Methods for Local Induction and Quantitative Analysis of Human Sweat. (18797)

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The demonstration by Conn(1), that the concentration of sodium in the sweat is affected by the systemic administration of steroid hormones has given a new significance and immediacy to the quantitative study of sweat composition. When due allowance has been made for the influence of the rate of sweat flow upon the concentration of sodium, as in the standardized test of Locke *et al.*(2), it may be possible to use this phenomenon for the clinical evaluation of patients with suspected disturbance of steroid metabolism. Further progress in the exploitation of this system for pharmacological and clinical ends would seem to require a greater command of its physiology than is now available. Such a development, as contrasted with the rapid growth of renal physiology, for instance, has been delayed by want of convenient and quantitative means for the induction, collection, and analysis of sweat.

As a means of inducing the required flow of sweat, the superficial injection of a small dose of some cholinergic drug offers a number of advantages: (1) convenience, (2) freedom from general disturbance of physiological state, (3) quantitation and timing of the stimulus to the glands, (4) opportunity for comparison of the responses of symmetrical sites to different stimuli or of the same site to repetitive stimuli. This form of stimulation has been used in numerous studies, but the investigation of the glandular response apparently has been confined to the qualitative detection of emergent moisture. Quantitation of the response to local stimulation requires the use of special procedures for the collection and analysis of small quantities of sweat, since the advantages cited above would disappear in the attempt to induce sweating by this means over an area greater than a few cm^2. The capillary tube technic of Lobitz and Osterberg(3) could be used for this purpose, and would allow observation of the response of a small number of glands; however, for studies in which it is sufficient to determine the average behavior of a larger number of glands (100 to 1000, depending on the length of the collection period), the methods to be described appear to be both simpler and more exact. Actually, they are at least as simple as the classical methods of harvesting sweat in ml quantities from large areas of skin. Because they allow the complete collection of sweat from an anatomically homogeneous region and the subdivision of the collection into short, accurately timed periods, the present methods are definitely superior to the large scale collections even for the study of the sweat response to general stimulation.

Procedure. The procedures described in the present report combine local stimulation of the glands with quantitative analysis of the sweat. The flow of sweat is induced by the subdermal injection of a cholinergic drug in a dosage which is sufficiently small to make the systemic effect inappreciable, yet is massive with respect to the cells in the neighborhood of the injection. The sweat is absorbed into preweighed discs of filter paper (S & S No. 589, Green Ribbon, 25 mm diameter); the amount collected, usually between .010 and .100 ml per disc, is determined by the increment in weight. Subsequently, each disc is extracted for analysis of either sodium or urea; other substances of interest could be determined by appropriate methods of micro analysis. Evaporation is prevented during

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1. Conn, J. W., *Arch. Int. Med.*, 1949, v83, 416.

2. Locke, W., Talbot, N. B., Jones, H. S., and Worcester, J., *J. Clin. Invest.*, 1951, v30, 325.

3. Lobitz, W. C., Jr., and Osterberg, A. C., *J. Invest. Dermat.*, 1945, v6, 63.

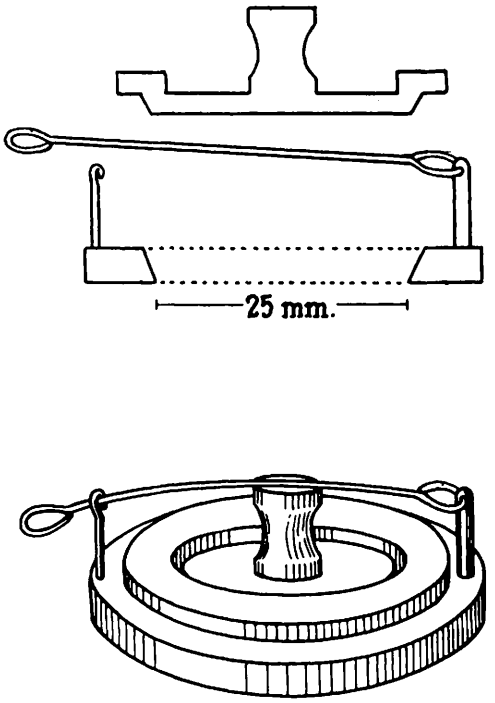

FIG. 1. Plastic Chamber for the Collection of Sweat.

