Table 2. Effects of sera and liver extracts from Ph and Sham rats on liver DNA synthesis

Conditions	No. of experiments (positive)	*Spec act ± SEM	P	%
PH sera vs Sham sera	15/20	+12.4 ± 4.3	< 0.01	+ 9.9
PH extract + sera (PH or Sham) vs Sham extract + sera (PH or Sham)	16/20**	+23.4 ± 6.8	< 0.01	+19.0

*Spec act, difference in specific activity. **Results using PH and Sham sera are combined.

(table 1). This stimulation was somewhat specific. When the effects on renal DNA synthesis of sera from PH were compared to sera from Sham, there was significant depression in 8 experiments (–11%, $p < 0.05$).

Could liver be the source of circulating hepatotropin? Liver extracts from PH placed alone in medium failed to stimulate. LaBrecque and Pesch[8] also did not find any increase in ^{3}H-thymidine incorporation into DNA of liver slices or isolated liver cell suspensions by their hepatic extract which had previously stimulated DNA synthesis when injected in vivo. They felt that this was because a longer time than that used for in vitro incubation was necessary to show stimulation. However, in our previous studies on the renotropic system[11], we showed stimulation of DNA synthesis by renal extracts only when sera was present. Although liver extracts from PH rats did not stimulate DNA synthesis in slices when alone, they did increase this parameter in the presence of PH sera or Sham sera (table 2).

Our assay uses incorporation of ^{3}H-thymidine into DNA as an estimate of hyperplasia. While the use of slices offer similar initial intracellular precursor pools for DNA, non-specific extracellular dilution of tracer by sera could increase isotope incorporation into DNA in the test slice despite no actual change in DNA synthesis. However, this would appear with slices from any organ, and sera from PH actually decrease DNA synthesis in rat kidney slices. Similarly, finding that neither extracts from regenerating nor control livers affect DNA synthesis alone and that extracts from regenerating livers cause a relative stimulation to hepatic DNA synthesis only in the presence of sera also suggests that our results are not secondary to non-specific isotope dilution by extracts.

These in vitro findings are consistent with a proposal[5] that it may take hours for stimulators to develop, but a considerably shorter period of time for them to stimulate DNA synthesis. As a first approximation, we suggest that after loss of liver mass, there is a rise in a humoral factor and a liver factor that occurs within 20 h. Since the latter works only in the presence of sera, it could activate the serum factor which, in turn, stimulates DNA synthesis. If the liver is the only organ containing specific activator, this could explain why PH sera enhance liver slice DNA synthesis not renal.

Pharmacology and function of the myoepithelial cell in the eccrine sweat gland[1]

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Summary. Both acetylcholine and a Ca-ionophore, A23187, are comparatively strong stimulants of eccrine sweat secretion in vitro. Nevertheless, the contraction of the secretory coil was seen only after stimulation with acetylcholine but not with alpha or beta adrenergic drugs or with A23187. It was thus inferred that the myoepithelial contraction may not be playing an indispensable role in the overall process of eccrine sweat secretion.

The secretory coils of human, monkey, cat and other mammalian eccrine sweat glands consist of 3 cell types: clear, dark and myoepithelial cells. Because of its resemblance to other fluid and electrolyte transporting cells, the clear cell is generally believed to be responsible for secretion of water and electrolytes by the sweat gland[3]. The function of the dark cell is unknown, although secretion of a mucoid substance in sweat is often regarded as being its function. The myoepithelial cell is spindle-shaped, lies on the basement membrane, and is filled with masses of myofilaments. It is rather infrequent that foot processes of 2 opposing myoepithelial cells are bound to each other, and for this reason the myoepithelial cells are generally regarded as discontinuous cells[3]. The function of the myoepithelial cell is not clear, but the following hypotheses have been postulated: a) myoepithalial contraction expulses the preformed sweat onto the skin surface; b) the myoepithelial cell is a suppotive structure for the tubule; c) myoepithelial contraction opens the intercellular channel of the secretory cells[3]. The present study is thus intended to clarify the role of the myoepithelial cell in eccrine sweat secretion using an isolated single segment of the secretory coil. An isolated eccrine sweat gland responds to a number of sudorific agents in varying degrees[4]. If there is a dissociation between myoepithelial contraction as analyzed photomicrographically

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3 R. A. Ellis, in: Handbuch der Haut- und Geschlechtskrankheiten, vol. 1: Normale und pathologische Anatomie der Haut. Ed. J. Jadassohn. Springer-Verlag, Berlin, Heidelberg, New York 1967.

