STUDIES ON SWEATING

IV. A New Quantitative Method of Assaying Sweat-delivery to Circumscribed Areas of the Skin Surface*

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I. Introduction

There is a need for a relatively simple method that would permit quantitative assaying of sweat delivered to the skin surface. None of the various methods available at present has been found entirely satisfactory, and so far none has been found suitable for the exclusive assay of "sensible" sweat, i.e. the sweat coming from the sweat ducts and collecting on the skin.

The limited number of procedures affording results in units of weight [Y. Kuno (1), G. E. Burch and collab. (2, a and b), J. S. Weiner (3)] does not include any method which would be sufficiently convenient for extensive use under the ordinary conditions of a clinical laboratory. Quite recently E. Cohen (4) devised a quantitative method for determining the amount of palmar sweat by weighing a square of blotting paper before and 1) involves the risk of a weight loss through evaporation before the weighing is ended (4); 2) hardly eliminates the possibility of an admixture of "insensible sweat", fatty matter, or cellular and horny particles. In addition, the technic is still too inconvenient and time consuming for a great number of examinations within limited periods of time.

In recent years we obtained accurate measurements by means of a special electrohygrometer, but its use was likewise hardly practicable for medical examinations on a large scale, since too much time was consumed by a single determination; moreover, the intricate construction of the apparatus necessitated frequent adjustments.

The only procedure known to us for examining the degree of sweating which can be employed with relative ease, is the application of reagents which produce color changes in the presence of water. *Their use also affords a most valuable means for direct examination of outpouring sweat, exclusive of "insensible" cutaneous perspiration.* Until the present time, however, color indicators could be used only for approximations for which more or less arbitrary standards (5, 6) were chosen.

Another handicap in the application of indicator-methods is the usually strong objection of both the subjects under test and those performing the examination, to the unavoidable discoloration of clothing, skin, etc., with staining products formed by the indicator-substances in use. Thus, the dark blue salt resulting from the reaction of ferric chloride with tannic acid in the presence of traces of water [method of J. J. Silverman and V. E. Powell (7)] often causes irreparable damage to clothing and leaves stains on the skin which are removable only with difficulty. Similarly, though to a somewhat milder degree, the purple product of the starch-iodine reaction [methods of Minor (8), W. C. Randall (9), and others] is apt to soil clothing.

One of the greatest disadvantages of almost all the indicator-methods employed thus

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far (i.e. the starch-iodine methods, the Quinizarine reaction, and the ferric chloride reaction with tannic acid, when the latter is incorporated in filter paper) is inadequate sensitivity. Apart from different degrees in sensitivity, i.e. differences in the minimum volume of water which is detectable with the various procedures, each of these methods may partially or completely fail to indicate the presence of sweat (water) on the skin, because of inherent chemical peculiarities. For example, alkali interferes with the starch-iodine reaction; and acid with the change in color of Quinizarine. Inadvertent effects of this kind may be produced by natural variations in pH which occur on the skin surface. Moreover, certain chemicals which may be applied to the skin for experimental reasons are capable of artificially inhibiting "positive" reactions. Thus, cationic wetting agents interfere with the starch-iodine reaction, while the usually strongly acid aluminum salts tend to prevent Quinizarine from turning blue. Conversely, the test reactions may be intensified artificially and thereby caused to indicate quantities of sweat beyond those really present. The Quinizarine reaction, for instance, is fallaciously intensified by cationic wetting agents.

Testing by means of a prism, which was suggested by M. G. Netsky (10), is advantageous because of its cleanliness, its instantaneous applicability without any preparatory measures, and the immediate perceptibility of the findings. However, only very rough impressions are obtained with regard to the *quantity* of sweat present. In our hands, the prism was useful for gaining quick preliminary impressions, but was valueless for exact and reliable recordings.

For all these reasons we attempted to quantitatively assay the degree of outpouring sweat by an indicator-method, which would: a) be sufficiently convenient for general use; b) permit quantitative evaluation of the amount of water in the sweat delivered to the skin surface; c) reduce to a minimum the possibility of erroneous results.

