The metabolism and hormonal responses of human eccrine sweat glands isolated by collagenase digestion

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1. Collagenase digestion of biopsies of human skin yields eccrine sweat glands that can be picked out under binocular light microscopy. The glands are viable as determined by the exclusion of Trypan Blue, the uptake of Methylene Blue, electron microscopy, the rate of lactate dehydrogenase release, ATP content and the rates of glucose oxidation and lactate release. 2. It is proposed that eccrine sweat glands engage in aerobic glycolysis, which accounts for the high content of lactate in sweat (15–60 mM) and the high lactate/pyruvate ratio (100:1) [Emrich & Zwiebel (1966) *Pfluegers Arch.* **290**, 315–319]. 3. Acetylcholine causes a 4-fold increase in cyclic GMP content, dilatation of the intercellular canaliculi and a reversible, atropine-sensitive, 2-fold increase in the rates of glucose oxidation and lactate release. 4. Isoprenaline causes a 2.5-fold increase in cyclic AMP content. Phenylephrine does not significantly alter cyclic nucleotide metabolism.

The development of methods for the isolation of human eccrine sweat glands so as to permit metabolic studies has been prompted by the following observations. First, eccrine sweat glands are exocrine glands that do not secrete a significant protein component (Sato, 1977). Secondly, sweat contains a lactate content of 15–60 mM (Emrich *et al.*, 1967). Finally, the commonest lethal inherited disease of caucasians is cystic fibrosis, which is an inborn error of exocrine-gland metabolism affecting 1 in 1600 babies (Nadler *et al.*, 1978). There has only been one report of a study *in vitro* on human exocrine glands and as the technique described depends on the post-mortem availability of parotid glands it precludes systematic study in cystic fibrosis (Mangos, 1981). Eccrine sweat glands demonstrate a marked abnormality of function in cystic fibrosis (di Sant'Agnese *et al.*, 1953), and as they are the only accessible exocrine glands not to be progressively destroyed by the disease they are the most suitable glands to isolate and study. Aspects of human sweat-gland function have been studied *in vivo* (Emrich *et al.*, 1967) and in biopsies of human skin (Prompt & Quinton, 1978), but these methods have obvious limitations. Attempts at harvesting human eccrine sweat glands by microdissection have foundered on their invisibility when viewed *in situ* (Wolfe *et al.*, 1970; Mangos, 1973; Sato & Dobson,

1973). Wolfe *et al.* (1970) and Mangos (1973) attempted to circumvent this problem by staining the glands before dissection with the supravital dye Methylene Blue. However, this is a powerful oxidizing agent that alters the cytoplasmic redox state (Brin & Yonemoto, 1958) and interferes with oxidative phosphorylation (Lehninger *et al.*, 1959), which inhibits secretion (Sato, 1977) and thus invalidates metabolic studies. Wolfe *et al.* (1970) and Sato & Dobson (1973) have microdissected unstained human glands, but the technique is so difficult and the yield so poor that it precludes systematic study. As this situation parallels that which applied to the islets of Langerhans before Moskalewski's (1965) description of collagenase digestion of the pancreas, it was decided to apply the same technique to human skin so as to isolate eccrine sweat glands, determine their viability and study the responses of glucose and cyclic nucleotide metabolism to acetylcholine, the major secretagogue, and α - and β -adrenergic agonists, which have a smaller effect (Sato & Sato, 1981*b*).

Experimental

Materials

Collagenase (type II), luciferase (type V), luciferase (type IV), acetylcholine chloride, atropine sulphate and ouabain were from the Sigma London Chemical Co., Poole, Dorset, U.K. ATP, NAD, NADH, lactate dehydrogenase and alanine aminotransferase were from BCL, Lewes, East Sussex,

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U.K. D-[U-¹⁴C]Glucose was from Amersham International, Amersham, Bucks., U.K. Glutaraldehyde (electron-microscopy grade) was from Taab Laboratories, Reading, Berks., U.K., and OsO₄ from EM Scope Laboratories, Ashford, Kent, U.K. All other chemicals were from BDH, Enfield, Middx, U.K., and of the highest purity available. Cyclic AMP and cyclic GMP ¹²⁵I radioimmunoassay kits were from NEN, Southampton, Hants., U.K. Bovine brain phosphodiesterase, prepared by the method of Klee & Krinks (1978), was a gift from Dr. D. Harrison of this Department. Microdissection was carried out by using an Olympus MLR-320-W binocular dissecting microscope and two pairs of stainless steel Dumont no. 5 microforceps.