4 K. Sato, Rev. Physiol. Biochem. Pharmac., in press (1977).

and the sudorific response in vitro to a given drug, then the role of the myoepitheliel contraction can be deduced indirectly.

Methods. A monkey palm eccrine sweat gland was used as a model of the human sweat gland. The sweat gland was dissected out from a biopsy specimen under a stereomicroscope[5]. The secretory coil was further isolated from the duct. The entire procedure was performed in a cold Krebs-Ringer Bicarbonate buffer (KRB, also containing 5 mM glucose and 0.5 g albumin, gassed with 5% CO_2 + 95% O_2, pH 7.4)[5]. Induction of sweat secretion from an isolated sweat gland in vitro was performed essentially as described in reference[5]. Photomicrographs of the isolated secretory coil segment were taken in separate sets of experiments in a thermostated (37 °C) microchamber placed on the mechanical stage of a microscope. The chamber consisted of a narrow groove 100 μm in depth, the top of which was covered with a small piece of ordinary

Summary of the in vitro secretory response to various sudorific agents

Stimulants	Concentration (in M)	Number of glands studied	Maximal sweat rate* (10^{-9} l/min gl) and
Mecholyl	10^{-6}	80	5.2 ± 0.25 (SEM)
A23187 (Ca^{++} ionophore)	3×10^{-6}	13	5.5 ± 0.44
Phentolamine (α-adrenergic agonist)	10^{-4}	24	1.1 ± 0.12
Isoproterenol (β-adrenergic agonist)	5×10^{-6}	21	1.9 × 0.15

* Sweat samples were collected every 10 min period and the maxima sweat rate in each gland was used for computation.

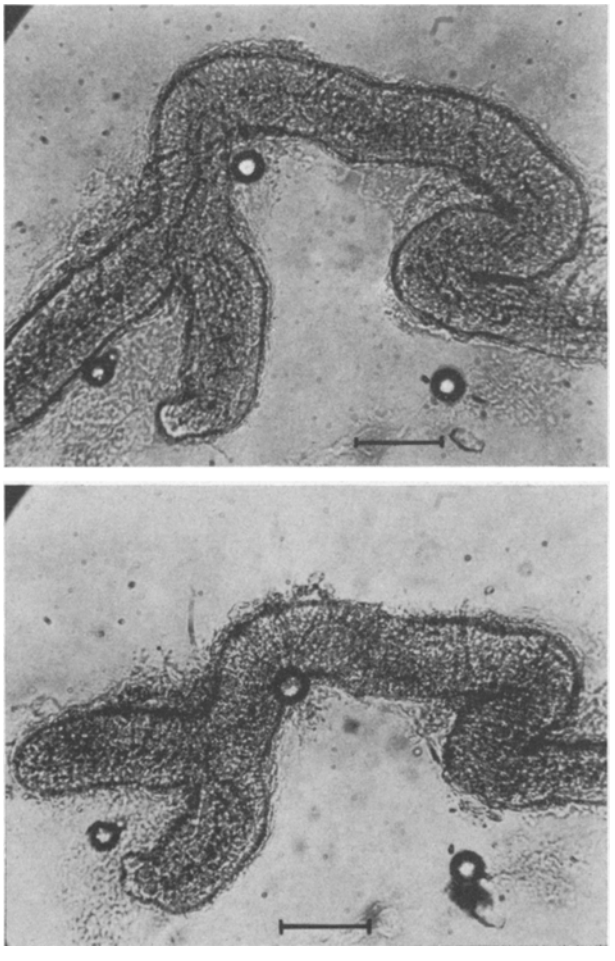

Fig. 1. *A* An isolated segment of the secretory coil before stimulation. *B* The same segment after stimulation with 10^{-6} M acetylcholine. The bar represents 100 μm.

