J. Physiol. (1966), **184**, *pp.* 203–214
With 3 *text-figures*
Printed in Great Britain

THE LOCAL TRAINING EFFECT OF SECRETORY ACTIVITY ON THE RESPONSE OF ECCRINE SWEAT GLANDS

BY K. J. COLLINS, G. W. CROCKFORD AND J. S. WEINER
From the M.R.C. Environmental Physiology Research Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1

(*Received* 5 *October* 1965)

SUMMARY

1. The influence of repeatedly raising the body temperature by radiant heat to a level at which acclimatization to heat is normally acquired was investigated in two series of experiments, the first without the subjects sweating, the second with sweating.

2. In a second investigation local sweat-gland activity was induced by drug injections on successive days without raising the body temperature.

3. These experiments show that the increased sweating capacity characteristic of acclimatization to heat is a result of sweat-gland activity and does not appear to be induced by or to depend on an elevated body temperature.

4. Secretory activity results in a loss of glycogen from sweat-gland cells on the first day of heat exposure but not after the glands have been 'trained' by acclimatization to heat.

5. The state of acclimatization has no influence on the threshold concentration of acetylcholine required to elicit sweating when injected intradermally.

INTRODUCTION

One of the physiological changes induced in man by repeated daily exposure to heat is the marked increase in sweat rate in response to a given thermal stress. This increased secretory activity poses the question whether the increased secretion is due to changes in the central nervous system, in the glands themselves or a combination of both. Recent work by Fox, Goldsmith, Hampton & Lewis (1962, 1964) and Brebner & Kerslake (1963) lends strong support to the theory that the increased sweating response is achieved mainly by an enhanced output from the glands in response to the same nervous stimulus, although some contribution from the hypothalamic temperature-regulating centre is not ruled out. Both groups of workers were able to inhibit sweating during heat acclimati-

zation, Brebner & Kerslake by first soaking the subjects in warm water before the heat exposures, Fox *et al.* by local cooling of the skin. Although these procedures affected the changes in sweating during acclimatization, exceptional local conditions are created which may not be applicable to the conditions prevailing during acclimatization. In view of this, two additional approaches have been devised to differentiate more clearly between the effects of repeated thermal stimulation of the thermoregulatory centre and repeated peripheral stimulation of the sweat glands.

It is a common observation that indirect cooling will inhibit sweating, the effect of cooling the hands on sweating having been reported on by Hill in 1921. More recently the effects of combined internal heating and external cooling have been reported in detail (Benzinger, 1961; Benzinger, Kitzinger & Pratt, 1963). The first approach used in the present investigation was to raise the body temperature to a level at which acclimatization to heat normally occurs whilst at the same time inhibiting sweating centrally by indirect cooling. In the second approach, local sweating was induced by intradermal injections of a sudorific drug into the forearm of resting non-sweating subjects with a normal body temperature. A preliminary account of these experiments has been published (Collins, Crockford & Weiner, 1963). In addition, evidence from the threshold responses to sudorific drug injections, local blood-flow changes and sweat-gland histochemistry from skin biopsy specimens has been brought together to try to clarify the nature of the peripheral changes produced by acclimatization.

METHODS

Acclimatization procedure. The deep body temperature of three male subjects was elevated to approximately 37·5° C by heating the upper half of the body with infra-red radiation for 100 min on 10 consecutive days. At the same time sweating was inhibited by immersing the subjects to the waist in well-stirred water at 10° C. Marked sweat-gland activity was induced when this procedure was repeated with the bath at 36° C. The body temperature was again raised by heating the trunk but not allowed to rise above the level reached on the corresponding day of the cold-bath series.

