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EXCITATION AND DEPRESSION OF ECCRINE SWEAT GLANDS BY ACETYLCHOLINE, ACETYL-β-METHYLCHOLINE AND ADRENALINE

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Acetylcholine is generally accepted as the neurohumoral transmitter to the eccrine sweat glands (Dale & Feldberg, 1934; Chalmers & Keele, 1952). The response of the sweat glands to intradermal injections of acetylcholine and related compounds has been the subject of numerous studies (for a review see Randall & Kimura, 1955) but it has only recently become apparent that the glands are not necessarily excited, and may in fact be depressed, by large or repeated doses of these drugs (Collins, Sargent & Weiner, 1958). No systematic investigation of the effects of these substances in different concentrations has hitherto been made. The finding that the excitability of the glands depends on the doses used may have some bearing on the study of those conditions in which the sweat glands cease to function, e.g. after prolonged sweating, in heat stroke and in dehydration, or when, as in acclimatization, the glands become more responsive.

Thaysen & Schwartz (1955) found that repeated intradermal injections of methacholine (acetyl-β-methylcholine) resulted in a gradually decreasing output of sweat until the glands became refractory to further stimulation. The decrease in sweating was ascribed to exhaustion of the gland cells and this was thought to resemble the natural sweat-gland 'fatigue' which ensues after prolonged exposure to heat stresses near the limits of tolerance (Gerking & Robinson, 1946). Thaysen & Schwartz (1955) found that thermally fatigued glands, like methacholine-treated glands, failed to respond to methacholine. In heat-induced fatigue the decline in sweat rate is associated with a sustained or rising skin temperature and a rise in salt output of the sweat (Robinson & Robinson, 1954). Dole, Stall & Schwartz (1951), however, failed to show any increase in the sodium concentration of sweat collected during repeated stimulation by methacholine when the rate of flow decreased. Some evidence

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has already been brought forward (Collins *et al.* 1958) that the depression by high concentrations of drugs does not involve prolonged activity of the sweat glands. Thus the analogy with thermally induced fatigue might not be exact.

Alternative explanations can be advanced to account for the depression by drugs, and these are considered in this paper.

(1) It is possible that the decline in the number of active glands is the result of physical damage by repeated injections.

(2) It is possible that large amounts of acetylcholine or methacholine may indirectly influence sweat gland activity by a local vasoconstriction, such as has been found in the salivary gland (Graham & Stavraky, 1953; Dirnhuber & Lovatt Evans, 1954). The vasoconstriction produced by excess acetylcholine may, in turn, be due to the release of an adrenaline-like substance from, for example, blood vessel walls (Kottegoda, 1953; Burn & Rand, 1958). Accordingly, the action of adrenaline injected alone and in combination with acetylcholine has been examined.

(3) It is also possible that when high concentrations of sudorific drugs are maintained by repeated injections, the sweat glands become accommodated to the action of the drug. This may be analagous to the 'densensitization' produced by acetylcholine at the motor end-plate (Thesleff, 1955; Katz & Thesleff, 1957).

In addition, studies have been made of the recovery from drug-induced depression. Sonnenschein, Kobrin, Janowitz & Grossman (1951) reported that following sweat-gland activity induced by intradermal injections of adrenaline a local inhibition of sweating developed which sometimes lasted for more than 24 hr. They noted a similar inhibition in six out of ten trials with small doses of acetylcholine. In Kuno's (1956) view this phenomenon is peculiarly associated with the direct action of these drugs on sweat glands. As little is known about a depression developing or persisting for days after injection, we sought for evidence of it in the course of these experiments.

METHODS

Sweat responses were investigated on the flexor surfaces of the forearms of four male subjects. Sweat-gland counts were made on the proximal two-thirds of the forearm, unless specified otherwise. At the laboratory temperature in which the experiments were conducted ($22 \pm 3°$ C) there was no spontaneous sweating on the forearm.

A standard procedure was adopted to test the activity and number of sweat glands evoked by thermal stimulation. The lower legs were immersed in a water-bath at 43–45° C for a given period, usually 1 hr. To test the responsiveness of the glands at intervals up to 7 days and more, two methods of stimulation were used. In one, indirect heating for 1 hr was employed and during the last 30 min, when sweating was profuse, prints were taken at set times from an exactly delimited area. The same area could then be tested in the same way on subsequent occasions. In the second method the chosen area was tested by injecting a small dose of methacholine (10 or 20 μg in 0·05 ml. saline) on each occasion.

Intradermal injections were given by 0·5–1·0 ml. tuberculin or Agla micrometer syringes fitted with 28-gauge $\frac{1}{2}$ in. (12 mm) needles; the tip of the needle usually penetrated about 2–3 mm into

the dermis. Drugs were injected in 0·05–0·5 ml. sterile normal saline (NaCl 0·9% (w/v)), each injection taking roughly 20–30 sec. Control injections of normal saline were frequently made throughout the experiments. The principle sudorific agents employed were acetylcholine chloride and methacholine chloride (Savory & Moore, acetyl-β-methylcholine chloride). Other drugs used during the course of these experiments were atropine sulphate, adrenaline chloride, procaine hydrochloride, histamine acid phosphate, tolazoline hydrochloride, hyaluronidase and neostigmine methylsulphate. All solutions for injection were prepared freshly for each experiment with aseptic precautions.

In some experiments three or more areas on the same forearm were investigated simultaneously. Diffusion of the drug into adjacent areas along cutaneous lymphatic vessels was prevented by adjusting $\frac{1}{4}$ in. (6 mm) rubber bands round the limb, according to the procedure recommended by Wada, Arai, Takagaki & Nakagawa (1952).

Active sweat glands were enumerated on shaved areas of the skin by the plastic-impression method of Thomson & Sutarman (1953). The plastic solution was prepared from 5 g polyvinyl formal and 1 ml. butyl phthalate made up to 100 ml. with ethylene dichloride. The viscosity was measured by a microviscosimeter and adjusted to 40–60 centistokes before use. Skin areas from which sweat-gland prints were taken were carefully blotted dry before each application of the plastic. The plastic was painted on the skin by a sable brush (No. 12, Winsor & Newton). Rapid drying (within 10–15 sec) was facilitated by a current of warm air. The dried plastic was removed from the skin by $\frac{3}{4}$ in. (19 mm) wide strips of Selloproof or Vinyl No. 12 transparent adhesive tape (Adhesive Tapes Ltd) and mounted on glass slides. A ball-point pen was found to be convenient for marking the skin before each print. A clear and reproducible identification mark could in this way be obtained on both the skin surface and the plastic film. The surface of the skin was unaffected by applications of the plastic at 3–10 min intervals during 1–3 hr. A local irritation and erythema sometimes occurred in experiments prolonged beyond 3 hr, particularly when the forearm was sweating profusely as in indirect heating experiments. Consecutive prints of the sweating response were examined by photometric projection (magnification $\times 40$). By orientating the slides in relation to skin markings and the sebaceous glands it was possible to identify the site of individual glands with accuracy and to follow the activity of each gland throughout an experiment. The numbers of active glands per square centimetre were tabulated by dividing the image on the screen into four areas, each representing 0·25 cm^2 (see Fig. 2).