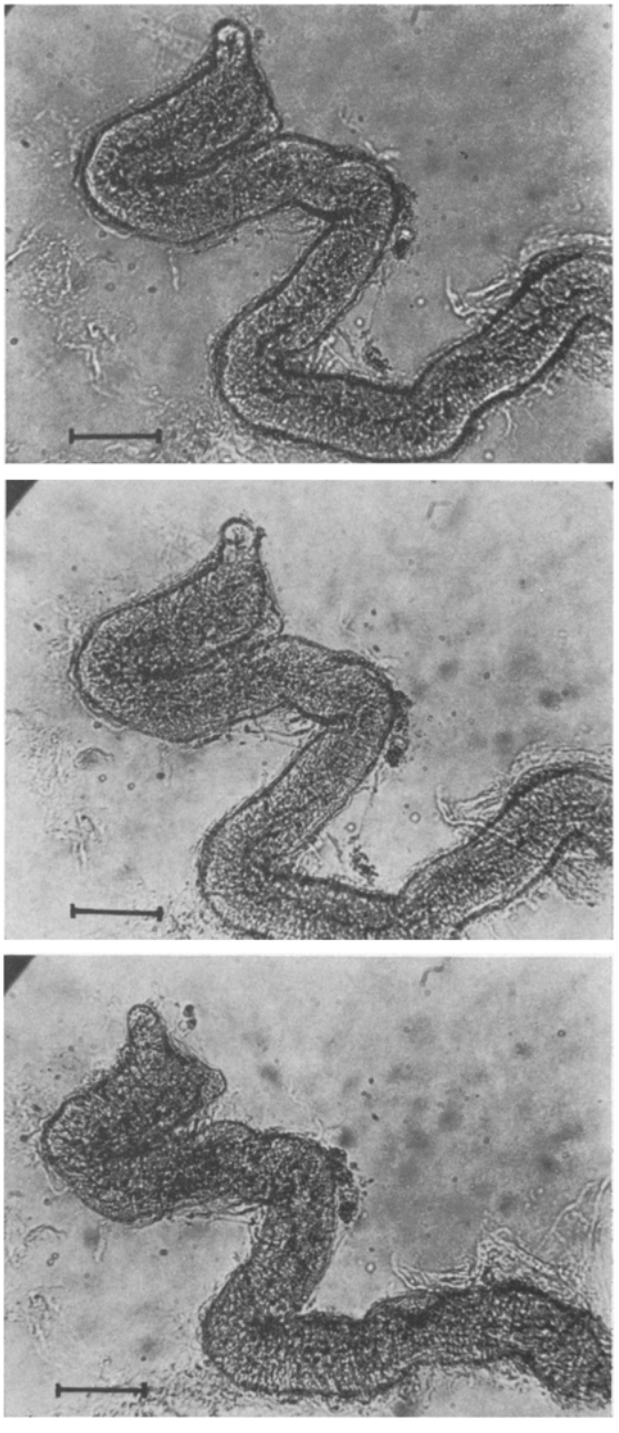

Fig. 2. *A* A segment of the secretory coil before stimulation. *B* 10 min after addition of 2×10^{-4} M A23187. *C* The same segment after the subsequent addition of 10^{-6} M acetylcholine.

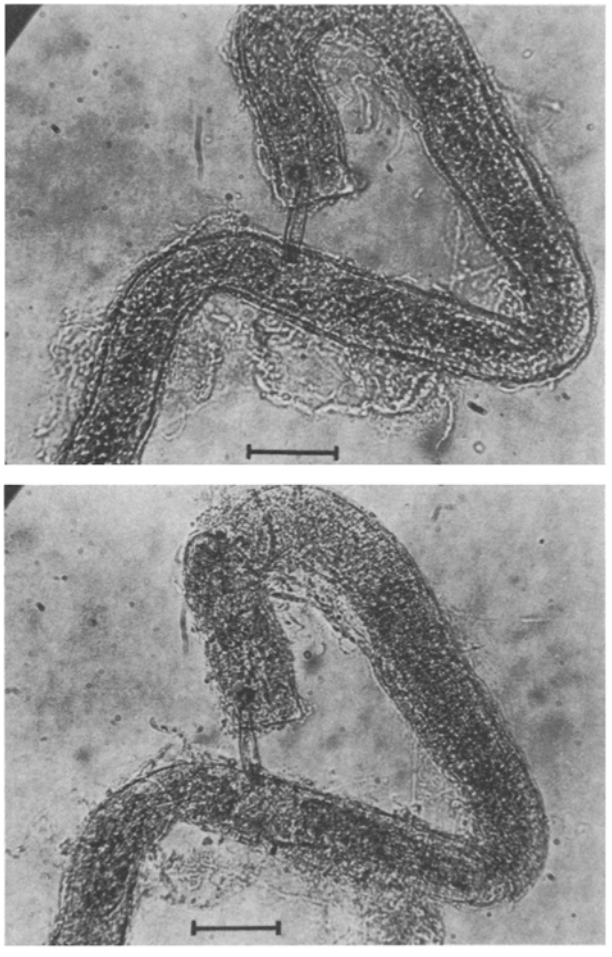

Fig. 3. *A* Before stimulation. *B* After stimulation with 10^{-4} M phenylephrine. Note the absence of the tubular contraction.

