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PATTERN OF SWEAT GLAND ACTIVITY ON THE FOREARM AFTER PHARMACOLOGIC STIMULATION

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Abstract. The pattern of sweat ducts on the forearm after injection with acetylcholine or atropine has been studied from photographic enlargements. Sweating was indicated by bromphenol-blue powder or by the impression of sweat drops in a plastic material which was applied to the skin. Most sweat ducts appeared to be located at the crosspoints of skin folds, though some spiralizing ducts ended in an unfolded area of the horny layer. Sweating was markedly inhibited by skin tension. It is suggested that the pliability of the folds of forearm skin and the presence of the sweat duct openings at their intersections cooperate in the maintenance of a well functioning skin.

Over the body surface the skin is furrowed in a highly specific pattern of folds and grooves. The skin of the grooves and the skin outside the grooves do not necessarily have the same composition. The more pliable horny layer of the skin of the grooves may be expected to have a higher water content than the horny layer of skin which does not have to fold when the skin is stretched and relaxed during normal use. Other factors may also influence the variable character of the skin folds and the adjacent skin.

The present investigation seeks to elicit information about the distribution pattern of sweat duct openings on the skin of the forearm. The patterns of grooves and sweat ducts are thought to be genetically determined (6). Yet, very little is known about the factors that generate these patterns. Kuno (5) states: "In the palm of the hand and the sole of the foot, the sweat pores open along the cutaneous ridges so that they take a linear arrangement", and: "On the general body surface, the sweat glands are scattered and no particular system can be found in their distribution." The number of sweat glands per cm² forearm skin is thought to be about 200, but figures deviating markedly from this have also been published (5). A new technique for visualizing grooves and sweat ducts has been developed during recent years (4, 7, 8). This technique has proved very useful for a variety of investigations, and we report here a quantitative study of the location of sweat ducts on the volar aspect of forearm skin.

MATERIAL AND METHODS

Sweating was stimulated locally on the volar aspect of the forearm of a healthy 51-year-old volunteer during the winter. In some experiments 0.02 ml 0.01% acetylcholine was injected; in other experiments 0.1 ml 0.001% atropine was injected.

Visualization by bromphenol-blue

Bromphenol blue powder was lightly dusted upon the skin. The composition of the powder was bromphenol-blue [corn starch] gum tragacanth in the ratios 1 : 8 : 8. This powder turns blue in the presence of liquid at pHs above 4.6 (3). Some minutes later, when the colour had developed sufficiently, a colour photograph was taken of the investigated skin area. The photograph was enlarged × 5.5.

Visualization by impressions in a replica

The skin was injected with the relevant solution and 30 sec later a plastic cast was made. An elastic rubber base impression material (KERR Light Bodied Permlastic) was prepared by mixing equal parts of base material and catalyst; this was spread over about 20 cm² skin. The material was allowed to harden on the skin for 5–10 min and was then carefully removed with forceps. The impression method has been described by others (1, 2, 4, 7, 8). A photograph was taken of the replica, which was illuminated in such a way that the folds and the impressions of the sweat drops were clearly visible. An area of 3 × 4 cm² around the injection site was enlarged × 5.5.