The actual volume of sweat secreted was not measured. The relative output of individual glands was however assessed from the size of the perforations in the sweat-gland prints. Thomson & Sutarman (1953) showed that for rates of sweating below 3·8 g/100 cm^2/hr the size of the holes in the plastic impression was directly related to the quantity of sweat produced. Preliminary experiments in which sweat rate was measured by the capsule method of Dole *et al.* (1951) indicated that this relationship was probably true for sweating up to 7 g/100 cm^2/hr induced by indirect heating. Skin temperatures were measured in certain experiments by a thermocouple and potentiometer.

RESULTS

Sweat-gland responses to indirect heating

A typical forearm sweat response expressed in terms of the number of active glands produced by indirect heating is shown in Fig. 1*a*. Sweating was usually first detected 5–15 min after immersing the legs in the bath. There were differences between the subjects both in the latent period and in the time taken for attaining the maximum number of active glands. The maximum numbers of active sweat glands for each subject are recorded in Table 1. The density of sweat glands in Subject IV, who rarely exhibited more than 80 active glands/cm^2, was less than in the other three subjects, but the output

per gland as measured by the area of the perforations in the sweat prints was larger. In three subjects counts on the same region of each arm were similar. The counts on the left arm of Subject II, however, usually exceeded those on the right. In all subjects the density of active glands at the wrist was about 30% higher than on the upper region of the forearm. There were considerable differences in the activity of individual glands. Glands which first showed

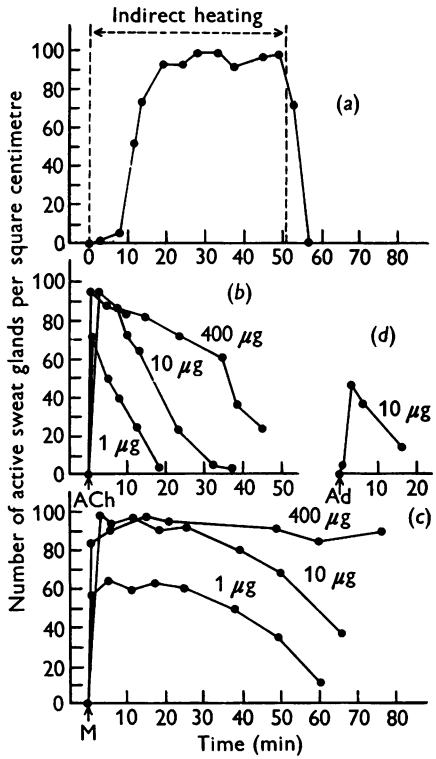

Fig. 1. Number of active sweat glands per square centimetre on the proximal two-thirds of the forearm (Subject I) during (*a*) heating the lower legs in a water-bath at 44° C, indirect heating; and after intradermal injections of (*b*) 1 μg, 10 μg and 400 μg ACh, (*c*) methacholine (M) 1 μg, 10 μg and 400 μg and (*d*) adrenaline (Ad) 10 μg.

activity tended to be among the most active throughout heating. As heating progressed both the number of active glands and the output per gland rapidly increased. When the legs were removed from the hot water-bath after 1 hr, sweating ceased within a few minutes. During the period of heating the oral temperature increased by approximately 1° C, with very little change in forearm skin temperature.

Sweating induced by indirect heating was completely suppressed in a small area of the forearm for at least 20 min by a single intracutaneous injection of

0·5 ml. procaine 1% (w/v). Injection of a test dose of acetylcholine into the procainized area elicited a vigorous sweat response.

In hot room experiments at 40° C dry bulb, 30° C wet bulb, sweating was absent in the injection area for more than 30 min after injecting 1 μg atropine/0·1 ml. Four hours after administration of 8 μg atropine/0·1 ml. in a cool environment (22° C), glands in the injection area remained unresponsive to indirect heating.

TABLE 1. The maximum number of sweat glands per square centimetre activated by indirect heating and by intradermal injections of sudorific drugs in the flexor surface of the forearm. All figures represent mean maximum values with standard errors; the number of determinations is given in parenthesis

	Subject I		Subject II		Subject III		Subject IV	
	L. arm	R. arm	L. arm	R. arm	L. arm	R. arm	L. arm	R. arm
Indirect heating	100±3 (11)	105± 7 (16)	101± 5 (5)	91± 8 (8)	108± 7 (7)	111±10 (4)	78±1 (3)	80± 2 (4)
(wrist)	129±6 (3)	125± 3 (2)	139±10 (3)	129± 8 (2)	145±10 (4)	157±13 (3)	88±3 (2)	—
Acetylcholine, 10 μg/0·05 ml.	93±3 (5)	106±12 (2)	80± 1 (2)	68±10 (4)	95 (1)	110± 8 (2)	77±4 (5)	73± 4 (4)
200 μg/0·05 ml.	95±4 (5)	88 (1)	92± 8 (3)	76± 9 (2)	106± 3 (9)	94± 7 (4)	73±4 (12)	72± 3 (7)
Methacholine 10 μg/0·05 ml.	98±3 (8)	103± 5 (5)	92±10 (4)	64± 2 (2)	109±7 (5)	101± 1 (5)	71±2 (3)	71± 4 (2)
200 μg/0·05 ml.	100±1 (2)	99± 7 (5)	97± 2 (3)	77± 3 (3)	106±13 (2)	110± 7 (5)	79±4 (3)	67± 4 (7)
Adrenaline 10 μg/0·05 ml.	47 (1)	28± 2 (2)	22± 9 (2)	45±11 (5)	88± 0 (2)	75±12 (4)	28 (1)	49±11 (2)

Sweat gland responses to single intradermal injections

Acetylcholine (ACh) in concentrations from 1 μg/0·05 ml. to 5 mg/0·05 ml. (2×10^{-5} to 10^{-1}) was injected during the course of these experiments. Threshold values, i.e. the smallest doses evoking sweat gland activity, were less than 1 μg/0·05 ml. for all subjects. Active sweat glands were confined to areas of vasodilatation in the injection wheal (1–2 cm in diameter) and along the course of cutaneous lymphatic vessels. No clear evidence of axon reflex sweating (Coon & Rothman, 1941) was obtained. A few active sweat glands 1–2 cm from the perimeter of the injection wheal were occasionally observed within 1 min of the injection, but this activity could be attributed to rapid diffusion of the drug. On the other hand, local pilomotor responses, which have also been imputed to axon reflex stimulation (Rothman & Coon, 1940), were regularly evoked with concentrations of 5–500 μg/0·05 ml.

The plastic impression method is not suitable for accurate measurement of the latent period of response to intradermal injections of ACh, which is reported to be between 5 and 30 sec (Coon & Rothman, 1941; Chalmers, 1950). Prints taken 15 sec after injections of from 10 μg to 1 mg ACh/0·05 ml. showed some active sweat glands, but doses less than 10 μg ACh/0·05 ml.

did not always evoke a response within that time. This suggests more rapid diffusion by higher concentrations of the drugs to sweat-gland receptor sites. Response curves for glands in the square centimetre contiguous to the site of injection are illustrated in Fig. 1*b*. As the dose increases from 1 to 400 μg/0·05 ml. the glands remain active for a longer period. A study of the prints showed that with these doses the response was uniform over the square centimetre. Those glands proximal to the site of injection remained active longest. Concentrations less than 10 μg/0·05 ml. did not usually stimulate all functional glands in the square centimetre. The output per gland declined before the number of active glands decreased.