II. ORIGIN AND PRINCIPLE OF NEW METHOD

The procedure on which we based our present studies was the one introduced by P. Boymond (11) for ascertaining the presence of water in pharmaceutical preparations, and which was subsequently utilized by L. Manuila (12) of Werner Jadassohn's group at the University of Geneva for studies of the axillary perspiration. The Boymond-Jadassohn-Manuila method offered us a tool of decisive value in our endeavor. These authors employed a powder mixture containing Bromthymol blue or Bromphenol blue as indicator substances, together with alkali.

The principle of the test is based on the color change of these pH indicators in aqueous alkaline media from slightly tan to dark blue. The presence of water is conditio sine qua non for the ionization which is responsible for the change in color; hence, the turn in color invariably reveals the presence of water.

Manuila covered the surface of greased ping-pong balls with the powder mixture and with gauze, placed the prepared balls for ten minutes in the axillary vaults, obtaining colored impressions of the sweat present which were used for purposes of comparison.

The method about to be described utilized the principle of this procedure modified in such a way as to make it suitable for tests on *all* areas of the skin surface and for *quantitative* estimation of sweat delivery. Sweat prints were taken from the skin area under investigation and compared with a series of standard-prints prepared with measured quantities of water (or sweat).

III. DESCRIPTION OF METHOD

a). Formula of Indicator-Powder and Preparation of Filter Paper Squares

Numerous pieces of 5 x 5 cm. of salt-free filter paper[1] were treated with an adherent powder mixture of the following composition:

Bromphenol Blue[2]	5.0 Gm.
Sodium Carbonate (pure, finely ground)	15.0 Gm.
Corn Starch	40.0 Gm.
Gum Tragacanth	40.0 Gm.

The powder was placed to a depth of $\frac{1}{2}''$ at the bottom of circular glass jars (about 10 cm. high and 8 cm. in diameter) with well fitting screw caps. The filter paper squares were vertically and loosely placed in a jar; after tightly capping the jar, the contents were vigorously shaken for 15 min. and then allowed to stand. The powder was replaced about every month by a freshly prepared mixture. Powder which assumed a distinct bluish tint was discarded. Immediately before use of the filter paper, the jar was again shaken for approximately 5 min., the squares were removed with ivory tipped tweezers and the excess powder was shaken off.

b). Preparation of Standard Prints for Comparison

Quantities of 0.1 to 50 mgm. of water, or pooled sweat, were dropped from a capillary pipette onto individual squares of sandpaper[3], each 2 x 2 cm. in size. The droplets were evenly distributed over the entire square with the shaft of a thin glass rod. Immediately before, as well as immediately after the addition of water or sweat, the squares were weighed on an analytical scale in order to ascertain the amount of water spread over the surface. Instantly after the second weighing, prints were made of the water pattern on the squares, by placing a sheet of the prepared filter paper on the surface of the sandpaper and keeping it there for 10 seconds. Moderate and uniform pressure was exerted on the filter paper with the flat base (5 cm. in diameter) of a mortar. All the prints (for the calibration as well as for the examination on the skin) were taken by the same (two) persons.

Series of prints were collected from 5 to 10 different squares of sandpaper sprinkled with the same, or almost the same quantity of water or sweat, in order to have on record possible variations in the pattern produced on different occasions by a given amount of water.

In a large series of control tests, the epidermal surface (2 x 2 cm.) of human cadaver skin (epidermis plus cutis) was used instead of sandpaper. The skin specimens were obtained immediately post-mortem. The post-mortem skin failed to produce a print on our prepared filter paper unless water or sweat was deliberately placed on its surface.

[1] Genuine Whatman Filter Paper, No. 2, W & R Balston, Ltd., England.

[2] Manufactured by Hartman-Leddon Co., Inc., Phila., Pa.

[3] 3M Imperial Flint Paper 1,10″, 3M Company Minnesota Mining and Mfg. Co., St. Paul 6, Minnesota.