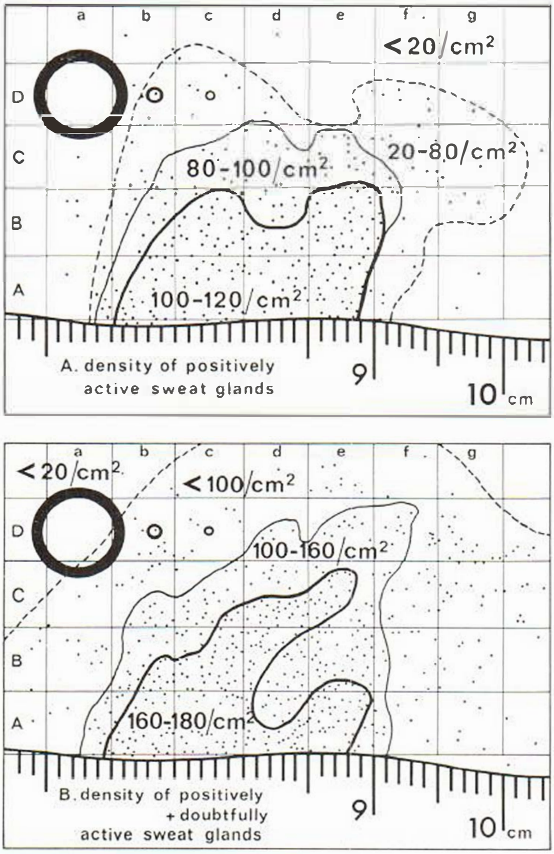

Fig. 1. Distribution of clearly active sweat glands (A) and of all possibly active sweat glands (B) in a photograph of forearm skin after injection of acetylcholine (bromphenol-blue powder). The densities of maximum and sub-maximum activity are also indicated.

Production of distribution maps from the photographs

The enlarged photograph was covered with a transparent plastic film. An area representing 3×4 cm² skin was divided into squares of 0.5×0.5 cm². The number of sweat glands inside each 0.25 cm² was marked on the transparency and counted.

In the bromphenol-blue photograph several sweat glands gave clear blue points; they produced sufficient sweat to cause a clear reaction of the indicator. Besides these there are also less clear points, which may have been produced by less productive sweat glands. Both sorts of reaction have been counted separately. The distinction between them is somewhat arbitrary.

In the photograph of the replica a distinction has also been made between active and less active sweat glands. These differences can be seen in Fig. 3. The apparent photographic difference between bubbles of about 1 nl and larger and bubbles smaller than 1 nl has been interpreted as a difference between "active" sweat glands and "partially-active" or "non-active" sweat glands; they have been counted separately. The heavy dots in Figs. 4, 5, and 6 represent the sites of "large pits" and small dots represent "small pits". Based on the results of a photograph of non-injected skin, an area of less than 20 "large pits" per cm² stimulated skin has been considered to represent the area of non-, or submaximally, stimulated sweat glands. An area with a density of 20 large pits or more has been considered as an area of sweat glands actively reacting to the injection. This frontier has been drawn. Based on these results, an area of less than 100 pits ("all pits") per cm² has been considered to be an area in which no reaction whatsoever has occurred following the injection. Similarly an area of less than 92 "small pits" per cm² (mean of 61 + S.D.) has been considered to be an area in which no reaction whatsoever has occurred. It is supposed that the application of the impression material upon the skin will have produced these pits artificially, and that their presence cannot be considered to be of significance for the investigation.

Next, the *epicentre* of the activity was determined and outlined. The outline of the epicentre was chosen arbitrarily to be about 10–20% less than the maximum density. Following acetylcholine injection it is about 2–3 cm²; following atropine injection the epicentre may be smaller.

The area of activity of the sweat glands has been taken as more extensive than the epicentre of maximum activity. Arbitrarily the outline of the *area of activity* has been drawn at a density half-way between the maximum density and the density which surrounded the area of no-reaction; e.g. in Fig. 5 B at 160/cm², this being half-way between 220 and 100/cm². The area of activity has not been outlined when it nearly coincided with the area of non-reaction (Fig. 4 A) or the epicentre (Fig. 5 A). The area of activity could not be outlined in this way when an epicentre was absent (Fig. 5 D). We have assumed that an outline at a density of 184/cm² in the investigated forearm skin (twice that of the frontier of the no-reaction area) will certainly delineate an area of increased activity. Such an area of increased activity has been outlined by a heavy line.

Density of intersections of skin folds

Photographs of casts of volar forearm skin under different conditions were investigated. The marking and counting of intersections of folds on a transparency is somewhat arbitrary. No difference between clearly-seen and not-clearly-seen intersections has been made as this difference was found to be very small in a preliminary investigation.