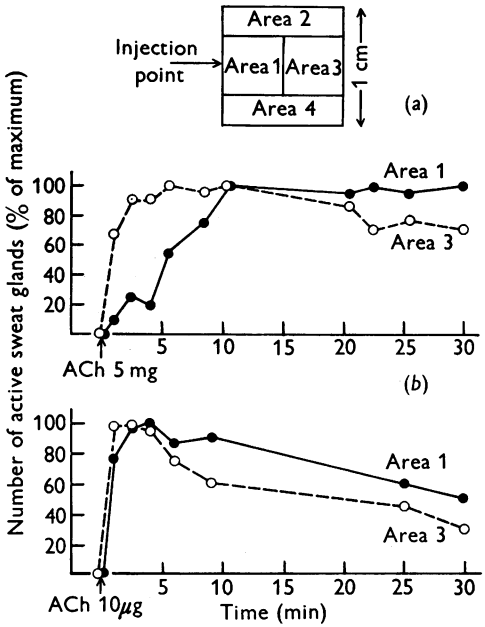

Fig. 2. Percentage of the maximum number of active sweat glands in Areas 1, ●, and 3, ○, (each 0·25 cm²) evoked by an intradermal injection of (*a*) 5 mg ACh in 0·05 ml. and (*b*) 10 μg ACh in 0·05 ml. Active glands were enumerated in four areas, each of 0·25 cm², in 1 cm² contiguous to the point of injection (see diagram). Subject II.

With very high concentrations of ACh, 2–5 mg/0·05 ml., a different pattern of response was observed in the injection wheal (Fig. 2*a*). In three out of the four subjects, many of the glands immediately adjacent to the injection point (Area 1) did not respond to the drug in these concentrations until 4–10 min after injection. The corresponding area distal to the site of injection near the edge of the wheal (Area 3) responded within 2 min. There was no inhibition in Area 1 after injections of equivalent volumes of less concentrated solutions of acetylcholine (e.g. 10 μg, Fig. 2*b*).

Acetyl-β-methylcholine (*methacholine*). Unlike acetylcholine, which is quickly

hydrolysed by cholinesterase, methacholine exhibits a prolonged local action on the sweat glands. The response (Fig. 1*c*) lasted considerably longer than that with ACh in equivalent concentrations (Fig. 1*b*). The slow destruction of methacholine renders its distribution in the cutaneous lymphatic vessels readily visible; narrow channels of vasodilatation with active sweat glands sometimes extended up to 12 cm from the injection wheal. No axon-reflex sweating or pilomotor reactions were observed with concentrations of 0·01 μg–1 mg/0·05 ml. Thresholds for the four subjects were found to be less than 0·1 μg/0·05 ml. The latent period of the response to doses smaller than 100 μg/0·05 ml. was similar to that for the same concentrations of ACh. Doses of methacholine greater than 10 μg/0·05 ml. evoked approximately the same maximum number of active glands per square centimetre as indirect heating (Table 1).

The transient depression in Area 1, previously noted when massive doses (2–5 mg/0·05 ml.) of ACh were injected, was also produced by methacholine in somewhat lower concentrations (400 μg-1 mg/0·05 ml.), but not with a first injection of 200 μg/0·05 ml. (Fig. 3).

Adrenaline. It has been firmly established that eccrine sweat glands in most human subjects can be stimulated by intradermal injections of adrenaline and related compounds (Haimovici, 1950; Sonnenschein *et al.* 1951; Chalmers & Keele, 1951). Forearm sweat glands in the four subjects of these investigations were activated by adrenaline 10 μg/0·05 ml. (Table 1), but the total number of glands stimulated was less than with acetylcholine or methacholine in the same concentration. The output of the glands was small and the response decayed rapidly (Fig. 1*d*). Three subjects were tested with 50 μg/0·05 ml. and gave a response even smaller than with 10 μg/0·05 ml. Both doses of adrenaline evoked pilomotor reactions. An intense local vasoconstriction developed 5–10 min after injection, which was at first limited to the injected area and later appeared along the course of cutaneous lymphatic vessels. Vasoconstriction in the injection area was still present 6–8 hr after an injection of adrenaline 50 μg/0·05 ml. There was a reduction in skin temperature in the vasoconstricted area of about 2–3° C relative to surrounding skin areas. If a second dose of adrenaline was injected into the adrenaline-treated area the response was considerably smaller than the first, usually less than half. The sweat response to methacholine 10 μg was not diminished by a previous injection of adrenaline 10 μg. The vasoconstricted area rapidly dilated on injecting the methacholine.

Diluting fluids. The effects of intradermal injections of diluting fluids were examined in two ways. Injections were made on the non-sweating arm in order to reveal possible sudorific actions and in other experiments during heat-induced sweating to detect inhibitory effects.

In all subjects 0·05–0·5 ml. sterile normal saline failed to stimulate sweating

nor was any inhibition of sweat-gland activity observed in the injection wheal during heating. Propylene glycol (frequently used as a vehicle in commercial preparations) was injected in concentrations of 7·5%, 15% and 30% (w/v) in normal saline. The two higher concentrations produced turgid wheals in which vasoconstriction persisted for several minutes. Local vasoconstriction in the wheal was produced by all intradermal injections, but this disappeared usually within a minute. No sweating was produced by injections of propylene glycol in saline. During indirect heating, however, 15% or 30% (w/v) propylene glycol reduced sweat-gland activity in the injection wheal for at least 1 hr.

Depression of sweat-gland activity by repeated injections

The effect of repeated injections of acetylcholine or methacholine. Single massive doses (2–5 mg/0·05 ml.) of ACh, as already noted, depressed sweat-gland activity near the injection site (Area 1) for several minutes. After two

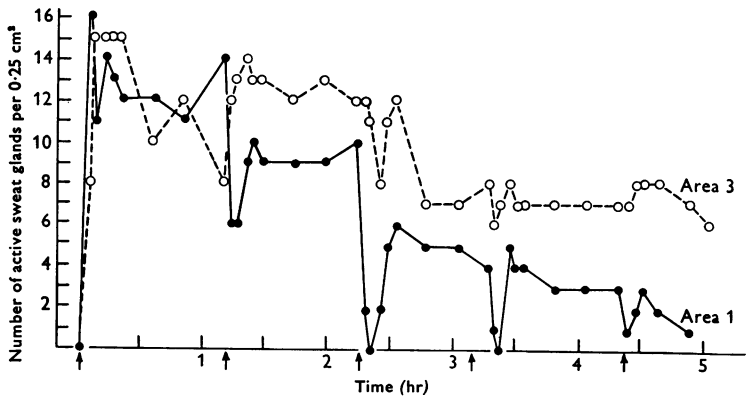

Fig. 3. Effect of five intradermal injections, at arrows, of 200 μg methacholine in 0·05 ml. at intervals of approx. 1 hr on sweat gland activity in two adjacent areas of 0·25 cm²; 1 (proximal, ●) and 3 (distal, ○) to the injection point. Subject IV.

injections of 1 mg ACh or 400 μg methacholine there was an even more pronounced depression. When methacholine 200 μg/0·05 ml. was injected repeatedly at intervals of 1 hr, there was a progressive decline in the number of active glands (Fig. 3). The first injection stimulated the same number of glands in Area 1 and Area 3. The second injection, however, immediately inhibited the activity of some glands in Area 1 but stimulated the glands in Area 3. With successive injections the number of functional glands in Area 1 was depressed more rapidly than in Area 3. The inhibition in Area 1 after each injection was followed by a partial recovery before the next injection.

