration upon thermal stimulation which suggest that sodium influx and potassium efflux are central to the secretory process. However, the changes in intracellular ionic concentration associated with fluid secretion by the epitrichial (apocrine) equine sweat gland, have not been investigated. In this study, the elemental compositions of the epithelia of both resting and active equine sweat glands have been investigated and compared with those found in man (McWilliams *et al.* 1987).

Four Shetland ponies (2 mares and 2 geldings) were exposed to a hot, humid environment (40°C dry bulb, 23°C wet bulb) in a climatic chamber for 4 h and the

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rate of cutaneous water loss was continuously monitored from the flank of each using the technique of McLean (1963). Skin specimens (0·37 mm in diameter) were obtained (Findlay & Jenkinson, 1960), without anaesthetic, from a shaved area on the contralateral side since sweating occurs synchronously on both flanks (Findlay & Robertshaw, 1965; Allen & Bligh, 1969). This procedure did not cause undue distress. An unstimulated (control) sample was taken immediately before each animal was led into the chamber. Subsequently, samples were taken at the onset of sweating, after 3 h of continuous sweating, and 12 and 24 h after the animals had returned to a cool environment.

Each skin sample was cryofixed, freeze-dried and vacuum-embedded in Araldite resin. Thin sections (100–200 nm) of 20 funduses and 25 ducts were cut dry on a diamond knife, and 20–30 energy dispersive X-ray (EDX) spectra were acquired from each epithelial profile studied. Care was taken to ensure that these were acquired only from intracellular sites. Spectra were analysed by the continuum normalization procedure (Hall, 1971) using sections of aminoplastic resin containing appropriate salts as standards (Roos & Barnard, 1984) to give data as mass fractions ($mmol\,kg^{-1}$) for the elements sodium, chlorine and potassium.

The continuum radiation recorded from our sections includes a contribution from the embedding resin, and so our mass fractions do not equate directly with those obtained from freeze-dried sections (e.g. Izutsu & Johnson, 1986). As the resin replaces tissue water (Ingram & Ingram, 1983, 1984; Meyer, Schmidt & Zierold, 1985) the specimen's mass will approximate, ignoring freeze-drying artefacts, to the mass of hydrated sections. The concentrations we report are therefore more comparable with data from fully hydrated sections.

Analysis of variance showed that, in both the fundus and duct, the data for each of the three elements from the control, 12-h post-heat and 24-h post-heat samples could be represented by a single population. These data were therefore pooled to give overall 'unstimulated' values for each animal. The data obtained from the two sets of samples obtained during sweating were similarly shown to belong to single populations and so were pooled to give mean 'active' values. The significances of any differences between the mean resting and active values were tested using the paired *t*-test and the results of this test confirmed using the non-parametric Mann–Whitney *U*-test.

All of the ponies started to sweat within 1 h of entering the hot environment and continued to do so throughout the entire experimental period.

Fig. 1 shows an electron micrograph of an unstained section of freeze-dried sweat gland fundus. Sections cut from bulk freeze-dried, resin-embedded blocks have lower contrast and resolution than do freeze-dried cryosections (Meyer *et al.* 1985), but sufficient detail could be discerned to allow the probe to be positioned accurately within the epithelium.

The mean concentrations of sodium, chlorine and potassium in the resting and active secretory funduses are presented in Fig. 2A. Activity caused a decrease in potassium concentration, and a rise in the concentrations of both sodium and