RESULTS

Visualization by bromphenol-blue powder

Bromphenol-blue positive sweat gland activity after injection of acetylcholine is shown in Fig. 1. In the epicentre of activity are 100–120 clearly active sweat glands and about 60 small or doubtful blue points per cm². The density of sweat

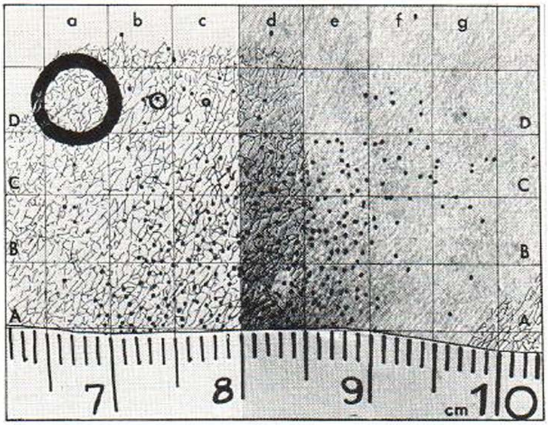

Fig. 2. The clearly active sweat glands are indicated by dots on a transparency laid over a photograph of forearm skin which had been stimulated by acetylcholine injection and whose sweat drops were visualized by bromphenol-blue powder. Injection in the area *A,d*. The pattern of skin folds has been drawn on the transparency in the squares underneath *a, b, c,* and *d*.

glands estimated by direct visualization of this skin surface by means of a magnifying glass was only $80/cm^2$. The density of the clearly active sweat glands (Fig. 1 A) decreases with distance from the epicentre. The density of the "doubtful" blue points also decreases in that direction (Fig. 1 B).

The bromphenol-blue powder accumulated especially in the small skin folds and thereby accentuated the pattern of these folds (Fig. 2 left). These folds were again drawn on a transparency; see the right hand side and the centre (*ABCD, d*) of Fig. 2. Most of the sweat glands are seen to be located near or at the intersections of the skin folds.

Visualization by the impression technique

In Fig. 3 a photograph of a part of a replica is presented. Light reflections in the pits demonstrate that they are bubbles imprinted in the replica. In this part of the replica four large bubbles ($\phi = 0.14$–0.23 mm), a small bubble ($\phi = 0.05$ mm), and a pair of smaller bubbles ($\phi = 0.04$ mm) are present. The volumes are quite different; large bubbles: 1.3–3.2 nl, the single small bubble: 0.06 nl, and each of the pair of small bubbles: 0.02 nl (1 nl is equivalent to about 1 μg sweat). The right hand one of the pair of small bubbles is situated at the end of the last convolution of the spiral sweat duct inside the black circle. The volume of this convolution (length 0.4 mm, diameter 0.015 mm) is roughly 0.06 nl. Although the pair of bubbles may indicate that only a part of the contents (air or liquid) of this spiral convolution came out of the duct during the preparation of the replica, they show the presence of a duct, and were counted as "small pits".

The results of three different experiments are shown in Figs. 4, 5, and 6. In Fig. 4 the skin was injected with acetylcholine, the arm was immediately bent, and the skin tension released. In Fig. 5 the skin was also injected with acetylcholine, but the arm was immediately stretched, and the skin tension increased. The densities of

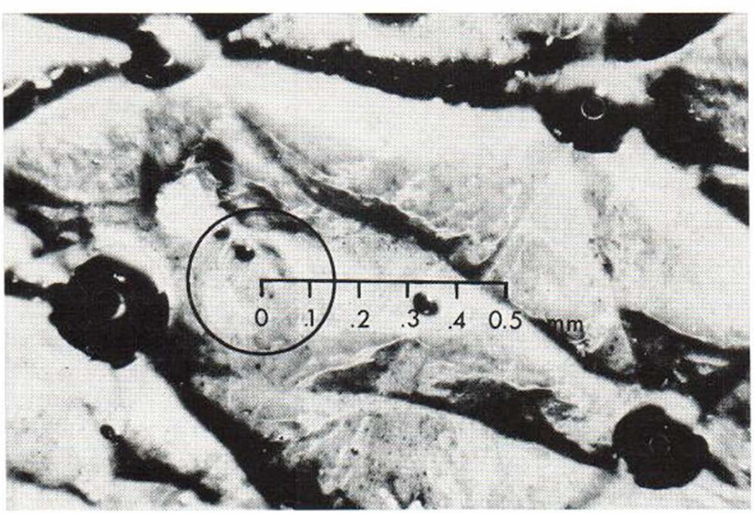

Fig. 3. Part of a replica of volar forearm skin after injection of acetylcholine. Four large bubbles have been formed by sweat drops of 1.5–3 nl volume, and are reflecting light. Inside the black ring a small bubble can be seen at the end of the spiral sweat duct; another bubble being present nearby at the inside of the convolution. Their volumes represent only 0.02 nl.