Physiological measurements. During the experiments the sublingual temperature was measured with a 30 S.W.G. copper/constantan thermocouple to an accuracy of $\pm 0{\cdot}02°$ C. The output of the thermocouple was determined by the null method using a potentiometer and light beam galvanometer. On two subjects of similar size (height 173 and 174 cm, weight 75·0 and 81·7 kg, respectively) oesophageal temperatures were taken on a number of occasions with a thermocouple embedded in a 2×22 mm stainless-steel tube attached to a 55 cm length of 2 mm o.d. polythene tubing through which the thermocouple wire ran. The thermocouple was introduced through the external nares to a distance of 45 cm (Ackas, Karvonen, Piironen & Ruosteenoja, 1962). Skin temperatures were measured at sixteen sites on the upper half of the body and on both thighs and calves with a roving thermocouple stretched across the open end of a V-shaped applicator. During each 100 min experiment skin temperatures were taken at 30, 60 and 90 min. The mean skin temperature for the experiment was determined by combining the three sets of readings and weighting the measurements according to the respective areas of the head, arms and hands, trunk, and

legs and feet (calculated by the method of Du Bois & Du Bois, 1916). The weight loss of the subject was determined by weighing to within ± 5 g before and after the experiments. In addition the activity of the sweat glands was followed throughout the exposure using a ventilated capsule, 1·5 cm in diameter, applied to the volar surface of the forearm, the same site being used throughout the investigation. Sweat-gland activity was continuously monitored with an infra-red water vapour analyser and the subject maintained in a state of low sweat-gland activity during the cold-bath experiments by appropriately reducing or increasing the intensity of radiant heat. The capsule was ventilated with dry nitrogen at 1 l./min and although it was impossible to observe the state of wetness of the skin under the capsule, the rapidity with which the water vapour analyser responded to changes in sweat rate suggested that evaporation was taking place in close proximity to the sweat-gland pores. The instrument was calibrated with 0·1 ml. distilled water immediately before and after each experiment. The two calibrations were consistent within ± 3 %.

Tests for acclimatization. The influence of the two periods of elevated deep body temperature on sweat-gland performance was determined on two subjects by a standardized hot-room test on the day before (T 1) and the day after (T 2) the cold-bath experiments, and finally on the day after the warm-bath experiments (T 3). The hot-room exposure lasted 3 hr in an environment of $46 \pm 0{\cdot}5°$ C dry bulb, $30 \pm 0{\cdot}5°$ C wet bulb with an air movement of 80 ft./min.

During the hot-room test the subjects step-climbed on a 12 in (30·5 cm) stool 18 times/min for 5 min in every 40 min. Pulse rate and weight were determined on entering the chamber and at the end of each 40 min period. Arm-bag sweat collections were made 5 min before each weighing. After weighing, the subjects replaced their sweat loss with water at 38° C. The sublingual temperature was followed at 5 min intervals throughout the exposure.

Just before the hot-room exposures, the sweating response to a 0·5 ml. intradermal injection of acetyl-β-methylcholine (methacholine, 2×10^{-4} g/ml.) was measured on the arm and leg using an unventilated capsule as described by Schwartz & Thaysen (1956). The same injection sites, identified by a spot of Indian ink injected intradermally, were used throughout.

For the third subject, 60 min of indirect heating with the legs immersed in well-stirred water at 43° C was substituted for the hot-room exposure. The course of the oral temperature during the indirect heating is shown in Fig. 1.

Drug-induced sweating. Using four non-sweating subjects seated in a room temperature of 20 to 23° C, sweat gland activity was induced at a normal body temperature by two intradermal injections of acetyl-β-methylcholine (10^{-4} g in 0·5 ml. of 0·9 % saline) given with an interval of 50 min on the volar surface of the forearm. This procedure, which produced sweating lasting about 100 min in the injected area, was repeated on 10 consecutive days. The sweat produced during the first 30 min after each injection was measured by the unventilated capsule method of Schwartz & Thaysen (1956). During the same period, control injections of saline were given in the other arm and the sweating response assessed by a drug injection on the first and tenth days.

Sweating threshold and sweat-gland glycogen. The threshold concentration of acetylcholine required to elicit sweating was measured by iodine-starch paper prints following intradermal injections of a series of 10-fold dilutions in 0·02 ml. volumes. Sweating thresholds were determined on the forearm and leg, before and after the bath experiments, and similarly when near-maximum heat acclimatization was produced in two subjects by 10 consecutive daily exposures to the hot-room procedure given above. During the hot-room acclimatization the subjects extended the work period to the maximum they could tolerate except on the first, fourth and tenth days, when a standard work-rate was maintained. On those 3 days, skin biopsies were taken on the forearm before the exposure and after 1·7 l. of sweat had been lost, using the rotary punch method of Urbach & Shelley (1951). The skin biopsies were fixed in Carnoy's fluid, serially sectioned, and stained for glycogen by the McManus-PAS

procedure with diastase control (McManus, 1948). Sections were also examined for ribonucleic acid content by staining with 1 % aqueous toluidine blue, controlled with ribonuclease (Pearce, 1960).