Methods

Preparation of human eccrine sweat glands. Slivers of apparently normal skin (5 cm × 0.5 cm) were obtained from the incisions of elective abdominal operations on healthy adults receiving surgery at the John Radcliffe Hospital. Ethical Committee permission has been granted for this procedure. The slivers were placed in the transport/dissection buffer (Na⁺, 144 mM; K⁺, 5.9 mM; Mg²⁺, 1.2 mM; Ca²⁺, 1.0 mM; Cl⁻, 128 mM; SO₄²⁻, 1.2 mM; PO₄²⁻, 1.2 mM; 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (Hepes), 25 mM; glucose, 5 mM; pH 7.4 at room temperature).

The skin was dissected into small pieces with scissors and incubated in 15 ml of bicarbonate-buffered medium (Krebs & Henseleit, 1932) containing glucose (75 μmol)/collagenase (type II, 5 mg) equilibrated with O₂/CO₂ (19:1) and shaken in a water bath (15–45 min; 37°C; 130 cycles/min). The incubation was washed twice by centrifugation (300 g/5 min) and the pellet resuspended in the transport/dissection buffer (described above) at room temperature. The final suspension was placed in a plastic Petri dish and sweat glands free of collagen fibres and adipocytes were picked out under the binocular microscope. The yield was approx. 10–20 glands per sliver of skin.

Perfusion of sweat glands. Sweat glands were perfused by an adaptation of the method of Cooper *et al.* (1973). A length of cotton button thread was passed through a 100 μl Oxford Sampler tip and held in place with a knot. The glands were naturally adherent to the thread and seven were placed routinely at intervals along it. Perfusion was made with a peristaltic pump (Pharmacia P-3) at a flow rate of 2 ml/h. The perfusate was bicarbonate-buffered medium (Krebs & Henseleit, 1932) containing 5 mM-glucose equilibrated with O₂/CO₂ (19:1) and additions of acetylcholine and atropine as described in the text and Table 2. It was found necessary to include a bubble trap immediately before

the Oxford Sampler tip and this consisted of a T piece attached to a second peristaltic pump. The whole apparatus was assembled in a constant-temperature cabinet maintained at 37°C. During the course of perfusion, 50 μl portions of eluate were collected and stored at 2°C before assay of lactate (see below). At the end of perfusion the glands were removed quickly and Trypan Blue exclusion and Methylene Blue uptake determined by the methods of Tenant (1964) and Wolfe *et al.* (1970) respectively.

Incubation of sweat glands: D-[U-¹⁴C]glucose oxidation. Pairs of glands were incubated in 0.15 ml of perfusate buffer (described above) equilibrated with O₂/CO₂ (19:1) and with additions of acetylcholine or being made up to 5 mM-D-[U-¹⁴C]glucose at a specific radioactivity of 0.15 Ci/mol as described in the text or in the Tables. The glands were incubated for 3 h in a small plastic tube placed in a scintillation vial as described by Keen *et al.* (1963) and shaken gently at 37°C in a water bath. D-[U-¹⁴C]Glucose oxidation was arrested at 3 h by the injection of 50 μl of 8% (v/v) HClO₄ into the plastic tube and the ¹⁴CO₂ trapped in Hyamine injected into the scintillation vial as described by Ashcroft *et al.* (1970). Quenching was corrected by the external-standard channels-ratio method and each sample was counted long enough to accumulate 2000 counts. After the completion of ¹⁴CO₂ trapping in Hyamine the HClO₄ supernatant was removed for ATP assay (see below).

Lactate dehydrogenase release and assay. Pairs of glands were incubated for 3 h as described above, following which the glands were removed into 150 μl of fresh bicarbonate-buffered medium and homogenized by exposing them to 10 s of ultrasonic vibration delivered by a Dawes Soniprobe set at position 1. The lactate dehydrogenase activity of the original 3 h incubation medium and homogenate were determined by the method of Bergmeyer & Bernt (1974).

Electron microscopy. Sweat glands were fixed in 4% glutaraldehyde/0.1 M-sodium phosphate (pH 7.2) for 1 h (Karnovsky, 1965). After an overnight wash in 0.1 M-sodium phosphate, pH 7.2 at 4°C, the glands were exposed to 1% OsO₄/0.1 M-sodium phosphate, pH 7.2, at 4°C for 1 h and then dehydrated in ethanol and two changes of propylene oxide (Smart *et al.*, 1981). Impregnation and embedding was achieved by using low-viscosity resin that was polymerized overnight at 60°C (Spur, 1969).