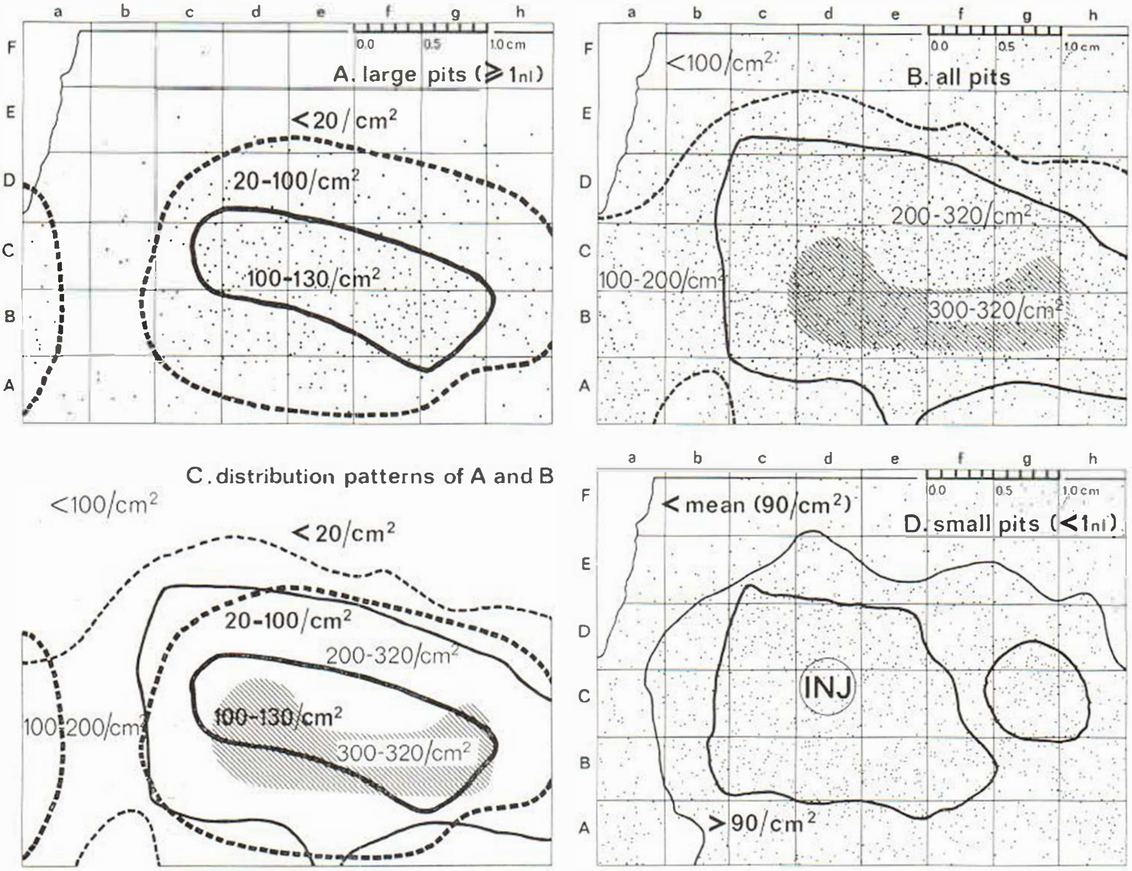

Fig. 4. Presentation of the occurrence of large pits (heavy dots in the figures) and small pits (small dots) in a photograph of a replica of volar forearm skin after sweat stimulation by injection of acetylcholine in the area *C, d.* The skin tension had been reduced as much as was possible. (A) shows only large pits (sweat glands at maximum activity). (D) shows only small pits (sweat glands at sub-maximum or without activity). (B) shows all pits. In (A) and (B) the epicentre of maximum activity has been indicated. Areas of sweat gland activity have been outlined (20–100/cm^2 in (A) and 200–320/cm^2 in (B)) and the areas of non-activity outlined (< 20/cm^2 in (A) and < 100/cm^2 in (B)). These patterns of (A) and (B) are superimposed in (C). The area of non-activity of small pits (submaximal active sweat glands) is indicated by "< mean (90/cm^2)" in (D). The heavy line in this figure outlines the density of more than 184/cm^2, and represents an area of sweat gland activity.