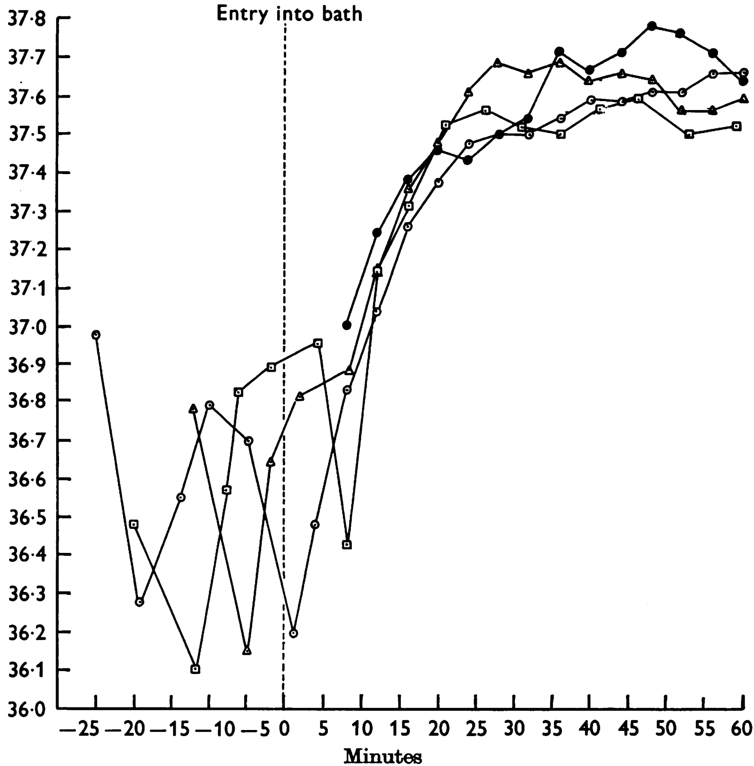

Fig. 1. The oral temperature of subject G.C.C. during the four indirect heating tests. T1 = □, T2 = ○, T2*b* = △, and T3 = ●. The subject entered the bath at time 0.

RESULTS

Heat acclimatization procedure. Fig. 2 shows the mean oral temperatures, at 10 min intervals, of three subjects undergoing two heat acclimatization procedures, first with sweat-gland activity suppressed and then with the sweat glands active. The somewhat lower oral temperatures during the first half of the warm-bath experiments were produced because evaporative heat losses prevented the subjects being heated sufficiently rapidly to attain the temperature reached at the corresponding time in the cold bath. This temperature difference between the two runs would of course tend to reduce the degree of acclimatization acquired in the warm-bath experiments. The body weight loss and the mean skin temperature of the upper half of the body are shown in Fig. 3.

The combined insensible sweat and respiratory water loss of the three subjects, determined by weighing at hourly intervals, was found to be

between 50 and 60 g/hr. In the warm-bath experiments but not in the cold, this non-thermal weight loss was more or less balanced by the cutaneous uptake of water over the immersed half of the body. The sweating which occurred in the cold bath was confined to the non-immersed areas of the