Known quantities of water or pooled sweat were dropped onto the skin either at random, or in a regulated pattern through the holes of a sieve suspended above the epidermal surface. The use of a sieve made it possible to mechanically distribute equal quantities of water over the desired area in evenly spaced patterns.

It was found necessary to spread a minute quantity of waxy material—comparable to the range in quantity found on healthy human skin (13)—over the surface prior to the addition of sweat or water. A weighed film of 0.2 to 0.9 mgm. of the residue of pooled ether collections from human skin-surface served this purpose.

All the prints obtained from both sandpaper and the human skin were assembled as standards for comparison with the sweat patterns of our test subjects (see Fig. 1).

Although the color of the prints is permanent without further processing, they were framed under glass like Kodachromes, in order to avoid contamination.

To facilitate the matching of test prints with the standard prints, the latter were arranged in successive 5 mgm. groups, i.e. in groups of patterns obtained with water quantities 1) up to five mgm.; 2) from five to ten mgm.; 3) from ten to fifteen mgm. and so forth.

c). Performance and Reading of Tests

When the amount of sweat delivery was assayed on one of the larger surfaces of the skin (forehead, trunk, extremities, etc.), the sites to be tested were defined by means of a stencil and then marked by an inked metal ring, 3.5 cm. in its inner diameter. Several cardboard-stencils were on hand, fitting the back, chest, or abdomen, etc. They were perforated in regular and symmetrical fashion by square holes (4 x 4 cm.). In this way it was possible to ensure symmetrical situation of two test areas, identification of the sites for repeated examinations, or comparison of the results on certain sites in different subjects, etc., depending on the purpose of the individual investigation.

A square of the prepared filter paper was held for ten seconds under moderate pressure on the test area. This was accomplished by means of the base of a mortar, in the same way (and by the same two persons) as in the printing of the standard records.

The prints obtained from the skin were matched with the standard patterns produced by known quantities of water.

To aid the comparison, a mask of white cardboard with a hole of 2 cm.^{2} was placed with its opening over the center and then successively over several other parts of the test print. Thus, each of the segments exposed was equal in size to a standard print.

It is obvious that perfect identity and consequently, perfect matching of any test print with the corresponding standard pattern were practically impossible. It was for this reason (and to avoid "pseudoaccuracy"), that the prints produced by unknown amounts of sweat were matched with the closest