Semi-thin (1 μm) sections were cut and stained with Azure A to locate areas of interest, which were further sectioned for electron-micrographic examination. Gold to silver (continuous interference colour scale 60–90 nm) ultra-thin sections were collected on to copper grids and stained with uranyl acetate/lead

EXPLANATION OF PLATE 1

A photograph of a sweat gland (a) and electron micrographs of a sweat gland incubated with ouabain (b) and with acetylcholine (c)

Magnification was: (a), 50 $\times$; (b), 5500 $\times$; (c) 12000 $\times$. Abbreviations: CC, Clear Cell; CD, collecting duct; IC, intercellular canaliculus; LG, lipofuscin-like granule; M, mitochondrion; N, nucleus; TJ, tight junction; SC, secretory coil.

citrate sequence. These were examined by using a Philips EM 301 at 60kV.

Lactate. To avoid contamination, acid-washed glassware or disposable plastic ware was used throughout and handled with plastic gloves or forceps. Lactate was determined in the perfusate by an adaptation of the method of Noll (1974), which is based on the lactate dehydrogenase-catalysed reduction of NAD to NADH with completion of the reaction ensured by the transamination of pyruvate. Eluate (10 μ l) was added to 15 μ l of 0.1 M-2-amino-2-methylpropan-1-ol, pH 9.9, at 30°C containing 0.36 units of lactate dehydrogenase, 0.1 unit of alanine aminotransferase, 0.7 mM-NAD and 4.5 mM-sodium glutamate. The mixture was incubated for 1 h at 30°C and 15 μ l was removed for NADH assay by the luciferase method (Stanley, 1971). Standard curves were linear over the range 0–400 pmol of lactate and control experiments showed that the reaction was complete in 10 min and that lactate in the perfusate was stable for up to 6 h at 2°C, within which time it was assayed.

ATP assay. ATP was determined in 10 μ l of 'HClO₄ supernatants' of incubated sweat glands by the luciferin/luciferase assay (Stanley & Williams, 1969). Control experiments with ATP in HClO₄ showed that standard curves were linear over the range 0–200 pmol and that ATP was stable in HClO₄ for up to 6 h, within which time it was assayed.

Determination of cyclic AMP and cyclic GMP. This was performed using NEN ¹²⁵I radioimmunoassay kits based on the protocols of Steiner *et al.* (1972) with greater sensitivity being achieved by prior acetylation of the cyclic nucleotide (Harper & Brooker, 1975). Pairs of glands were pre-incubated in 40 μ l of perfusate buffer equilibrated with O₂/CO₂ (19:1) at 37°C for 15 min and then made 10 μ M with respect to acetylcholine, isoprenaline or phenylephrine, as described in Table 3. After further incubation for 10 min, 250 μ l of boiling 50 mM-sodium acetate, pH 6.2, was added and the incubation mixture was boiled for 5 min. After cooling to room temperature the glands were homogenized by exposure for 10 s to ultrasound delivered by a Dawes Soniprobe set at position 1 and the homogenate was centrifuged (10 000 g/2 min). Portions (50 μ l) were removed for acetylation before ¹²⁵I radioimmunoassay in duplicate for cyclic AMP and cyclic GMP. Control experiments showed, first, that the addition of phosphodiesterase decreased the cyclic AMP and cyclic GMP contents of the homogenates to zero; secondly, that the method of arresting cyclic AMP and cyclic GMP metabolism described here gave results comparable with those for the addition of 200 μ l of boiling 0.1 M-HCl to the incubated glands, boiling for 5 min, followed by freeze-drying and reconstitution in 50 mM-sodium acetate, pH 6.2

(Sato & Sato, 1981a); thirdly that the content of cyclic AMP and cyclic GMP in the homogenates was stable at 2°C for 12 h, within which time it was assayed; and fourthly, that the sensitivity of the radioimmunoassay kit could be increased by halving all the volumes in the manufacturer's instructions.

Protein assay. This was determined by the method of Lowry *et al.* (1951).