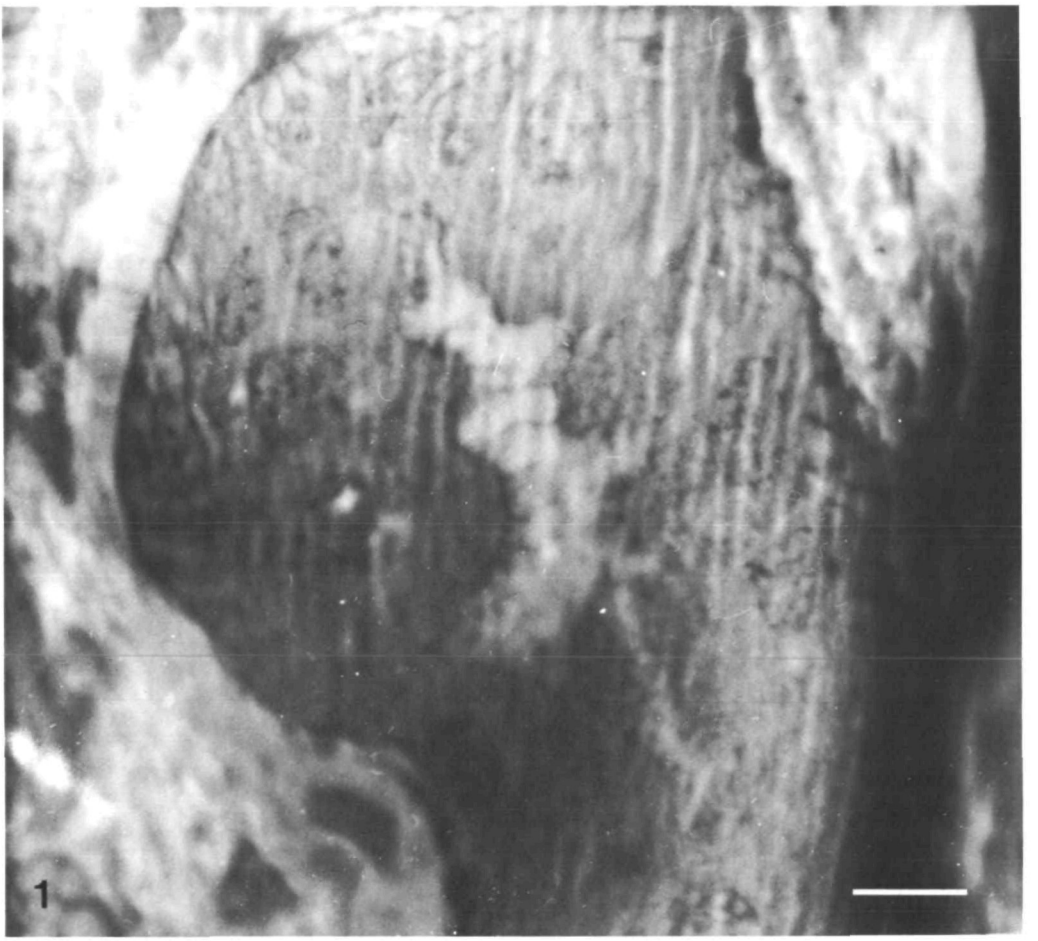

Fig. 1. Electron micrograph of a cryofixed, unstained, dry cut section of a fundus from an equine sweat gland. Scale bar, 5 μm.

chlorine. Activity also caused similar changes in the elemental composition of the ductal epithelium (Fig. 2B).

Dilation of the intercellular spaces occurs in both the duct and fundus of the thermally stimulated equine gland (Montgomery *et al.* 1982). However, the changes in composition reported here are not due to extracellular elements making a greater contribution to the spectra acquired from the active glands, as activity had no effect upon the concentration of phosphorus (data not shown) measured from either cell type.

The magnitude of the fall in potassium concentration in the fundus (23 %) compares with that reported in previous microanalytical studies of sweat glands: 25 % in the thermally stimulated human gland (S. M. Wilson, H. Y. Elder, A. M. Sutton, D. McEwan Jenkinson, F. Cockburn, I. Montgomery, S. A. McWilliams & D. L. Bovell, in preparation) and 38 % in the pilocarpine-stimulated murine