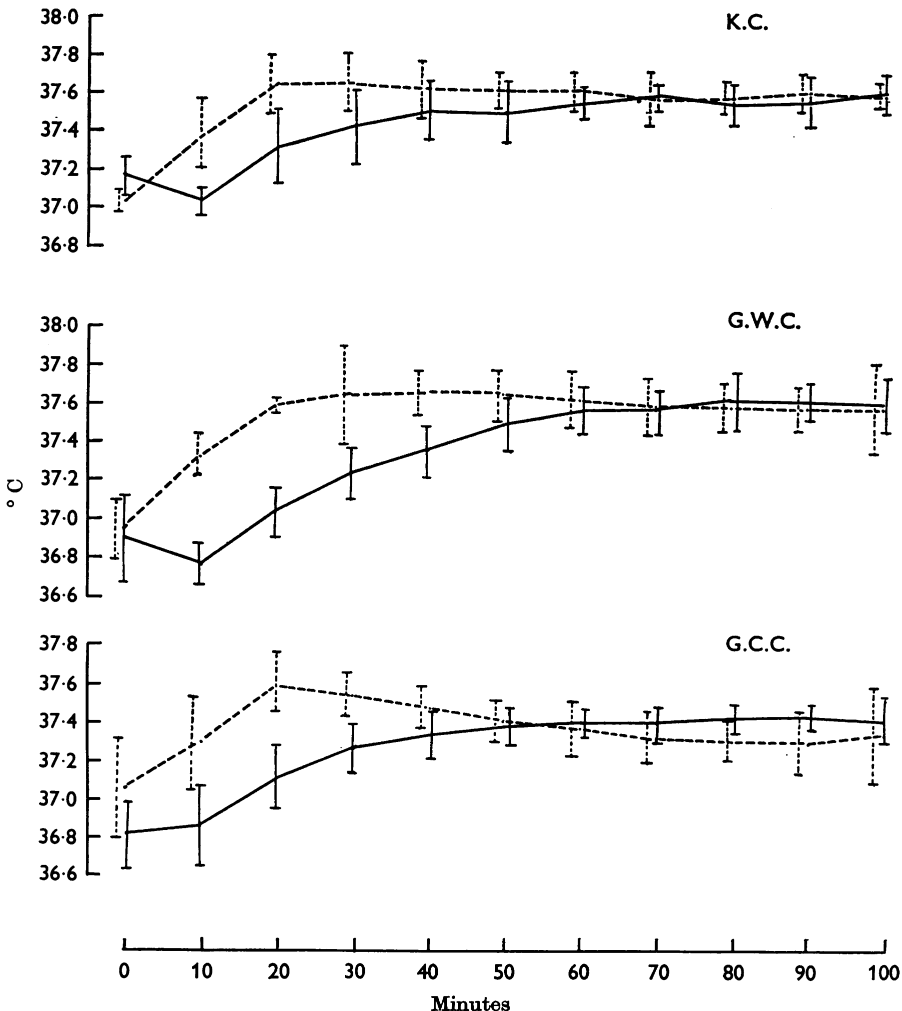

Fig. 2. The mean oral temperature at 10 min intervals of the three subjects during the cold-bath experiments (interrupted line) and warm-bath experiments (continuous line). The standard deviations are indicated by the bars.

body and the weight losses cannot therefore be directly compared with those of the warm-bath experiments in which the immersed portion of the body also contributed to the sweat loss. The forearm sweat responses of all three subjects were, however, monitored during the cold-bath experi-

ments and showed a low constant loss of 50, 55 and 56 mg/10cm²/10 min, respectively. In the warm-bath experiments, the forearm sweat losses increased from 310, 251 and 386 mg/10cm²/10 min on the first day to 883, 563 and 628 mg/10 cm²/10 min after 9 or 10 days.