sweat impressions are clearly lower than in Fig. 4. In Fig. 6 atropine was injected and the impression taken under normal tension.

Density of intersections of skin folds

On two replicas of skin under normal tension, 424 and 422 intersections of skin folds per cm^2 were counted. The replica of stretched skin (under extra tension), from which Fig. 5 was taken, yielded 388 such intersections per cm^2. The replica of unstretched skin (without extra tension, bending the arm as far as possible), from which Fig. 4 was taken, yielded 456 intersections per cm^2. The number of intersections for each 0.25 cm^2 of a photograph was fairly constant; its relative standard deviation being 2%.

By stretching and relaxing the skin, the surface area was found to be increased by stretching, and decreased by relaxing by about 10% in each case. The corresponding difference in the densities of the intersections of folds was 8%. Therefore the density of intersections of skin folds of the forearm can be taken as about 420/cm^2.

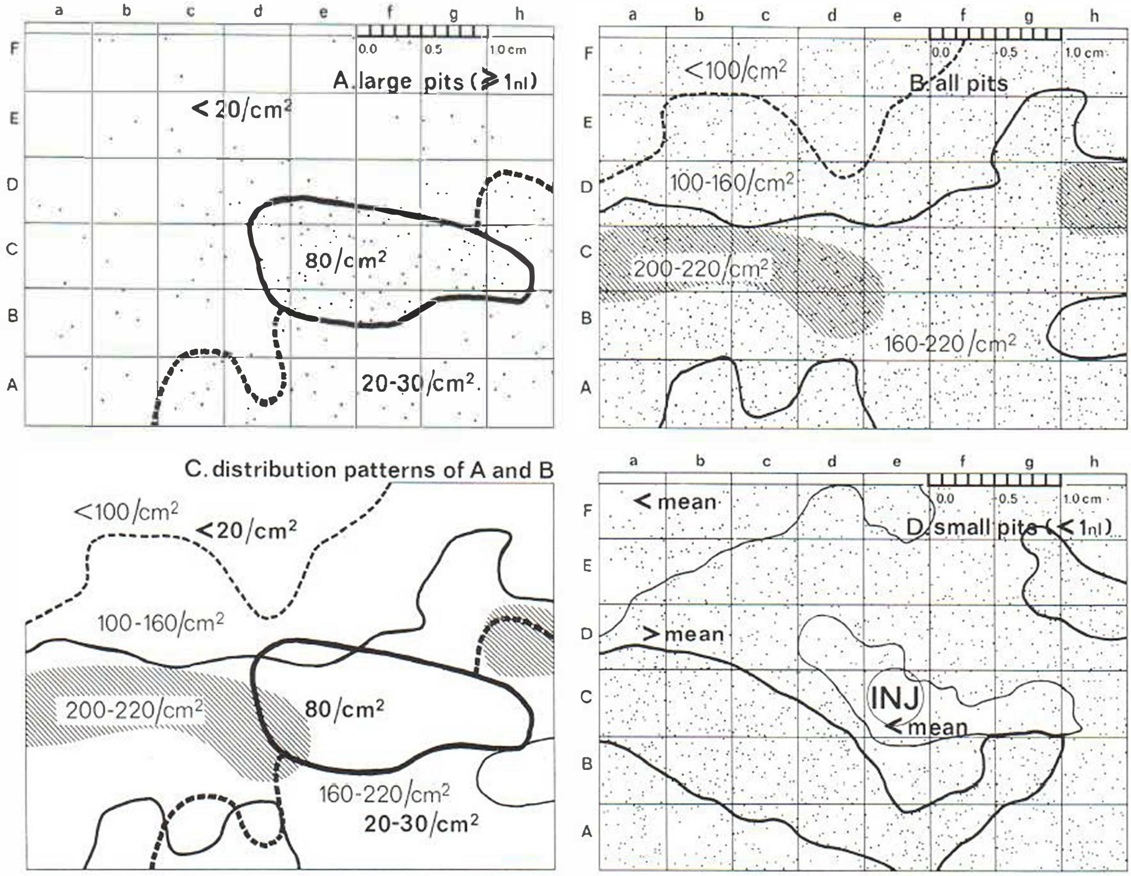

Fig. 5. Identical presentation as in Fig. 4. In this experiment the skin was given as much tension as could be produced by hand. The epicentre in (B) is split in two areas. Small pits do not occur in the epicentre of maximum activity (area of 80/cm² in (A) and "< mean" in (D) coincide).

DISCUSSION AND CONCLUSIONS

Both techniques require a certain minimal amount of water, but the impression technique is more sensitive although less specific. Conclusions have therefore been drawn from the results of both techniques together.

The principal shortcoming of the impression technique is the significant background density of large and small pits, but mostly the density of small pits, due to mechanical artifacts and the presence of hairs. Furthermore, the photographs do not reveal all pits in the Permlastic material. Some pits are covered by a horny layer scale from the skin. Removal of the scales was not attempted.

The occurrence of large pits following sweat stimulation is influenced markedly by the choice of the hardening period, and thus by the choice of the impression material. For the material used the following calculation is relevant. The investigated skin produced 0.2 mg water per cm² and per minute after injection of acetylcholine; so on average each sweat gland produced about 1 nl sweat if 100 sweat glands per cm² were active. Thus under our circumstances the Permlastic impression material hardens at such a rate that the sweat secretion of active sweat glands is stopped after about a minute. Of course the exact length of time cannot be predicted, being dependent on many circumstances (e.g. mixing preparation time of the material, humidity at the skin surface and during the experiment, etc.). Yet it suffices for a distinction to be made between sweat glands which are active during the whole period of that minute, and others which are not. This is especially obvious from the re-