the collection by a plastic unit, which consists of a Bakelite ring and a Lucite cover (Fig. 1). The important details in the design of this unit are the bevelled vertical junction between cover and ring, the flat overhang of the cover which is pressed tightly against the ring by an arc of spring wire, and the chamber of 1 mm depth that remains to accommodate the disc when the unit is closed. A satisfactory fixation of the ring to the skin may be obtained with a thin film of Duco Household Cement, providing that the ring is held firmly in place until the cement is dry, a matter of about 20 minutes. Once securely fixed, the ring may be left in place for at least 3 days. Before the test the required number of filter paper discs are placed in glass-stoppered weighing vessels (Pyrex, 30 ml capacity) and each vessel with its contained disc, is weighed to a precision of ± 0.03 mg. Thereafter each disc remains enclosed in its own vessel, except for the time of collection. The vessels are kept covered with paper hoods, and are handled with care to avoid contamination of the rim or stopper. At the completion of a test the vessels are returned to the balance room, allowed to come to temperature equilibrium, and reweighed. If the stimulus is to be local, the test area enclosed by the ring is first irrigated with distilled water, dried with analytical grade filter paper and then left open to allow observation during the injection. A 25 gauge, 1½ inch needle is introduced into the skin outside of the ring and passed in a horizontal direction until its tip lies just under the skin at the center of the area. Through it the stimulating solution is injected in a volume of 0.1-0.5 ml. The needle is withdrawn, a disc quickly set in place and the unit closed. Thereafter, at intervals of 10-30 minutes for a total of 90-120 minutes, the discs are replaced.† In case it is desired merely to collect the entire output in one long period, it is convenient to employ two discs at the same time; the water holding capacities of about 0.10 ml per disc are additive under these conditions. For the most part Mecholyl (acetyl-β-methylcholine chloride) in a dose of 0.2-2.0 mg has been used to stimulate the sweat glands; comparable results have been obtained with Prostigmine methylsulfate (0.1 mg), and Furmethide (furtrethonium iodide, 0.2 mg). Although procaine alone does not cause the excretion of sweat under the conditions of this test, it potentiates the action of Mecholyl and in addition renders the injection entirely painless. For these reasons it has usually been included in the solution for subdermal injection: Mecholyl 25 mg, procaine 20 mg (2 ml of a 1% solution), and sterile water to 12.5 ml. A volume of 0.25 to 0.50 ml of this solution will provoke a brisk local sweat response in the average subject.

After the test is completed and the weights of the samples have been determined, the discs are extracted for chemical analysis. An extract, suitable for analysis of sodium with a Perkin-Elmer flame photometer (Model 52A), is given by the following procedure. Each vessel receives 25 ml of $LiNO_3$ solution (75

† Water evaporates from a moist disc at about 1 mg/min; since transfer of disc from plastic chamber to closed weighing bottle can easily be completed within 2 seconds, evaporative loss may be neglected.