Results and discussion

Indices of viability

The method of collagenase digestion described here has been found to be very reliable. The only important variable is the length of exposure to collagenase that is required; the younger the donor, the shorter the period of time. Glands removed on isolation, or after perfusion or incubation, were found invariably to exclude Trypan Blue or take up Methylene Blue. Plate 1(a) shows a photograph of a sweat gland at a magnification of $\times 50$. It shows the secretory coil, collecting duct and a few adhering collagen fibres. Plate 1(b) is an electron micrograph of a gland at a magnification of $\times 5500$ after incubation in bicarbonate-buffered medium for 20 min in the presence of 10 mM-ouabain. It shows three Clear Cells in an extremely good state of preservation. The central cell nucleus, mitochondria and glycogen granules are seen, as are two systems of intercellular canaliculi with attendant tight junctions in a closed, non-secretory state. Plate 1(c) is an electron micrograph at a magnification of $\times 12000$ of a gland after incubation in bicarbonate-buffered medium for 20 min in the presence of 1 μ M-acetylcholine. In addition to the features displayed in Plate 1(b), it shows a dilated intercellular canaliculus feeding into the collecting duct. Electron microscopy shows therefore that the glands are intact and respond morphologically to secretagogue and ouabain in the way common to all fluid secretory glands (Diamond & Bossert, 1967). In Plate 1(c) lipofuscin-like granules can also be seen.

Table 1 illustrates some aspects of the composition of sweat glands. It shows that on isolation the glands have an ATP content of 6.23 pmol/ μ g of protein, which compares well with that found for other isolated exocrine gland cells [e.g. parotid acinar cells (Kanagasuntheram & Randle, 1976)] and that this is maintained over incubation for 3 h and is not significantly decreased by 1 μ M-acetylcholine. The rate of release of lactate dehydrogenase by the isolated glands is 3.5% per h, which compares well with that found for other isolated gland cells [e.g. parotid acinar cells (Kanagasuntheram & Randle, 1976)].

Table 1. *Aspects of the composition of sweat glands*

Pairs of glands were incubated and assayed for ATP content as described in the text. The ATP content after incubation for 3 h was not significantly altered by the addition of $1\text{ }\mu\text{M}$ -acetylcholine as determined by Student's *t* test ($P > 0.3$). The protein content of glands and the rate of lactate dehydrogenase release from the glands was determined as described in the text. The rate of D-[U- ^{14}C]glucose oxidation was determined as described in the text. ** $P \leq 0.01$, when the value with $1\text{ }\mu\text{M}$ -acetylcholine is compared with that from unstimulated glands.

Parameter	Number of subjects	Number of pairs of glands studied per subject	Conditions of assay	Mean $\pm$ S.E.M.
Protein content ($\mu\text{g/gland}$)	4	6	Isolation	3.8 ± 0.7
ATP (pmol/gland)	6	3	Isolation	23.7 ± 5.3
ATP (pmol/gland)	6	3	Incubation for 3 h	25.3 ± 4.9
ATP (pmol/gland)	6	3	Incubation for 3 h + $1\text{ }\mu\text{M}$ -acetylcholine	18.6 ± 4.5
Lactate dehydrogenase content (m-units/gland)	3	6	Incubation for 3 h	44.7 ± 6.7
Lactate dehydrogenase released (%/h)	3	3	Incubation for 3 h	3.5 ± 0.7
Lactate dehydrogenase released (%/h)	3	3	Incubation for 3 h + $1\text{ }\mu\text{M}$ -acetylcholine	3.6 ± 0.7
CO_2 evolved (nmol/gland per h)	6	6	Incubation for 3 h	0.200 ± 0.034
CO_2 evolved (nmol/gland per h)	6	6	Incubation for 3 h + $1\text{ }\mu\text{M}$ -acetylcholine	$0.425 \pm 0.057^{**}$

Glucose metabolism

Table 2 illustrates the rate of release of lactate during perfusion. With unstimulated glands the rate was constant for over 5 h. This rate was more than doubled by $1\text{ }\mu\text{M}$ -acetylcholine. These findings are comparable with those reported by Sato & Dobson (1973), thus showing that collagenase digestion does not alter the response to acetylcholine. The increased rate with acetylcholine was maintained for at least 30 min and was restored to the unstimulated rate within 15 min either by withdrawal of acetylcholine or by the addition of $1\text{ }\mu\text{M}$ -atropine. This suggests that the effects of acetylcholine are specific and physiological. The 2-fold stimulation of lactate production by acetylcholine is accompanied by a similar stimulation of glucose oxidation, as illustrated in Table 1.