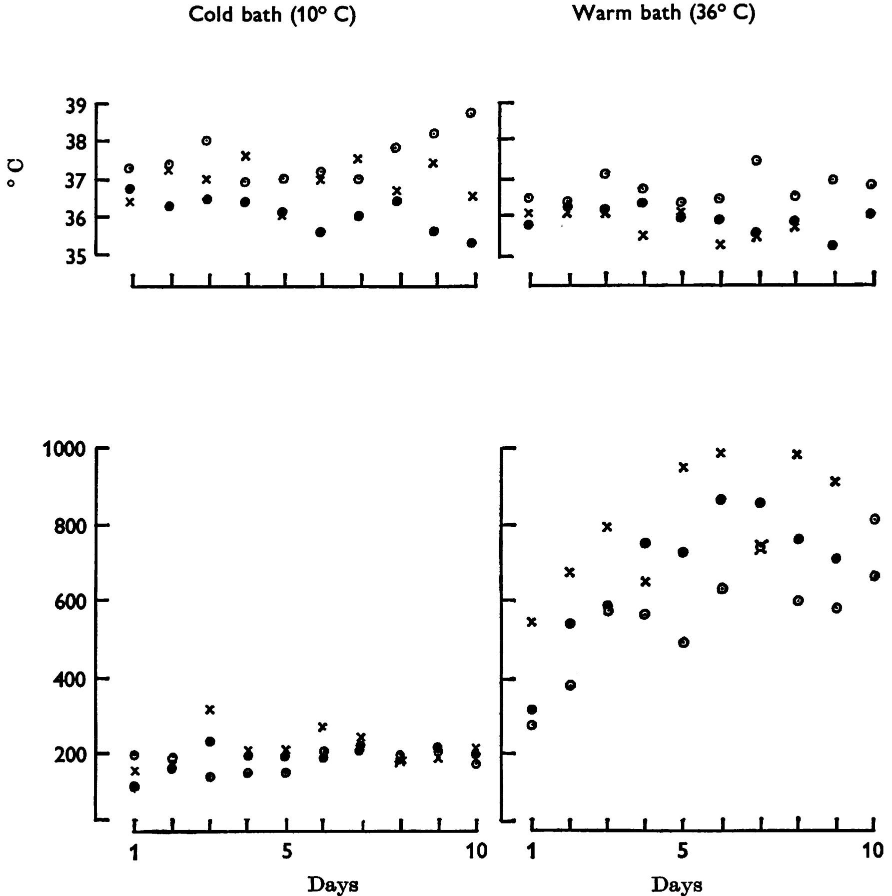

Fig. 3. Above, the mean skin temperature of the upper half of the body of the three subjects during the cold- and warm-bath experiments. Below, the total weight losses for each experiment of the three subjects, ● = G.W.C., ○ = K.C. and × = G.C.C.

The results of the uniformity tests before (T 1) and after (T 2) the cold-bath series, and after the warm-bath series (T 3), are shown in Table 1.

The total body sweat loss in T 1 and T 2 was almost identical, the percentage changes for the three subjects being +2·7 %, +5·3 and −1·9. However, between T 2 and T 3 there was a large increase in sweat rate, the percentage increases being +15, +29 and +64 %. The experiments were

all made during the winter months and it is unlikely that changes in ambient temperatures influenced the sweat response. None of the subjects indulged in active sports during the investigation. On the third subject, G. C. C., a much larger increment in sweat rate (64 %) was obtained in T 3 than in the other two subjects. This was probably because indirect heating was used for the test, a method which produces a smaller but more reproducible rise in oral temperatures compared with the hot-room routine. As 2 weeks elapsed between the cold- and hot-bath experiments on this subject, a second test (T 2*b*) was performed before the warm-bath experiments.

TABLE 1. Uniformity tests of the sweating capacity of three subjects. T 1 before the cold-bath, T 2 after the cold-bath, T 2*b* before the warm-bath and T 3 after the warm-bath experiments

	T 1	T 2	T 2*b*	T 3	
Total weight loss (g/hr)	442	454	—	521	K. C.
	430	453	—	585	G. W. C.
	270	265	250	410	G. C. C.
Arm capsule sweat rate (mg/10 cm²/10 min)	63·9	78·9	—	104·7	
	43·8	45·0	—	67·2	
	49·4	34·6	42·7	70·8	
Leg capsule sweat rate (mg/10 cm²/10 min)	26·7	24·2	—	45·9	
	16·3	17·4	—	24·2	
	26·8	40·1	35·3	73·9	
Arm-bag collection (ml./hr)	8·3	11·6	—	14·6	
	4·1	8·5	—	16·2	
	—	—	—	—	

The minimum concentration of an intradermal acetylcholine injection required to elicit sweating on the arm or leg (10^{-7} g/ml. in subjects G. W. C. and K. C., 10^{-8} in subject G. C. C.) did not change after either of the acclimatization procedures.