Larger doses of methacholine (2 mg/0·5 ml.) injected at 60 or 100 min intervals (as in the experiments of Thaysen & Schwartz, 1955) gave comparable results. The area of depression now extended over the whole square

centimetre contiguous with the injection puncture. In the square centimetre beyond that the sweat glands were fully active.

Repeated injections of smaller doses were given (Fig. 4) for comparison with the effects of the high dose levels just described. 10 μg methacholine/0·05 ml. and 200 μg methacholine/0·05 ml. injected in opposite arms at first stimulated a similar number of sweat glands. With repeated administration, however, the small doses produced a different effect. After five injections of 10 μg at 20–30 min intervals, approximately the same number of glands as those initially stimulated were still functioning. Five injections of 200 μg, in contrast, produced a gradual depression until only 40% or less of the glands in

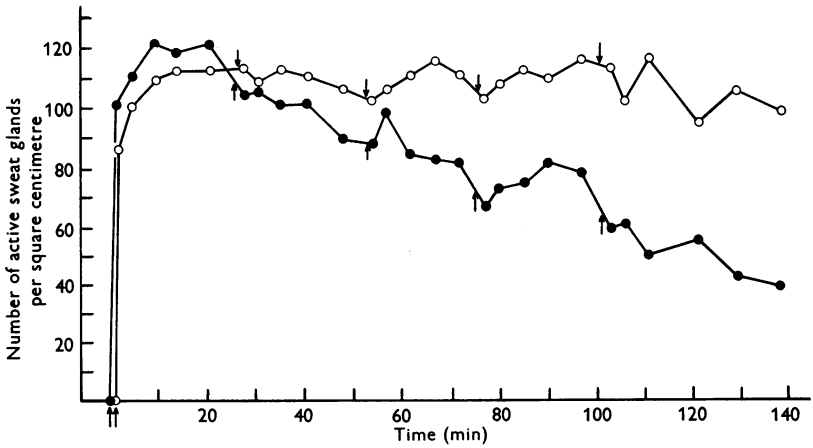

Fig. 4. Number of sweat glands per square centimetre activated in two separate areas of the forearm by repeated intradermal injections at arrows of methacholine in small doses, ○, (10 μg in 0·05 ml.) and large doses, ●, (200 μg in 0·05 ml.) at 20–30 min intervals. Subject III.

the area were active. Even after fourteen consecutive injections of 10 μg methacholine during 6½ hr, 70% of the glands in the square centimetre adjacent to the point of injection were still active. This reduction in the number of active glands took place almost entirely in Area 1, whereas in Area 3 (in contrast to the action of repeated doses of 200 μg), glands remained active throughout. Similar results were obtained with ACh, but the depression developed more slowly than with methacholine.

During drug-induced depression, the output of each active gland gradually diminished until the gland ceased to respond. The rate at which secretory activity decreased was by no means uniform for all the glands stimulated by the drug. There was a marked fall in output from glands in Area 1 before the output per gland in Area 3 decreased. In an experiment in which six injections of methacholine 400 μg/0·1 ml. were given at half-hour intervals, the activity of each gland in an area of 1 cm^2 was expressed as the sum of the pore areas

of that gland on consecutive prints. The activity of the glands was found to increase with the distance from the point of injection (Fig. 5).

Mechanical effects of repeated intradermal injections. Experiments were performed to assess the effects of mechanical traumata, such as repeatedly puncturing the skin or distending the skin with the injected fluid. Repeated administration of normal saline 0·05 ml. or 0·5 ml. did not impair the reaction of the sweat glands to subsequent treatment with ACh or methacholine. Similarly, if single doses of drugs, previously shown to produce no depression, were

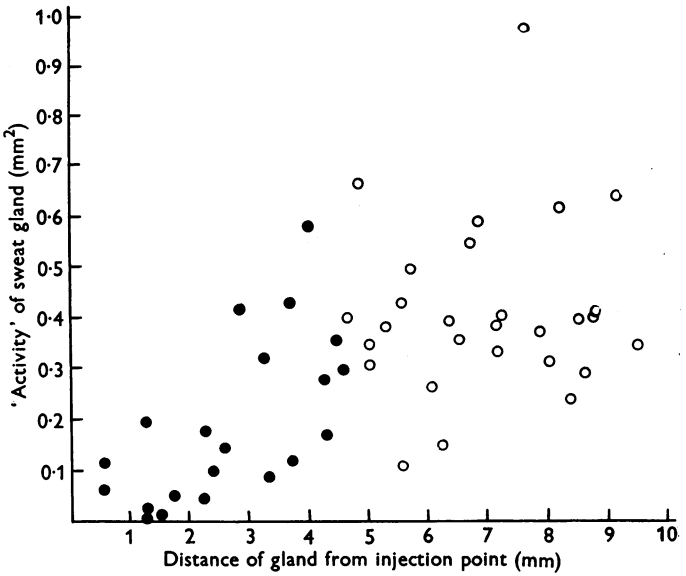

Fig. 5. Relationship between the 'activity' of sweat glands and the distance of the glands from the point at which six intradermal injections of 400 μg methacholine in 0·1 ml. were given at ½ hr intervals. The 'activity' is expressed as the sum of the pore areas (mm²) of each gland on twenty prints taken during the 3 hr experiment. ●, glands in 0·25 cm² proximal (Area 1); ○, glands in 0·25 cm² distal (Area 3) to the injection point. Subject I.

followed by five injections of saline during 3 hr, a second injection of the drug at the end of that period stimulated the same number of glands as the first. Further, the depression brought about by ACh or methacholine in high concentrations still occurred when, instead of repeatedly administering the agent through a single injection site, the consecutive punctures were made at different sites around the edge of 1 square centimetre. Several injections of the drugs into the same area produced only slight local oedema. When 12 i.u. hyaluronidase was combined with methacholine 400 μg/0·1 ml. there was no local accumulation of fluid, but repeated injections of this mixture produced a depression which was similar to that caused by injections of methacholine 400 μg/0·1 ml. alone (Fig. 6).

The influence of local vascular changes and of adrenaline. Perfusion experiments on various animal preparations by Hunt (1918) and many later workers have shown that ACh exhibits a dual vasomotor effect, small doses augmenting and large diminishing the blood flow. One explanation for the different effects of small and large doses on sweating responses might therefore be that these responses are influenced by local vascular changes. Blanching was usually observed in the injection wheal immediately after injection, but this was replaced within a few minutes by a lasting vasodilatation. Small and large doses of ACh or methacholine did not, however, produce any obvious differences in vascular reaction. This would appear to indicate that the depressant action of these drugs does not depend on vasoconstriction. Even though there is no vasoconstriction, the possibility that an adrenaline-like

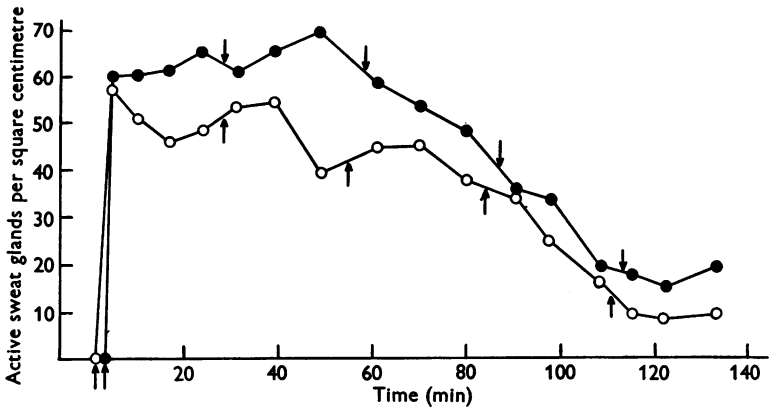

Fig. 6. Effect of hyaluronidase on the depression of sweat-gland activity by repeated injections of methacholine. Injections (at arrows) of 400 μg methacholine combined with 12 i.u. hyaluronidase (●) and of 400 μg methacholine alone (○). Subject IV.