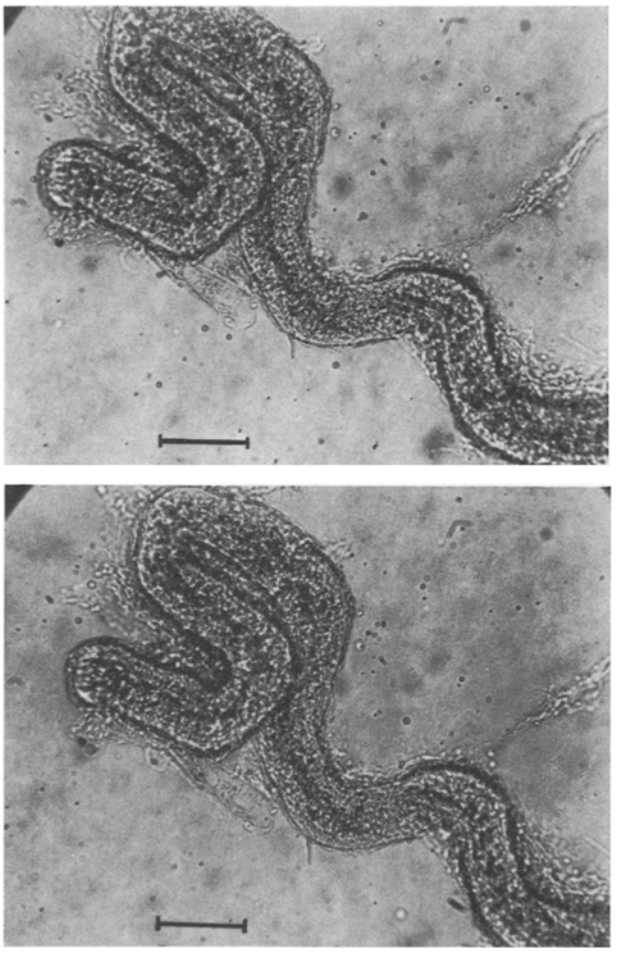

Fig. 4. *A* Before stimulation. *B* After stimulation with 5×10^{-6} M isoproterenol. Note the absence of tubular contraction.

thin cover glass. The isolated segment of the secretory coil (60 µm in tubular diameter) was placed in the slit of the chamber. The warmed KRB was continuously infused from one open end of the slit, and the overflowed KRB was removed from the other end by continuous suction. Polaroid photomicrographs were taken at regular intervals before and after the addition of drugs to the perfusate.

Results and discussion. The table summarizes the mean ± SEM secretory response to various sudorific agents. Note that both Mecholyl and A23187 gave similarly high sweat rates. The possible mechanism of the effect of A23187 will be elaborated in a separate publication[4]; however, briefly we assume that the observation might indicate the importance of the role of Ca^{++}-influx into the cell in triggering the secretory process in the secretory cell. Both adrenergic α- and β-agonists induced sweat secretion in vitro but to a much smaller extent than did Mecholyl (or acetylcholine) and A23187. Figure 1A is a segment of the secretory coil at rest, and figure 1B is the same segment during stimulation with acetylcholine (ACH). The bifurcation near closed end of the coil is a rare anomaly, but it was a helpful landmark of the secretory coil. The lumen of the secretory coil was nearly collapsed before stimulation. When ACH was added to the medium, the secretory coil immediately began to contract to $^2/_3$ to $^3/_4$ of the original length (figure 1B). A few sec were required for the contraction to reach its peak, but thereafter the coil remained in the contracted state as long as ACH was present in the bath. The lumen of the secretory coil dilated only slightly during stimulation, but usually it did not exceed 10 µm. The tubular contraction was found to be completely reversible either by washout of the medium or by the addition of atropine. The Ca^{++} ionophore, A23187, another strong stimulant of sweat secretion, was used at a high concentration, 2×10^{-4} M, to induce a rapid sudorific response[4]. No contraction was observed (figure 2A, B) but the subsequent addition of ACH caused forcible contraction of the tubule (figure 2C). Similar photographic analyses were performed for α- and β-adrenergic agents, which showed no tubular contraction (figures 3A, B, 4A, B). Since the tubular contraction is most likely due to myoepithelial contraction, it can be concluded that: a) the myoepithelial cell responds only to cholinergic stimulation; b) myoepithelial contraction is not a prerequisite for inducing or maintaining sweat secretion in vitro and perhaps also in vivo; c) the amount of 'preformed sweat' is so small before stimulation that the initial myoepithelial contraction may not expulse a significant amount of sweat.

5 K. Sato, Am. J. Physiol. *225*, 1147 (1973).