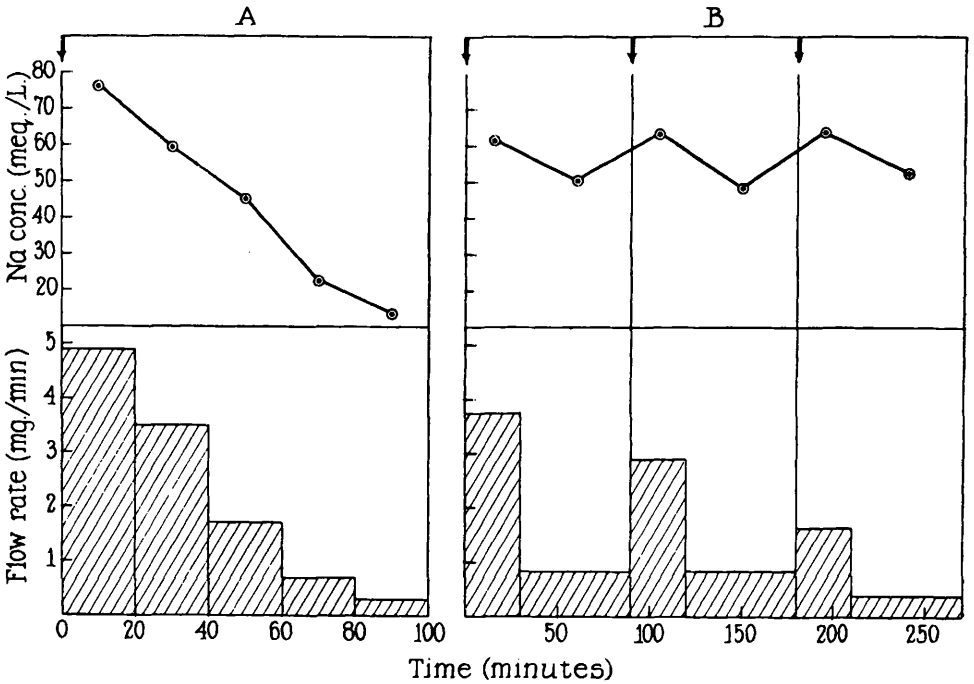

FIG. 2. The Local Excretions of Sodium and of Water Following a Single and Following Multiple Subdermal Injections of Mecholyl, 1 mg and Procaine, .8 mg. (Exp. A and B were performed on the same subject, but on different days).

meq/L); it is stoppered, shaken until the disc fragments, then decanted through porous filter paper (S & S No. 588—1H, 7 cm) to remove the pulp. Although complete extraction can be obtained without fragmentation of the disc when 12 or more hours are allowed for diffusion, the filtration cannot be avoided if the atomizer has a fine capillary, because a few fibers inevitably become detached from the disc. When the discs are to be analyzed for urea, rather than sodium, the extraction proceeds differently. To each vessel 0.1 *N* H_2SO_4 is added in volume of from 2 to 6 ml, depending on the estimated content of urea. The flasks are allowed to stand at room temperature overnight with occasional swirling, and then decanted to separate the extract from the disc. The determination of urea can be effected by a modification of the colorimetric method of Archibald(3). Aliquots of the extracts, 1 ml in volume, are pipetted into matched Pyrex cuvettes (10 x 75 mm size); equal volumes of standard solutions of urea in 0.1 *N* H_2SO_4 (6 to 24 γ/ml) and of 0.1 *N* H_2SO_4 alone are treated similarly to serve as standards and blanks, respectively. To each tube is added 1 ml of an acid mixture (conc. H_2SO_4, 180 ml; conc. H_3PO_4, 540 ml; water to a total volume of 1 liter), and 0.3 ml of a 3% solution of α-isonitrosopropriophenone in ethyl alcohol. The cuvettes are closed with rubber stoppers and shaken vigorously to mix the contents. In order to maintain the closure while the tubes are heated, a wide rubber band is stretched from the bottom to the stopper of each tube; this remains in place more securely if a small solid glass rod projects from the center of each stopper. After this preparation the tubes are immersed in a boiling water bath, from which light is excluded, and heated for 2 hours. They are then cooled to 20°C and their optical densities at 540 mμ determined in a spectrophotometer. From these data the urea concentrations of the unknown solutions are calculated in the usual manner.