substance is released under the influence of ACh (Kottegoda, 1953) and that the adrenaline exerts the inhibitory effect needs to be examined, since we have seen that adrenaline has a depressant effect. Three observations show that adrenaline is not involved. (1) when adrenaline depressed sweat activity in single doses and more markedly in repeated doses, there was always a lasting vasoconstriction. (2) When adrenaline was used to enhance the action of ACh there was vasoconstriction. Thus the depression induced by repeated injections of ACh 2 mg/0·5 ml. was enhanced by the addition of 10 μg adrenaline. When 10 μg adrenaline was combined with only 100 μg ACh/0·5 ml.—a dose of ACh which, alone, did not depress sweating—marked depression developed (Fig. 7). In experiments on two of the subjects vasoconstriction did not persist, but the areas were dilated for several minutes after each injection and constriction gradually developed before the next injection was given. In these subjects the decline in sweat gland activity

exceeded that in the area treated with ACh by itself only when there was obvious vasoconstriction, that is, in the period just before an injection of the adrenaline-acetylcholine solution. (3) When a sympatholytic agent such as tolazoline is combined with ACh or methacholine in high concentrations we should expect the depression to be prevented if adrenaline is involved. However, when 100 μg tolazoline was combined with repeated injections of 400 μg methacholine or of ACh 1 mg/0·1 ml. the depression developed just as rapidly as in the absence of tolazoline. In fact there was usually a more pronounced vasodilatation when tolazoline was present, showing again that the depression occurs both in the presence of an adrenaline antagonist and of

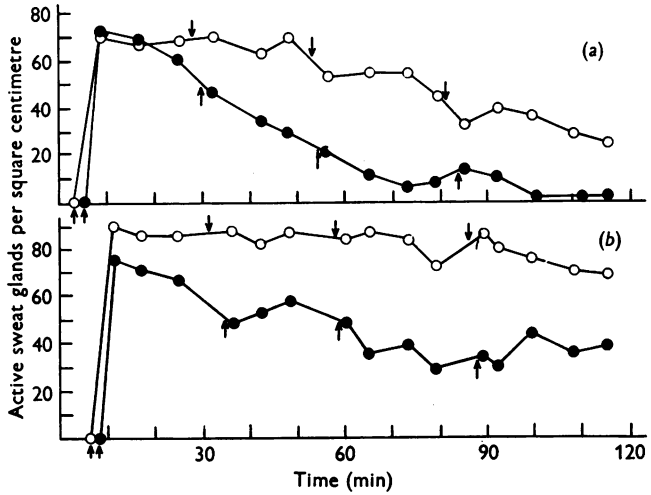

Fig. 7. Effect on sweat-gland activity of repeated intradermal injections (at arrows) of ACh combined with adrenaline. (*a*) 2 mg ACh combined with 10 μg adrenaline (●); 2 mg ACh alone (○). (*b*) 100 μg ACh combined with 10 μg adrenaline (●); 100 μg ACh alone (○). Subject IV.

vasodilatation. Tolazoline 100 μg/0·1 ml., alone, did not stimulate the sweat glands.

The effect of anticholinesterase. In an environmental temperature of 22° C an intradermal injection of neostigmine 5 μg/0·1 ml. elicited a local sweat reaction which was less intense, but of longer duration, than the response to ACh. It has been suggested that neostigmine and other anticholinesterases stimulate the glands, not only by allowing the ACh produced at nerve endings to build up to a threshold value for stimulation, but also by a direct excitor effect on the glands (Chalmers, 1950; Janowitz & Grossman, 1950). From experiments on two subjects at 20° C it was found that neostigmine 5 μg/0·1 ml. produced no response when injected after procaine 0·5% (w/v)/0·2 ml. and a small response after 0·2 ml. normal saline. At 23° C there was

again no response to neostigmine after procaine, but in both subjects there was vigorous sweating when neostigmine was injected after normal saline. These observations suggest that the sweat reaction to neostigmine 5 μg/0·1 ml. was due to potentiation of subthreshold amounts of transmitter and not to direct stimulation by the drug.

When neostigmine was injected together with ACh the duration of both vasodilator and sweat responses increased, so that the responses resembled those given by methacholine. It has already been shown that inhibition of sweat-gland activity was usually provoked more rapidly with large doses of methacholine than with ACh. If this is due to a difference in the rate of destruction of the two drugs by cholinesterase, then by delaying the removal of ACh cholinesterase inhibitors should augment the action of the drug and

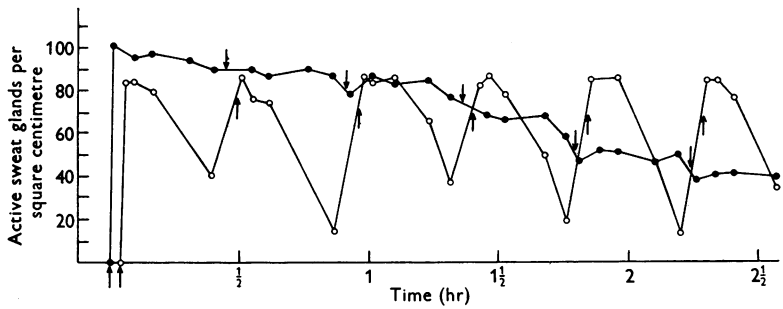

Fig. 8. Depression of sweat-gland activity by repeated intradermal injections of ACh combined with neostigmine. Injections (at arrows) of 20 μg ACh combined with 5 μg neostigmine (●) and of 20 μg ACh alone (○). Subject I.

exacerbate the inhibitory effect. When 5 μg neostigmine was combined with only 20 μg ACh/0·1 ml., the action of the ACh was at first potentiated, and then there ensued a progressive decline in the number of active glands similar to that produced by large doses of ACh or methacholine (Fig. 8).

In further experiments (Fig. 9) it was established that five injections of ACh 100 μg/0·1 ml. at 20 min intervals could produce a sustained sweat response. If after two such injections of 100 μg ACh, 5 μg neostigmine was added to each of the following three injections, then the number of active glands rapidly decreased. Repeated administration of neostigmine 5 μg/0·1 ml. alone did not depress the number of active glands (Fig. 9).