Sudorific drug injections. The sweat rates on a forearm site in four subjects injected daily for 10 days with methacholine are given in Table 2. All four subjects showed an increased sweat rate after 9 or 10 days of drug stimulation, the response to the drug injection having increased by 39, 33 and 98 and 64 %. Control experiments on the same subjects performed at the same time showed that after 10 days of 0·9 %, w/v, saline injections, the response to a standard injection of methacholine changed by + 10, − 3, + 16 and + 2 %, respectively.

Natural or artificially induced acclimatization to heat is a reversible process and it is therefore necessary to establish that the methacholine response is also reversible. Standard drug injections were therefore given 8 days after the end of the acclimatization period. The results (Table 2) indicate that three of the four subjects had lost most of the 'acclimatization' by the eighteenth day.

Two of the four subjects were then heat-acclimatized (days 22–32) using a severe 10-day hot-room routine. The sweating response to the standard drug injection was assessed on day 33 and again after a lapse of 46 days without exposure to heat. The results of these tests (Table 2) show that the percentage increase in sweating produced by the drug injections (39 and 33 % for subjects K and G, respectively) is notably smaller than that produced by a hot-room acclimatization procedure (123 and 127 %, respectively).

TABLE 2. Intradermal methacholine-induced sweat responses in the forearm (mg/10 cm²/hr)

	Day	Subject K	G	W	J
Methacholine injections (days 1–10)	1	378·4	396·4	137·3	344·5
	2	344·0	361·9	130·7	351·7
	3	424·8	427·3	129·4	403·6
	4	482·7	385·0	147·3	491·1
	9	673·7	467·3	282·0	632·1
	10	526·9	525·7	270·6	565·0
Percentage increase (days 1–10)	—	39 %	33 %	98 %	64 %
No treatment (days 11–18)	18	478·3	377·1	175·6	318·1
Heat acclimatization (days 22–32)	33	846·1	901·3	—	—
Percentage increase (days 18–33)	—	123 %	127 %	—	—
No treatment (days 34–80)	80	373·6	416·7	131·0	270·8
Saline injections (days 1–10)	1	349·9	253·3	140·9	270·9
	10	388·0	246·0	164·4	278·0
Percentage change	—	+10 %	−3 %	+16 %	+2 %

The threshold drug stimulus for sweat-gland activity was determined before and after hot-room acclimatization. Again no change in threshold could be detected on either the arm, back, chest or leg.

Glycogen content of sweat glands. Serially sectioned skin biopsies taken on days 22, 25 and 32, i.e. the first, fourth and last days of the hot-room acclimatization, were examined for glycogen in two subjects. There was a substantial loss of glycogen from the cells of the secretory portion of the gland after heat exposure on the first day, possibly a slight loss after heating on the fourth day, and no loss after heating on the tenth day. All the specimens taken before exposure contained large quantities of glycogen. Change in cytoplasmic basophilia (RNA content) were looked for but no obvious differences could be detected. Atrophy and vacuolization of pale cells were not a significant feature of any of the sections and there was no overt evidence of hypertrophy in the secretory coils after acclimatization. (Colour micrographs may be obtained on loan from the authors.)

DISCUSSION

In the warm- and cold-bath experiments oral temperature was used as an indication of the temperature of the hypothalamus, where the central thermal receptors are usually assumed to be. It was considered that the relation between the two temperatures would remain the same in the two thermally different situations. The basis for this assumption is that although the temperature of the blood returning from the legs must differ in the warm and cold baths it is unlikely that, after having passed through the heart and lungs, the temperature of the blood reaching the sublingual site of measurement would differ from that reaching the brain. Nevertheless, it is feasible that if the radiant heat load was unequal in the two bath series, differences in heat exchange between the venous return and arterial supply to the head could alter the oral/hypothalamic temperature relation. The almost identical mean head skin temperatures in the cold and warm baths, 36·8 and 36·7° C, respectively, indicate that such change as may occur in the oral/hypothalamic temperature relation is likely to be small compared with the elevation of oral temperature produced by the bath procedure. Oesophageal temperatures which were taken on two subjects (K. C. and G. W. C.) tended to fluctuate more than the oral temperatures, but the mean oesophageal values for the two subjects in the warm-bath, 37·45° C (S.D. 0·166), and cold-bath series, 37·36° C (S.D. 0·196), compared with mean oral temperatures of 37·44° C (S.D. 0·126) and 37·58° C (S.D. 0·095), respectively, confirm that an elevated central temperature was achieved in both bath series. The assumption that during the cold-bath experiments the hypothalamus was maintained at a temperature which is normally associated with some degree of acclimatization to heat therefore seems to be justified.