Cyclic nucleotide metabolism

Table 3 illustrates the response of cyclic nucleotide metabolism to secretagogues. Acetylcholine raises the cyclic GMP content over 4-fold, which is comparable with the effects of muscarinic cholinergic stimulation at other sites (Goldberg & Maddox, 1977). The unstimulated cyclic AMP content is similar to that reported for the monkey paw eccrine sweat gland [28 ± 2.4 fmol/gland (Sato & Sato, 1981a)] and rises 2½-fold with β -adrenergic stimulation. This is less than the 6-fold rise described for the isolated monkey paw gland, which might be a consequence of the collagenase digestion, but is more likely a reflection of the much greater response *in vivo* of palmar glands than thermoregulatory glands to β -agonists (Dobson &

Table 2. *Effect of acetylcholine $\pm$ atropine on output of lactate by human eccrine sweat glands*

Human eccrine sweat glands (7) were perfused at 37°C with bicarbonate-buffered saline equilibrated with O_2/CO_2 (19:1) containing 5 mM-glucose. Over the periods shown three collections of $50\text{ }\mu\text{l}$ of perfusate were made over 1.5 min intervals and assayed in triplicate. Each value is the mean ($\pm$ S.E.M.) of three such perfusions and three collections at each time point. The number of subjects studied is shown in parentheses. *, $P < 0.001$ for effect of acetylcholine; †, $P < 0.001$ for effect of discontinuing acetylcholine or addition of atropine.

Perfusion	Time (min)	Lactate output (nmol/gland per 60 min)
Control	28–32.5	0.96 ± 0.05 (9)
	58–62.5	0.96 ± 0.03 (27)
	298–302.5	0.94 ± 0.07 (9)
$1\text{ }\mu\text{M}$ -Acetylcholine	88–92.5	$2.41 \pm 0.10^*$ (27)
after 75 min	118–122.5	$2.37 \pm 0.22^*$ (9)
$1\text{ }\mu\text{M}$ -Acetylcholine at 75 min; discontinued at 105 min	118–122.5	$0.84 \pm 0.06^\dagger$ (9)
$1\text{ }\mu\text{M}$ -Acetylcholine at 75 min; $1\text{ }\mu\text{M}$ -atropine at 105 min	118–122.5	$0.88 \pm 0.06^\dagger$ (9)

Sato, 1972). This difference in response is attributed to the very different function of the two sets of glands, that of the palmar ones being to generate surface tension and so strengthen grip (Kuno, 1956). The lack of a statistically significant response of either cyclic nucleotide to phenylephrine reflects the

Table 3. *Cyclic nucleotide metabolism*

Pairs of glands were incubated in 40 μ l of perfusion buffer at 37°C for 10 min and then made up to 10 μ M of the secretagogue. After a further 10 min incubation, metabolism was arrested by the addition of 250 μ l of boiling 50 mM-sodium acetate, pH 6.2, and cyclic nucleotide assay was performed as described in the Experimental section. *** indicates that the content was significantly different ($P \leq 0.001$) from the unstimulated value when analysed by Student's *t* test. *n* refers to the number of subjects studied, each observation having been made on five pairs of glands per subject. Results are means $\pm$ S.E.M.

Condition of incubation	Cyclic AMP (fmol/gland)	Cyclic GMP (fmol/gland)
Unstimulated	19.9 $\pm$ 2.90 (<i>n</i> = 9)	3.50 $\pm$ 0.59 (<i>n</i> = 9)
10 μ M-Acetylcholine	22.33 $\pm$ 2.67 (<i>n</i> = 7)	14.75 $\pm$ 2.07 (<i>n</i> = 7)***
10 μ M-Isoprenaline (β)	49.42 $\pm$ 2.67 (<i>n</i> = 7)***	5.00 $\pm$ 0.99 (<i>n</i> = 3)
10 μ M-Phenylephrine (α)	22.03 $\pm$ 3.22 (<i>n</i> = 3)	5.66 $\pm$ 1.23 (<i>n</i> = 3)

poor response of sweat glands to that secretagogue (Sato & Sato, 1981b).

General discussion and conclusions

These studies demonstrate that collagenase digestion of skin yields eccrine sweat glands that are viable by the criteria of morphology, ATP concentration, lactate dehydrogenase release, Trypan Blue exclusion, Methylene Blue uptake and the responses of lactate release, glucose oxidation and cyclic nucleotide metabolism to secretagogue. This viability was maintained in buffers made free of albumin because of the findings of Berridge & Patel (1968) that 2% bovine serum albumin increased the rate of secretion in insect salivary glands 60-fold. The response of glucose metabolism to acetylcholine was studied because of the high content of lactate in the sweat and because of Sato's findings (1977) in the very similar monkey paw gland that exogenous glucose is the primary fuel of secretion, ketone bodies and non-esterified fatty acids being unmetabolized. The rate of glycolysis was determined by lactate production and the rate of oxidation by $^{14}\text{CO}_2$ production. The latter technique ignores endogenous glycogen metabolism, but as the experiments were performed over 3 h and as Sato (1977) has shown that endogenous glycogen stores can only sustain secretion for 10 min, this is acceptable.