Effect of atropine. Thaysen & Schwartz (1955) have shown that thermally-induced fatigue of the sweat glands can be prevented by intradermal infiltration with atropine, but similar experiments were not performed to establish whether atropine could 'protect' the glands from the depressant effect of large doses of methacholine. This point has been examined in four experiments with almost identical results. One of these studies is illustrated in Fig. 10. A selected area on each arm of the subject was tested a few hours before the

experiment, either by injecting small doses of methacholine (20 or 40 μg/0·1 ml.) or by indirect heating to determine the number and disposition of functional sweat glands. At the start of the experiment one area was injected with atropine 4 or 8 μg/0·1 ml. and the other with 0·1 ml. normal saline. Approximately 10 min afterwards both areas were injected with methacholine

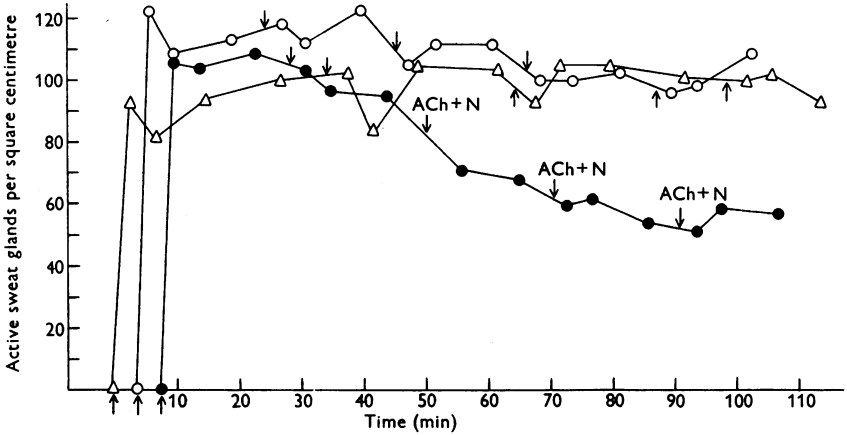

Fig. 9. Sweat-gland responses to intradermal injections (at arrows) of ACh and neostigmine. Five injections of 100 μg ACh (○); four injections of 5 μg neostigmine (△); two injections of 100 μg ACh followed by three injections of 100 μg ACh combined with 5 μg neostigmine (ACh + N, ●). Laboratory temperature 22° C. Subject III.

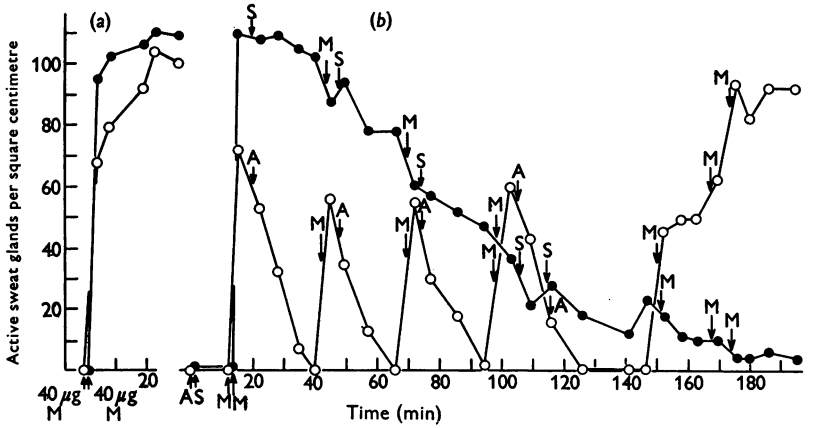

Fig. 10. Effect of repeated intradermal injections of methacholine and atropine on sweat-gland activity. (*a*) the number of active glands evoked by a test dose of 40 μg methacholine (M) in 0·1 ml. in an area on each forearm 2 hr before Expt. (*b*), in which the previously tested areas were injected (at arrows) with 4 μg atropine (A) in 0·1 ml. and 400 μg methacholine (M) in 0·1 ml. (○); 0·1 ml. normal saline (S) and 400 μgM in 0·1 ml. (●). Subject III.

400 μg/0·1 ml., and then again at 30 min intervals. In the area pre-treated with atropine, injections of atropine were repeated before each methacholine injection, and concurrently equal volumes of normal saline were injected in the area on the control arm. The area treated with atropine exhibited short bursts of sweating after each dose of methacholine while the control area showed the expected gradual decline in the number of active sweat glands. After the fourth injection of methacholine, a period of about 40 min was allowed for the effects of the atropine to wear off and both areas were then tested with three consecutive doses of methacholine 400 μg/0·1 ml. The response to the ultimate injection of methacholine in the previously atropinized area was almost equal to that given by the original test dose of 40 μg. In Area 1, where depression was usually most marked, the number of active glands and the mean output per gland was the same before and after treatment with atropine. Conversely, in the area on the control arm which had not been atropinized, these last injections of methacholine provoked near-anhidrosis.

Recovery of sweating responses after intradermal injections

Single injections. The number and exact location of functional glands in selected forearm areas were determined before the injection; these same areas were then retested by indirect heating, 4 hr, 1 day, 2 days and 1 week after the injection (Fig. 11). The active glands were located during successive tests with an error of not more than 2 glands/0·25 cm^2. On testing at intervals following the intradermal injection of 0·05 ml. normal saline there was no loss of responsiveness to indirect heating (Fig. 11*a*).

The effect of adrenaline injections (10 μg/0·05 ml.) was investigated in two subjects. In one there was no inhibition of the sweat response. In the other (Subject III, Fig. 11*b*) there was likewise no decline in activity after 4 hr but a decreased sweat response occurred 1 day after injection. Complete recovery of the number of active glands did not take place until after 1 week. Injections of ACh 10 or 200 μg/0·05 ml. did not inhibit responses to subsequent heating tests (Fig. 11*c*, *d*). With ACh 400 μg/0·05 ml. there was no depression after 4 hr but a fall occurred in the number of active glands in Area 1 after 1 day, with full recovery by the second day (Fig. 11*e*).

Four hours after injections of methacholine 10, 200 or 400 μg/0·05 ml. there was again no depression of the response to indirect heating (Fig. 11*g*, *h*). After 1 day, however, there was a significant decrease in the number of functional glands; the loss of responsiveness was proportional to the dose of methacholine injected. Two days after injection the area treated with 10 μg methacholine gave almost a normal response. After injections of 200 or 400 μg methacholine full activity was not re-established until after 1 or 2 weeks. Pre-treatment with atropine 8 μg/0·05 ml. before 200 or 400 μg methacholine did not prevent subsequent depression, but shortened its duration (Fig. 11*k*).

Repeated injections. Control experiments showed that five consecutive injections of 0·05 or 0·5 ml. normal saline did not depress the responsiveness of sweat glands tested 24 hr later, whether either indirect heating or an injection

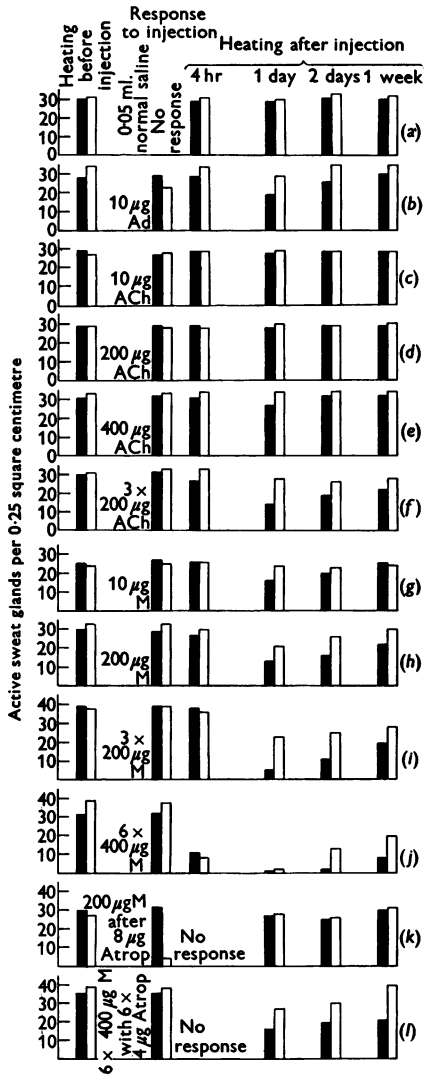

Fig. 11. Maximum number of active sweat glands stimulated by indirect heating 4 hr before and at intervals of 4 hr, 1 day, 2 days and 1 week after intradermal injections of sudorific drugs (Subject III). Black columns represent the maximum number of active glands in 0·25 cm² proximal (Area 1) and white columns glands in 0·25 cm² distal (Area 3) to the injection point. All injections of adrenaline (Ad), acetylcholine (ACh), methacholine (M) and atropine (Atrop) in 0·05 ml. saline. When repeated injections are given, the response in column 2 represents the maximum number of active sweat glands evoked by the first injection of the sudorific drug.