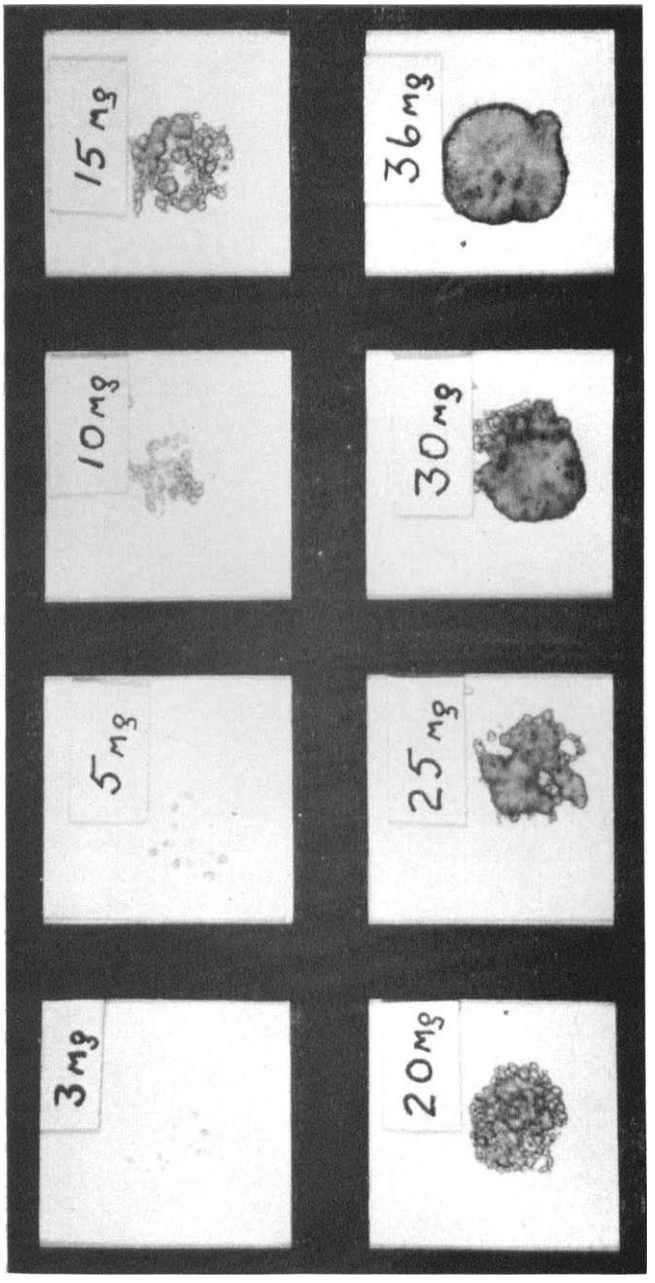

FIG. 1. Examples of standard prints produced by different amounts of water on filter paper prepared with Bromphenol blue powder.

pattern of the standard prints and purposely recorded in the 5 mgm.-group which included these matching standard prints. Hence, the readings of the test prints were designated by the following "equivalent" amounts of water:

Up to 5 mgm. per 2 cm.^{2}	(Group I)
5–10 " " " "	(Group II)
10–15 " " " "	(Group III)
15–20 " " " "	(Group IV)
20–25 " " " "	(Group V)
25–30 " " " "	(Group VI)
More than 30 mgm. per 2 cm.^{2}	(Group VII)

Evidently, the highest figure of one group is identical with the lowest figure of the following group, i.e., it was possible to designate "borderline prints" by either one of the two groups.

Readings in excess of 30 mgm. could not be distinctly differentiated, but at best only approximated. The confluescence of the larger drops resulted in a degree of blotting which produced rather similar prints, even when the difference in the effective quantities of water (or sweat) were relatively great (see Fig. 1).

IV. DISCUSSION OF METHOD

Comparison of the method just described with the gravimetric method of Cohen (4) showed rather close agreement in the results[4].

In comparative examinations with other indicator-methods (Quinizarine[5], ferric chloride-tannic acid[6], starch-iodine, Bromo-Acid) our method proved superior in sensitivity to all the other procedures.

It must be admitted, however, that when a surface (skin or sandpaper) was experimentally prepared with a strongly acid solution, e.g. aluminum chloride, subsequent sweating or added droplets of water partially or completely failed to produce the *blue* stain on Bromphenol blue paper. The blue stain was, nevertheless, replaced by a bright brownish-yellow color, which distinctly indicated the presence of water droplets. The appearance of the yellowish color, therefore, excluded the danger of erroneously "negative" readings which would result from excessive acidity. This effect, moreover, can be produced only by artificially (experimentally) decreasing the pH on the skin to ranges below the natural limit.

Both the Quinizarine and starch-iodine reactions are modified in a similar

[4] In 39 tests performed on symmetrical sites which were neither visibly exfoliating nor pathologically greasy, the results obtained by Cohen's method (modified only by applying the blotting paper to the skin for 10 seconds) did *not* deviate from our results in 15 tests; deviated from our results by less than ± 3.0 mgm in 13 tests; by less than ± 6.0 mgm in 9 tests; by -7.1 mgm in 1 test; and by $+11.6$ mgm in 1 test.

[5] Extensive trials were carried out in our laboratory with filter paper impregnated with a powder mixture containing Quinizarine.