The results obtained were that at 1 μ M-acetylcholine, the glands oxidized 0.071 nmol of glucose/gland per h and metabolized to lactate 1.14 nmol of glucose/gland per hour. The value for glucose oxidation reported here is twenty times that reported by Wolfe *et al.* (1970), but this can be accounted for by their use of Methylene Blue. If it is assumed that there is a yield of 1 mol of ATP/mole of lactate produced and 6 mol of ATP/mol of CO_2 produced, then the energy derived from glycolysis and glucose oxidation is approximately the same. The flux through lactate dehydrogenase is therefore 16 times that through pyruvate dehydrogenase. This ratio of 16:1 is in contrast with that seen, for example, in working heart muscle, which is 0.5:1

[calculated from the data of Neely *et al.* (1972)]. That this high lactate production *in vitro* is a reflection of the situation *in vivo* and not a consequence of poor oxygenation is established by a number of facts. First, the rate of lactate production *in vitro* is actually less than that seen *in vivo*. The maximum sweat rates seen *in vivo* are 0.3 μ l/gland per h, containing 15 mM-lactate; this gives a rate of lactate production of 4.5 nmol/gland per h (Emrich *et al.*, 1967). This is double that seen *in vitro*, which may indicate that 1 μ M-acetylcholine is a suboptimal concentration. Secondly the sweat gland is a coil of only one to two cells thickness, which ensures full exposure to the medium. Thirdly, when tissues are maintained under suboptimal conditions, there is a progressive increase in the rate of aerobic glycolysis (Fujita, 1928). This is not the pattern seen in perfused sweat glands and the basal rate of lactate release does not rise after 5 h of perfusion. It must be concluded therefore that the rate of lactate release *in vitro* is physiological.

The rate of glucose oxidation is taken to be physiological for two reasons. First, it rises in response to acetylcholine. Secondly, it is of the order of magnitude to be expected. *In vivo* at maximum secretion, a sweat gland produces 0.3 μ l of sweat/h, the sweat containing 140 mM- Na^+ (Schulz *et al.*, 1965). If it is assumed that under these conditions the bulk of the ATP generated is hydrolysed to fuel fluid secretion and if it is assumed that fluid secretion is subserved by the action of the ($\text{Na}^+ + \text{K}^+$)-stimulated ATPase with the consumption of 1 mol of ATP for 3 mol of Na^+ transported (Frizzell *et al.*, 1975), then it follows that the rate of lactate production *in vivo* accounts for 32% of the energy expenditure, the rest being accounted for by oxidation. Glycolysis *in vitro* accounts for 47% of the energy generated, the rest being accounted for by oxidation. The value for *in vivo*, however, almost certainly underestimates the actual lactate production, as the loss to the bloodstream has not been quantified. The values for *in vivo* and *in vitro* may therefore be even closer.

These findings place the sweat gland in the category of tissues such as the renal medulla and the retina, which engage in aerobic glycolysis (Krebs, 1972). The teleology in the case of the sweat gland is unclear, but it should be noted that the pH of sweat is 5 (Emrich & Oelert, 1966) and that this may be due to the diffusion of lactic acid into the lumen, where it dissociates. This may have useful bacteriostatic effects in the ducts and in the skin analogous to that exerted by the lactate generated by lactobacilli in the contiguous vagina. This may account for Sato's observation (1977) that secretion is dependent on exogenous glucose. The mechanism of aerobic glycolysis may lie either in the inhibition of NADH uptake by the mitochondria or of pyruvate dehydrogenase. These two mechanisms could be distinguished by the activation of pyruvate dehydrogenase by dichloroacetic acid (Whitehouse & Randle, 1973). Either mechanism would result in a reduced cytosolic NAD/NADH couple. It follows therefore that if the hypothesis that sweat glands engage in aerobic glycolysis is correct, then the [lactate]/[pyruvate] ratio of sweat should be raised. Emrich & Zweibel (1966) reported that it rose to 100:1 during high rates of secretion.

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