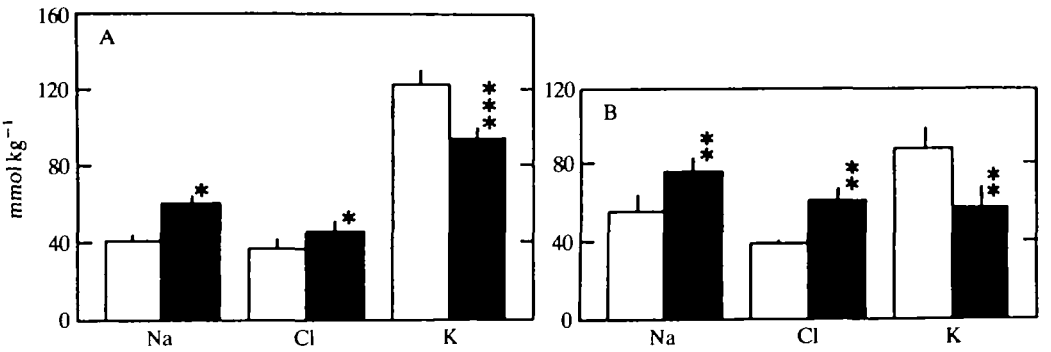

Fig. 2. Mean concentrations, presented with their standard errors ($N = 4$ in all cases), of sodium, chlorine and potassium, recorded from the resting (open bars) and active (filled bars) fundus (A) and duct (B) of the equine sweat gland. * $P < 0{\cdot}05$, ** $P < 0{\cdot}02$, *** $P < 0{\cdot}01$.

plantar gland (McWilliams *et al.* 1988). Furthermore, this technique has revealed an essentially similar fall in other vertebrate exocrine tissues (reviewed by Izutsu & Johnson, 1986). However, the secretory cells of the cockroach salivary gland gain potassium during activity (Gupta & Hall, 1983), suggesting a different mechanism of fluid production in insect tissues.

The increase in the sodium content of the secretory cells during activity (48 %) compares with that reported in our human study (74 %, S. M. Wilson, H. Y. Elder, A. M. Sutton, D. McEwan Jenkinson, F. Cockburn, I. Montgomery, S. A. McWilliams & D. L. Bovell, in preparation), but is lower than the increase seen in the rat gland (139 %, McWilliams *et al.* 1988). Previous microanalytical studies of other secretory tissues also report an increase in the cellular sodium content during activity, but the magnitude of this response is variable (Izutsu & Johnson, 1986), probably because the assay of such light elements by X-ray microanalysis presents considerable difficulties (Hall, 1971; Hall & Gupta, 1983).

It is well documented from electrophysiological and ion flux studies that salivary and pancreatic acinar cells lose potassium (Burgen, 1956; Darke & Smaje, 1972; Petersen & Singh, 1985) and take up sodium (Petersen, 1970*a*,*b*; Poulsen, 1974) during activity. Our findings provide evidence of similar transport phenomena in sweat glands.

In salivary glands the influx of sodium drives the inward movement of chloride against its electrochemical gradient (Case, Hunter, Novak & Young, 1984; Petersen & Maruyama, 1984), and X-ray microanalysis has demonstrated an increase in the cytoplasmic chlorine concentration during activity in the canine submandibular gland (Sasaki, Nakagaki, Mori & Imai, 1983), although not in the rat parotid (Izutsu & Johnson, 1986). Sato & Sato (1987) found that secretion from the simian sweat gland was dependent upon extracellular chloride and could be inhibited by either furosemide or bumetanide, suggesting a similar underlying mechanism. In our study, cytoplasmic chlorine in the active fundus rose above the

resting concentration, suggesting that sodium/chloride cotransport may also operate in the equine sweat gland. The extent to which this model may be applied to sweat glands in general is uncertain, since Quinton (1981) found that furosemide did not affect the rate of secretion from human sweat glands.

Equine sweat, like that of another herbivore, the cow (Jenkinson & Mabon, 1973) contains high concentrations of potassium. How this enters the sweat is unknown. The fundus may simply secrete a potassium-rich fluid, as in the rat footpad gland (Sato & Sato, 1978) or, alternatively, the ductal epithelium may secrete potassium into a primary secretion, as in the salivary glands (Young, 1979). The duct of the equine gland showed changes in the cytoplasmic concentrations of sodium, chlorine and potassium essentially similar to those in the fundus. This provides strong evidence that the ductal epithelium transports at least some of these ions. Elevations in the intracellular concentrations of both sodium and chlorine were observed in the coiled duct of the active human gland (Wilson *et al.* 1988), a tissue where sodium chloride transport occurs (Schulz, 1969; Quinton, 1981, 1983).

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