of a standard small dose of methacholine (10 or 20 μg/0·1 ml.) was employed as the test procedure. In contrast, an area which received five injections of methacholine 400 μg/0·1 ml. was still inactive on the third day when a test dose of 20 μg methacholine was given, and recovery was not complete even by the ninth day (Fig. 12). In another skin area no immediate decline in the number of active glands occurred after five injections of methacholine 20 μg/0·1 ml., but on the third day activity was much depressed and the full number of functional glands did not recover until the ninth day.

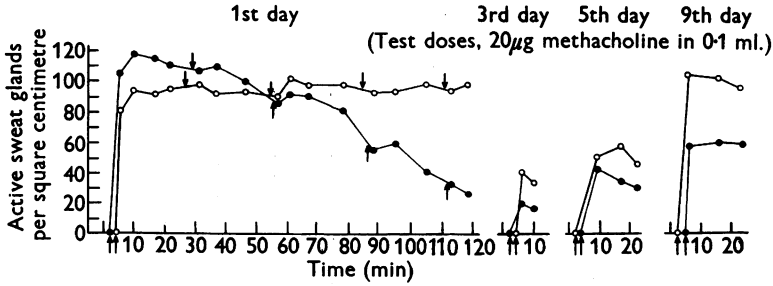

Fig. 12. Sweat-gland responses to test doses of methacholine after repeated intradermal injections of methacholine. Five injections of 400 μg methacholine in 0·1 ml. (●) and five injections of 20 μg methacholine in 0·1 ml. (○) were given at arrows on the first day, and the two areas then tested by 20 μg methacholine in 0·1 ml. on the 3rd, 5th and 9th days. Subject I.

Since single, small, test doses alone caused depression 1 day after administration (Fig. 11*g*), indirect heating appeared to be the more satisfactory method of testing. Some results of heating tests on areas repeatedly injected with drugs are shown in Fig. 11*f*, *i*, *j*. The sweating produced by three consecutive injections of ACh 200 μg/0·05 ml. at 30 min intervals ceased 2 hr after the first injection. Two hours later (that is 4 hr after the first injection) the glands responded normally to indirect heating. On the following day, however, there was distinct inhibition in Area 1 (an effect which could not be elicited by a single injection of ACh 200 μg/0·05 ml.) and recovery took more than 1 week (Fig. 11*f*). Similarly, three consecutive injections of methacholine 200 μg/0·05 ml. produced a normal maximum response in Areas 1 and 3 after 4 hr but depression after 1 day (Fig. 11*i*). Inhibition persisted in both areas for more than 1 week. When larger doses of methacholine were used, e.g. six injections of 400 μg, the glands failed to respond maximally to heat even after 4 hr, and the depression was more marked after 1 day (Fig. 11*j*). The duration of the depression which developed after 1 day appeared to depend on the dosage of the sudorific drug initially injected and not on the number of injections *per se*. Methacholine was more effective than ACh in producing the depression and large doses more effective than small. Atropine was found to

protect the glands to some extent against the depressant effect of multiple injections, just as it did with single injections (Fig. 11*l*).

It is clear that 1 day after a single injection, drug-stimulated glands enter into a state of depression, the persistence of which depends on the dose given, but this depression was not present 4 hr after the injection. It is therefore a slowly developing depression but one which can be accelerated by multiple injections of drugs in high concentrations.

Since 24 hr after single or multiple injections sweat-gland activity was found to be depressed, the question arises as to whether the glands had at all recovered from the immediate depression following large and repeated doses described above (p. 608). The usual depression was induced by five consecutive injections of methacholine 400 μg/0·1 ml. at 30 min intervals. After this,

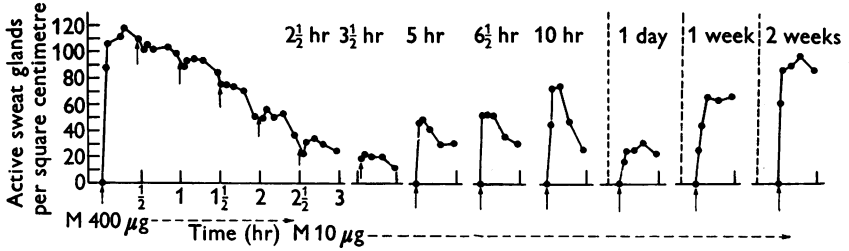

Fig. 13. Recovery of sweat-gland activity after depression by five intradermal injections at arrows of 400 μg methacholine (M) in 0·1 ml. Sweat-gland responses were tested by intradermal injections of 10 μg methacholine in 0·1 ml. at 2½ hr and at intervals up to 2 weeks after the first injection of 400 μg methacholine. Subject III.

small test doses of methacholine 10 μg were administered (Fig. 13). The first and second tests, at 2½ and 3½ hr after the first injection of 400 μg, showed that the depression still persisted. After 3½ hr there was a gradual recovery in activity, until at 10 hr over half the original number of active glands were again functioning. The next test at 24 hr then showed the marked inhibition (to which attention has already been drawn), followed by a slow recovery during the course of the next 2 or 3 weeks.

These observations suggest that there are two quite distinct phases of maximum depression in sweat-gland activity. The first occurs immediately after multiple injections of massive doses of the sudorific drug. The second is fully developed roughly 24 hr after injections. Recovery from the first phase of depression takes place within a few hours; but complete restoration of the number of active glands was not in fact realized even after 10 hr. This was probably due to the simultaneous development of the second depressant phase.

Associated with the slowly developing secondary phase of depression there was a local inflammatory reaction which became apparent some hours after the final injection of large doses of the drugs and was most evident after 24 hr.

The slow recovery of sweat-gland activity in the injected area was also accompanied by local scar formation and subsequent desquamation after 2 or 3 weeks.

DISCUSSION

Sweat-gland stimulation by intradermal injection of a sudorific drug involves diffusion of the drug to receptor sites, and constitutes a much less efficient means of stimulation than the natural release of transmitter substances at nerve endings. Chalmers (1950) found that the latency of response to ACh administered intradermally was 30–60 sec for near-threshold doses (10^{-6}); the delay in response could not be reduced below 10 sec even with very high concentrations, up to 10^{-2}. In contrast, nerve stimulation produced responses within 1 or 2 sec. We have found that single massive doses of ACh or methacholine may in fact delay the activation of sweat glands near the injection site (Area 1) for several minutes. One possible explanation for this long latency is that the sweat-gland receptors are rapidly desensitized to the action of the drug. A factor which should be considered is the vehicle in which the sudorific agent is injected; for, as we have shown, concentrations of propylene glycol exceeding 15% (w/v) can inhibit sweat-gland responses. Our results make it clear, however, that high concentrations of ACh or methacholine have a specific inhibitory effect.