[6] Of all the indicator-methods we tried, ferric chloride-tannic acid when applied directly to the skin, proved to be the most sensitive. However, this indicator combination when modified for *printing* was far less sensitive than our method with Bromphenol Blue.

way by acidity and alkalinity respectively, but in contrast to the bright brown of the Bromphenol Blue, the brownish tints replacing the ordinary color-reaction of these tests are much too indistinct to rule out misinterpretation of the results.

With regard to undesirable staining, Bromphenol blue involves less danger of staining clothing, skin, etc. than any of the other procedures employed to test sweating by color-reactions, since Bromphenol blue stains are easily removable.

A relatively minor disadvantage of the new method is a moderate irritation of the upper respiratory mucosa, sometimes felt by the investigators during preparation of the powder and filter paper. This difficulty was obviated by the use of a respirator ("gas mask") by the most sensitive operators.

The sandpaper appeared to furnish a suitable model-surface, since the multiplicity of the different surface angles allowed the partition of added water or sweat into droplets of the desired size and distribution. The adequacy of the sandpaper was confirmed by the fact that *practically identical patterns were obtained from sandpaper and fresh cadaver skin when equal amounts of water or sweat were used.* We feel, therefore, that in order to simplify the preparation of standard prints, sandpaper alone would be entirely satisfactory.

It was interesting to note that the added droplets did not remain on the surface of cadaver skin, unless traces of ether-soluble material were spread over the surface. The waxy coat prevented the sweat (or water) from diffusely soaking into the keratin. However, if the applied waxy film exceeded physiologically encountered quantities (i.e. 0.5 mgm. per $cm.^2$), again formation of droplets did not take place, since the water was emulsified by the waxy coat.

It must be admitted that the distribution of droplets, artificially generated on sandpaper or post-mortem skin has no relation to the natural pattern of sweat droplets. However, test patterns obtained from skin *in vivo* by any "printing" method are also liable to represent artificial distortions of native sweat droplets. During absorption by the paper, the droplets undergo disfiguration, and enlargement and very commonly become confluent. Examinations through a stereoscopic wide field microscope, prior to the taking of prints, have convinced us that these alterations of the native patterns are unavoidable. It was also apparent from these examinations that the sweat droplets must reach a certain size to produce a discernible print.

We felt justified in assuming that the quantities of sweat on the skin in vivo would produce patterns comparable with the standard patterns produced by the equivalent amount of water or sweat, since repeated standard prints obtained with the same volume of water were very similar. Furthermore, there was no noticeable difference between the standard patterns produced by water and those produced by sweat when equal amounts of the liquids were used. In addition, the prints obtained from post-mortem skin after evenly spacing the droplets by means of a sieve, were similar to the prints obtained after random dispersal of the same volume of water (or sweat) over the specified area of either cadaver skin or sandpaper. In order, however, to further obviate the possibility of erroneous readings, no attempt was made to estimate any test pattern pre-

cisely, but each was approximated within 5 mgm. by placing it in the 5 mgm.-group which included the best matching standard print.

The physico-chemical explanation for the observed phenomenon—namely, that equal quantities of water distributed by whatever means over the specified area of sandpaper or post-mortem skin produce very similar prints—appears to us beyond the scope of this paper.

To date, some 5,500 tests have been carried out in 40 subjects. Most of the tests were performed several times in the same persons under comparable conditions. There was satisfactory agreement among the results of these repeated tests, as will be shown in our subsequent report (14). The data also revealed a constant and characteristic distribution of the amounts of sweat on different areas of the body surface. By and large, the values were similar to those obtained with more intricate procedures by several previous investigators. These observations, as well as the comparative control examinations with the new gravimetric method of Cohen are regarded as indications of the efficacy of the method for assaying "sensible" sweat.

V. SUMMARY

1. A relatively simple method is described which permits rapid and sufficiently accurate quantitative estimation of sweat delivery to small circumscribed areas of the skin-surface.

2. In this method a powder mixture containing Bromphenol blue (introduced by Boymond, Jadassohn and Manuila for the detection of water or sweat) is incorporated in filter paper. Colored test prints are obtained on the filter paper and matched with standard prints produced by measured quantities of water or sweat.

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