4. Archibald, R. M., *J. Biol. Chem.*, 1945, v157, 507.

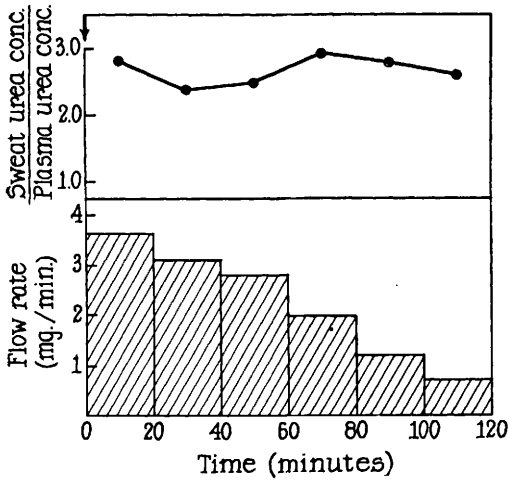

FIG. 3. The Local Excretions of Urea and of Water Following a Single Injection of Mecholyl 2 mg and Procaine 1.6 mg.

Success in application of these procedures of collection and analysis requires a meticulous chemical technic to avoid contamination; the glassware must be clean and be protected from dust in storage; all manipulations, except handling the outside of containers, must be performed indirectly with forceps or a clean glass rod. Even with great care in these respects, however, it was found that the blank value for sodium was excessive; this difficulty, which presumably arose from the extraction of sodium from glass surfaces, was eliminated by coating all glassware with silicone.‡ With attention to all details, and with siliconed glassware, the blank values for sodium and for urea should be less than .2 μeq. and .5 γ, respectively. For comparison, the amounts of sodium and of urea contained in 10 mg of sweat with concentrations of these substances in a low normal range (30 meq./L and 500 mg/L, respectively) are .3 μeq. and 5 γ; this quantity of sweat approximates the smallest amount to which the present method may be usefully applied.

Results. Typical responses to local stimulation of the sweat glands are illustrated by the graphs (Fig. 2 and 3). It may be seen that the rate of sweat flow reaches an early maximum and then declines; that this decline is due both to removal of the stimulus and to fatigue of the glands is suggested by the fact that reinjection of the drug provokes another, but lesser, output of sweat. In more detailed studies on the rate of water excretion after a single injection, it has been found that the excretion commences within 5 seconds after the injection, rises to a maximum rate within the first 20 minutes, and thereafter declines exponentially with a half time of 10 to 30 minutes.

The concentration of sodium varies in parallel with the rate of flow; this relation is similar to that found with thermal sweating (2), and, because of the more intense stimulation obtained with local injection, is demonstrable over a greater range (Fig. 2A). It should be noticed, however, that it may be affected by the preceding functional activity of the glands, since different relations between concentration and flow rate are found with repeated injections into the same area; for instance, in Fig. 2B the concentrations are as high after the third injection as after the first, despite lower rates of flow from the area. Urea, unlike sodium, is excreted at a concentration which exceeds that of the plasma and which is independent of the rate of sweat flow over wide limits (Fig. 3); with rates of excretion which are at the lower limit of the present methods there appears to be a further increase in the concentration.

Summary. Procedures are described for the stimulation of sweat by local injection of a cholinergic drug, the quantitative collection of the output from a definite area during consecutive periods, the determination of the volume and of either the sodium or the urea concentration in each sample. In their present form, these procedures are applicable to quantities of sweat between 10 and 200 mg; with slight modifications of the methods, these limits could be extended.

‡ Clean glassware by boiling in detergent solution, followed by boiling in sulfuric acid-dichromate cleaning mixture. Rinse thoroughly with distilled water; dry in oven. Expose surfaces to 2% solution of silicone (DC200, 350 Ctsk viscosity) in CCl_4; drain off excess. Dry at 100°C for 12 hours, then bake at 300°C for 30 minutes. This gives a durable coating, which remains indefinitely if exposure to detergents is minimized in subsequent cleanings.

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