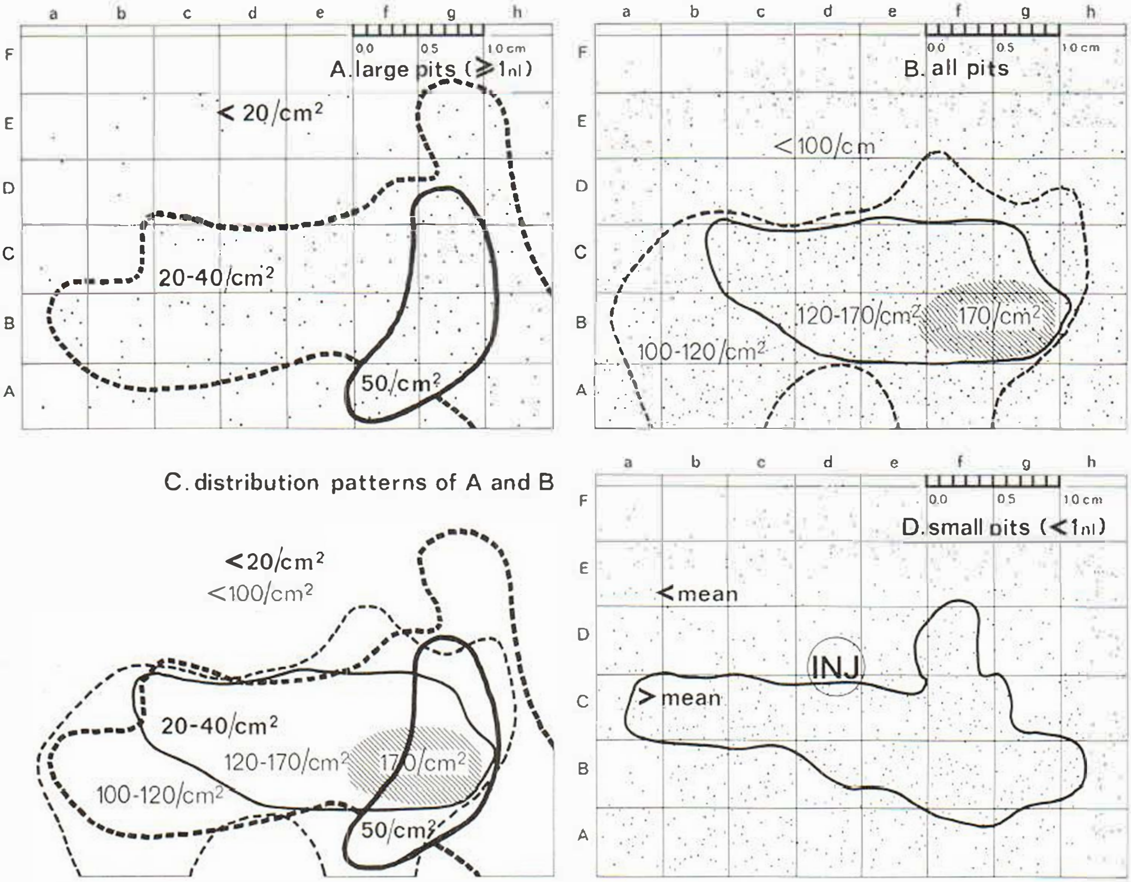

Fig. 6. Identical presentation as in Fig. 4. In this experiment atropine was injected in the area *C–D, d*. The epicentre of maximum activity is found to be displaced from the site of the injection.

sults of Fig. 6. In this respect the method differs from previously published methods (5, 8).

The densities of fully-active sweat glands *in* the epicentre of activity around the acetylcholine injection site (in Figs. 4 A and 5 A respectively at *C,d* and *C,e*) do not differ appreciably in various experiments with the same forearm skin but a correction must be made for tension (see captions to Figs. 4 and 5). The effects of tension can be seen both in and around the epicentres of activity (Figs. 4 and 5).

According to Fig. 5 D the skin tension inhibited the delivery of sweat around the injection site completely, unless the discharge of sweat was fully active during the whole period of the investigation; small pits representing partially-active sweat delivery were not found in the epicentre of large pits (compare with Fig. 5 A). Finally the resulting area of activity of the sweat glands appears to be about the same in skin with and without tension; (see Fig. 4 B and 5 B, areas of more than 100/cm^2.) The conclusion therefore is that skin tension will reduce the activity of sweat secretion. The same observation from different experiments has been reported by Warndorff (9).

Following atropine injection the epicentre of activity is found to be displaced from the site of injection (*C-D,d*), especially for the large pits (Fig. 6 A). It can be assumed that the sweat glands nearer to the injection site were already effectively discharged before the minute of the investigation started and that the more remote sweat glands had not yet started to discharge sweat at the beginning of the investigation. As a result, an increased density of small pits has been found at both sides of the epicentre of large pits.

There were more intersections of skin folds than pits following acetylcholine injections. Thus not every intersection of folds correlates with a pit though from the photographs of the replicas the reverse appeared to be very nearly true. Sweat duct openings on forearm skin are arranged in lines, coinciding with skin folds (1). The same is obvious from a bromphenol-blue print (Fig. 2). As the number of sweat glands on forearm skin is at least $200/cm^2$ [see also Kuno (5)], and the number of intersections of folds is roughly twice the number of sweat ducts, some connection may be supposed. Another commonly known fact is that a dry skin is not flexible and supple. The combination of these facts leads to the following supposition.

The presence of the sweat duct openings at the intersections of skin folds in the forearm helps in the maintenance of the suppleness of the skin of the folds, especially in winter and in other circumstances when the amount of sweat is low, and that the sweat will flow only through the folds. As a result, the permeation of water through the horny layer of the folds will be increased compared with the horny layer area outside the folds. The difference between both permeabilities can be supposed to be more significant in wintertime and in "dry skin" conditions when the upper horny layer outside the folds is dry and scaling and will not allow water to be transported outwards. The "dry skin" appearance will presumably disappear in summertime because of the raised activity of the sweat glands which will also moisten the skin outside the folds.

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