A comparison of the total body weight losses (Table 1) obtained on the three subjects in the standard tests before (T 1) and after (T 2) the cold-bath series shows that repeated daily elevation of the brain temperature without sweat-gland activity does not produce any marked change in the sweat response to the tests. Capsule collections (Table 1), although variable, confirm that none of the subjects showed a noticeable increase in sweating capacity on either the arm or leg. When the experiment was repeated, but sweating permitted, repeated daily exposure resulted in an increased total body sweat response to the standard test, and capsule collections confirm this.

Repeated local drug stimulation of the sweat glands in a resting subject at a normal body temperature results in a gradual enhancement of the sweat response. The increase in sweat response to the drug during the 10-day period is not unlike that associated with heat acclimatization

although somewhat delayed (cf. Table 2). The control sites in which saline was injected daily show a negligible change in sweat response in subjects G and J, but a more pronounced change in subjects K and W. In the case of subject W there is reason to suspect that some degree of heat acclimatization was acquired between days 1 and 10. No such explanation can be given for subject K. In all four subjects, after a lapse of eight days the sweat response of the drug-treated area fell towards the original level and after a further 62 days there was no sign of acclimatization.

Both the bath and drug experiments therefore indicate that the increased sweating capacity which is characteristic of heat acclimatization is for the most part only acquired as a result of sweat-gland activity and is not dependent on an elevated body temperature. This finding which has been reported earlier (Collins *et al.* 1963) is in agreement with the work of both Fox *et al.* (1962, 1964) and Brebner & Kerslake (1963).

The finding that sweat-gland training plays an important part in acclimatization to heat does not rule out a central participation, the most plausible evidence for which is that sweating begins at a lower body temperature in the acclimatized individual (Fox, Goldsmith, Kidd & Lewis, 1963). This earlier onset of sweating could be produced by a greater sensitivity of the glands to the nervous information reaching them. However, determination of the threshold concentration of acetylcholine required to elicit sweating showed no change as a result of either the bath experiments or even after full acclimatization in the climatic chamber. These results are contrary to the findings of Chalmers & Keele (1952), who found a difference in the sweat-gland threshold between winter and summer determinations. The most likely explanation for this discrepancy is that the above workers did not accurately duplicate the thermal state of the subjects on the two occasions.

More specific evidence of a change in the sweat gland itself during acclimatization has come from the histochemical study. On the first day of heat exposure, sweating induced a marked reduction in cell glycogen, but by the fourth and tenth days there was no change in cell glycogen after an identical bout of sweating. This substantially agrees with the observations of Dobson (1960) on sweat-gland glycogen changes during repeated episodes of profuse sweating in hot humid conditions. It therefore appears that changes are taking place either in the secretory cell and/or in the gland as a whole which enable sweat to be secreted without recourse to the cell's reserves of glycogen. Lactate, the anaerobic break-down product of glycogen, is present in sweat concentrations which diminish in the first of serially collected samples as acclimatization proceeds (Weiner & van Heyningen, 1952) and this may be related to the fact that the blood supply to the gland is probably lowest in the unacclimatized subject and at the beginning of a

single period of heat exposure. It is therefore tempting to suggest that an improved blood supply to the sweat glands is a major feature of acclimatization. However, stimulation of the blood supply to an area of the arm on 7 successive days by a rubefacient (tetrahydrofurfuryl nicotinic acid ester) failed to produce any change in the sweat response to a standard sudorific drug injection. Furthermore, although exposure of the skin to radiant heat in the cold-bath experiments produced a marked cutaneous dilatation coupled with only slight sweat-gland activity, there was no noticeable change in secretory capacity. It therefore appears that if changes in cutaneous blood supply are an important aspect of the increase in sweating capacity then the changes occur in the blood vessels of the gland itself and that these vessels either do not normally respond to thermal stimuli or if they do cannot be 'trained' by such stimuli unless the sweat glands are also activated.