Although human eccrine sweat glands can be stimulated by intradermal injections of adrenaline (not by systemic administration; Chalmers & Keele, 1951) there is no evidence for an adrenergic component in the nerve supply to the glands. Arguments against an adrenergic component are (*a*) that atropine can completely suppress thermal or mental sweating while adrenaline-blocking agents such as dibenamine do not (Chalmers & Keele, 1951); and (*b*) that amine oxidase activity is absent from human skin (Hellmann, 1955). Adrenaline does not act by the release of ACh, since atropine does not block its sudorific action (Sonnenschein *et al.* 1951). In our experiments the adrenaline response differs from that of ACh or methacholine in its smaller magnitude and shorter time course and by the presence of vasoconstriction. We have presented some evidence that the accompanying vasoconstriction may underly the small response to adrenaline.

The decrement in sweat-gland activity after prolonged and copious sweating in very hot environments is customarily described as 'fatigue' of the sweat apparatus. Thaysen & Schwartz (1955) assume that the decline in sweat-gland activity following injections of large quantities of methacholine is analogous to this natural fatigue. Our evidence, on the other hand, indicates that the two processes are not entirely similar. The depression induced by drugs does not necessarily entail prolonged or intense secretory activity. Dole *et al.* (1951) showed that during repeated injections of methacholine the sodium

concentration does not increase with the fall in output, as is the case in thermal sweat-gland fatigue (Robinson & Robinson, 1954).

To account for the depressant effects of large or repeated doses of ACh or methacholine administered intracutaneously, other explanations may be advanced. First, it is conceivable that gross mechanical damage may be inflicted on dermal structures, particularly by repeated injections. Data given by Barer (1954) indicate that an interstitial pressure in the skin exceeding 100 mm Hg may be expected from single intradermal injections of 0·05 ml., but this pressure rapidly declines. Local anhidrosis can be produced experimentally by intradermal injections of normal saline which increase tissue pressure sufficiently to constrict sweat gland ducts (Shelley, Horvath & Pillsbury, 1950). It is difficult to accept such an explanation for the depression by injections of readily diffusible solutions, especially as the depression is in no way alleviated by hyaluronidase. Small doses of the drugs involving repeated skin punctures and the injection of the same volume of fluid do not cause a decline in activity. Also, repeated administration of normal saline does not impair the reaction of the sweat glands to subsequent treatment with ACh or methacholine. Depression by large doses of drugs is unlikely to be due to osmotic disruption of sweat gland cells, since all solutions were close to isotonic. In addition, atropine may protect the glands from immediate inhibition by large quantities of sudorific drugs. This suggests some specific interaction between the drugs and the 'receptor' sites.

A second hypothesis to explain the reduction in sweat response is that large quantities of ACh or methacholine impair the local blood supply to the glands, possibly through the release of an adrenaline-like substance. An adrenaline antagonist, tolazoline, as we have shown, does not however prevent the decline in sweat-gland activity. The fact that adrenaline enhances the depression (despite its intrinsic sudorific effect) seems to be due to its intense vasoconstrictor action. Unless such vasoconstriction is present, adrenaline combined with ACh does not augment the depression. There was, however, no lasting vasoconstriction in areas injected with large doses of ACh or methacholine alone. Adrenaline vasoconstriction may have the effect of maintaining a high local concentration of ACh.

Since mechanical traumata or changes in blood supply do not seem likely causes for the depression, a third hypothesis may be suggested. The decline in sweat-gland activity by drugs in maintained high concentrations may be some form of accommodation or 'desensitization', analogous to that produced at the motor end-plate (Thesleff, 1955). This 'desensitization' is associated with large doses but not with prolonged secretory activity. Small doses of methacholine (10 μg/0·1 ml.) can maintain activity with little reduction in output for up to 6 hr. Thesleff (1955) suggests that the receptor system at the motor end-plate is gradually transformed into an inactive state from which

there is gradual recovery after complete withdrawal of the drug. There is a corresponding slow recovery of sweat-gland activity over some hours after depression by large doses of ACh or methacholine. The fact that the gland receptors are involved is well supported by (*a*) the action of atropine in protecting the glands from the depressant effects of high doses, and (*b*) the finding that neostigmine may allow small doses of ACh to produce depression. Whether natural fatigue is also a consequence of 'desensitization' it is not possible to say without further experiments.

One feature of the reduction in sweat-gland activity by sudorific drugs, which does not appear to be related to either desensitization or natural fatigue, is the subsequent long-lasting partial inhibition, which may persist for up to 3 weeks after injection. There is a latent period during which the inhibitory reaction develops; the depression is most marked after 1 or 2 days. Injections of normal saline or small doses of ACh do not produce this late-developing depression. Sonnenschein *et al.* (1951), who have noted a similar phenomenon in some, though not all, subjects after single intradermal injections of adrenaline or ACh, postulate that the effect is due to an inherent inhibitory action of the drugs or to locally produced metabolic end-products. Our experiments indicate that the depression can be diminished to some extent by simultaneous injections of atropine. Shelley, Horvath, Weidman & Pillsbury (1949) and Shelley *et al.* (1950) have demonstrated that a variety of minor epidermal injuries including iontophoresis, maceration, ultra-violet light and the application of chemicals to the skin may produce a long-lasting local anhidrosis. These injuries give rise to abnormal epidermal keratinization, with plugging of the sweat ducts and sweat-retention vesicles during intense sweating. The anhidrosis develops after a variable period of from hours to several days following injury, with recovery after some days or weeks. These and many similar experimental observations indicate that the function of sweat glands may be impaired by procedures which affect the integrity of the skin. While sweat-retention vesicles were not observed during the anhidrosis, it remains possible that high concentrations of drugs may produce secondary changes in the sweat glands or ducts. From the cytological and histochemical work of Dobson, Formisano, Lobitz & Brophy (1958) it should be possible to detect such secondary changes if they occur.

In conclusion, we postulate that two components are involved in the depression of sweat-gland activity by large amounts of sudorific agents. First, there may be desensitization of the glands, the onset of which is rapid with slow recovery over many hours. Secondly, persistent local effects of the drug may result in an injury reaction which inhibits the normal function of the glands over a period extending to days or weeks after injection.

SUMMARY

1. The responses of eccrine sweat glands in the human forearm to intradermal injections of acetylcholine, methacholine, adrenaline and to indirect heating were investigated by the plastic impression method.

2. Repeated administration of acetylcholine or methacholine in high concentrations progressively reduced the number of glands which were active and their output; in low concentrations they did not.

3. The drug-induced failure of sweating did not entail prolonged or intense secretory activity as in thermal sweat-gland 'fatigue'. There was a partial recovery of response during 10 hr following the depression.

4. The decline in activity following repeated injections did not appear to involve physical damage to the glands, increased intracutaneous pressure or local vascular impairment.

5. Atropine prevented the subsequent failure of the sweat response, and neostigmine promoted the depression even by small amounts of acetylcholine.

6. It is suggested that the sweat glands become accommodated or 'desensitized' to the action of sudorific drugs in high concentrations.

7. The responses to intradermal injections of adrenaline were smaller than those evoked by acetylcholine or methacholine. The sudorific action of adrenaline appeared to be counteracted by its vasoconstrictor effect and the vasoconstrictor action intensified the depression produced by high concentrations.

8. Single or multiple injections of acetylcholine, methacholine or adrenaline induced a local anhidrosis which was most marked after 1 or 2 days and which persisted for up to 3 weeks.

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