It has been suggested that the discomfort associated with exposure to thermal stress is, in the case of heat exposure, produced by the elevated body temperature and may even be derived from the central temperature sensor (Benzinger, 1963). In the bath experiments reported here there was little, if any, discomfort associated with the cold-bath experiments after the first 10–15 min, whereas in the warm bath the subjects definitely felt uncomfortable during the whole exposure. In the warm bath, however, the raised body temperature was associated with sweating and an increased pulse rate. It therefore appears that the discomfort associated with an elevated body temperature is a product of a functioning heat-loss mechanism and is not derived from an elevated body temperature *per se*.

REFERENCES

Ackas, E., Karvonen, M. J., Piironen, P. & Ruosteenoja, R. (1962). Intramuscular rectal and oesophageal temperature during exercise. *Acta physiol. scand.* **54**, 366–370.

Benzinger, T. H. (1961). The diminution of thermoregulatory sweating during cold-reception at the skin. *Proc. natn. Acad. Sci. U.S.A.* **47**, 1683–1688.

Benzinger, T. H. (1963). Peripheral cold and central warm reception, main origin of human thermal discomfort. *Proc. natn. Acad. Sci. U.S.A.* **49**, 832–839.

Benzinger, T. H., Kitzinger, C. & Pratt, A. W. (1963). *Temperature—Its Measurement and Control in Science and Industry*, pp. 637. New York: Reinhold Publishing Corporation.

Brebner, D. F. & Kerslake, D. McK. (1963). The effect of soaking the skin in water on the acclimatization produced by subsequent heat exposure. *J. Physiol.* **166**, 13–14*P*.

Chalmers, T. M. & Keele, C. A. (1952). The nervous and chemical control of sweating. *Br. J. Derm.* **64**, 43–54.

Collins, K. J., Crockford, G. W. & Weiner, J. S. (1963). The role of glandular activity in the increased sweat response of heat acclimatization. *J. Physiol.* **169**, 12–13*P*.

Dobson, R. L. (1960). The effect of repeated episodes of profuse sweating on the human eccrine sweat glands. *J. invest. Derm.* **35**, 195–198.

Du Bois, D. & Du Bois, E. F. (1916). A formula to estimate the approximate surface area if height and weight be known. *Archs. intern. Med.* **17**, 863–871.

Fox, R. H., Goldsmith, R., Hampton, I. F. G. & Lewis, H. E. (1962). The mechanism of the increase in sweating capacity induced by heat acclimatization. *J. Physiol.* **162**, 59–60*P*.

FOX, R. H., GOLDSMITH, R., HAMPTON, I. F. G. & LEWIS, H. E. (1964). The nature of the increase in sweating capacity produced by heat acclimatization. *J. Physiol.* **171**, 368–376.

FOX, R. H., GOLDSMITH, R., KIDD, D. J. & LEWIS, H. E. (1963). Blood flow and other thermoregulatory changes with acclimatization to heat. *J. Physiol.* **166**, 548–562.

HILL, L. (1921). Cooling and warming of the body by local application of cold and heat. *J. Physiol.* **54**, cxxxvii–cxxxviii.

MCMANUS, J. F. A. (1948). Histological and histochemical uses of periodic acid. *Stain Technol.* **23**, 99–108.

PEARCE, A. G. E. (1960). *Histochemistry, Theoretical and Applied*, 2nd edn, pp. 204–206. London: Churchill.

SCHWARTZ, I. L. & THAYSEN, J. H. (1956). Excretion of sodium and potassium in human sweat. *J. clin. Invest.* **35**, 114–120.

URBACH, F. & SHELLEY, W. B. (1951). A rapid and simple method for obtaining punch biopsies without anesthesia. *J. invest. Derm.* **17**, 131–134.

WEINER, J. S. & VAN HEYNINGEN, R. E. (1952). Observations on lactate content of sweat. *J. appl. Physiol.* **4**, 734–744.