was subtracted from the final reading so that this increased conductivity measured in Mhos was directly related to the sodium chloride concentration. Fig. 2 was prepared from determinations of known concentrations of sodium chloride. There is a linear relationship between conductance§ and sodium chloride concentration.

The standard error was calculated from 20 determinations on a standard solution containing 125 me/L. The dilutions were made with the micropipette in a manner identical to the handling of actual sweat. The deviations of these determinations were used to calculate the standard error of the mean. The standard error of the mean was 3.8 x 10^{-7} Mhos which corresponds to less than 4 milliequivalents. These determinations were done with a dip cell. The accuracy has been further increased by the use of the Henry cell.

Summary. The fact that the ionic content of rapidly secreted sweat consists almost entirely of sodium chloride make feasible use of conductivity measurement to determine the sodium chloride content of micro quantities of sweat. This method has a high degree of accuracy and is at the same time simple and rapid.

§ Conductance is the reciprocal of ohms and is expressed in mhos.

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A Method for Collection of Micro Quantities of Rapidly Secreted Local Sweat.* (22092)

HARRY H. LEVEEN.
Department of Surgery, Chicago Medical School and Michael Reese Hospital.

In many diseases it is recognized that disturbances in salt and water metabolism occur(1). It is not known whether the salt retention or losses which are seen as manifestations of some diseases are caused by variations in the minute to minute blood levels of mineraloid hormone activity. These gaps in our information arise from the fact that there is no simplified clinical and investigative tool for testing the overall activity of the salt hormones of the adrenal cortex and other organs. Urinary hormone assays of aldosterone can at best measure only gross changes which occur over a 24-hour period. It is a painstaking laboratory examination which can be done only in a few specially equipped research laboratories. The demonstration by Conn(2) that the concentration of sodium or chloride in perspiration mirrors the mineraloid activity of the adenal cortex provided the basis for the achievement of such a practical test. Most investigators have used hot rooms and other forms of thermal stimuli to produce diaphoresis, and have collected the sweat from an extremity in a water tight envelope made of plastic or thin rubber. The difficulties inherent in this method of sweat collection are obvious. Aside from the difficulties of collection, the electrolyte concentration of sweat has been shown to be dependent upon the rate of sweating. For this reason, it is best to measure the electrolyte concentration at the maximal rate of sweat production(3). It is also essential to be able to take frequent collections of small samples which are unmixed with a large dead space volume of a different concentration. Dole *et al.*(4) have devised a small chamber which contained an absorbent filter paper. Sweat was induced in the skin under the chamber by the injection of a parasympatheticomimetic drug in this area. While this has greatly simplified the collection of

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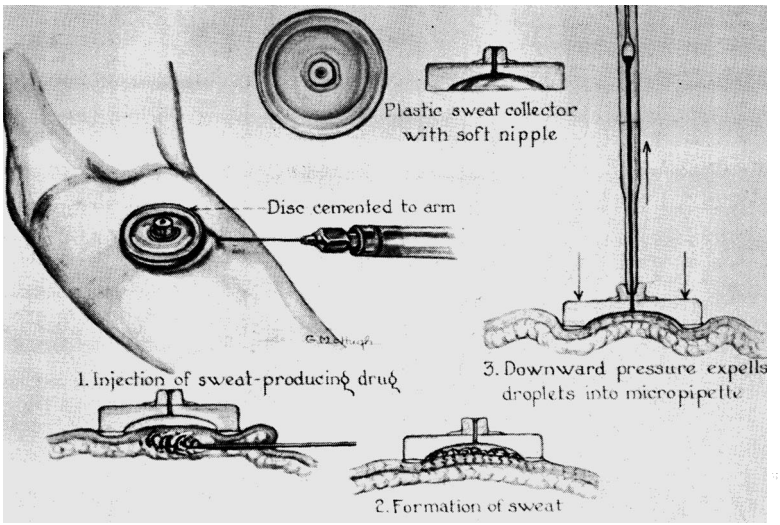

FIG. 1. Demonstration of manner of induction and collection of small quantities of sweat. The design is somewhat different than illustrated in the top center cross-section above. The walls of the cavity are convex rather than concave.

small amounts of sweat, only 10 mg of sweat are collected in about 20 minutes. Even with the best precautions in handling, and with precautions in the prevention of evaporation, it is difficult to achieve a high degree of accuracy when handling such minute quantities. The filter paper is then pulped to extract the sodium from it and hence a high degree of dilution of the collected sweat is required.

Materials and methods. Sweat is induced by local injection of cholinergic drugs. Although we originally used only acetyl beta methyl choline the solution suggested by Dole is excellent. Its formula has been slightly modified by the use of saline rather than distilled water in a more convenient amount: Acetyl beta methyl choline (Mecholyl), 25 mg; procaine, 20 mg; sterile isotonic salt solution, 10 ml. Sweat is collected in a small round plastic disc with a central cavity. A capillary hole communicates with the center of the concavity. The diameter of the concavity is one inch and its depth ⅛ inch. This covers a collection area of 5 cm^2 of skin when the collection chamber is cemented onto the skin. The sweat which collects in the concavity can be seen through the transparent plastic, but is protected from evaporation until collection is desired. On the outer flat surface a small circular piece of rubbery vinyl chloride plastic (Tygon or Koroseal) is cemented so that it coincides with the capillary hole in the lens. The completed assembly is shown in Fig. 1. The design now used is slightly different than illustrated. With concave dome-like collection chamber as illustrated, the sweat and bubbles were sometimes trapped at the periphery of the chamber by occlusion of the capillary hole by the skin. If the sweat collecting chamber has converging (convex) side walls, this does not occur. The vinyl chloride plastic teat is made to accommodate the tip of a λ pipette (λ = .001 ml). The type of pipette best suited for this purpose is one which is calibrated to contain rather than to deliver the required amount. The pipette is then washed out with the diluting fluid to make certain the transfer is complete. For routine work 10 λ pipettes are used although they may be purchased in any size up to 500 λ. Graduated pipettes are ideal for determining secretory rates. Although not recommended for this use, ordinary hemoglobin pipettes are 20 λ (20 cmm or .02 ml) and some have graduations at 10 λ and 20 λ.

Sweat is collected from a smooth and flat flexor surface of an extremity or more usually the back. If there is abundant hair, the site should be first shaved. The skin is then cleansed with a 1/1000 solution of aqueous

zepharin and thoroughly rinsed with distilled water. The skin is then dried by blotting and rubbed off with acetone. This process degreases the skin and allows for better adhesion of the plastic disc. The chamber is cemented onto the skin by moistening the outer edge of the disc with "Duco Cement." When the rim is tacky (not fluid), it is pressed against the prepared skin site and held for 3 to 5 minutes.

After fixation of the disc, the skin outside the disc punctured with a fine gauge 1½-inch needle, the point of which is directed toward the center of the disc 0.5 ml of the diaphoric substance is injected subcutaneously (Fig. 1, 1,) with a tuberculin syringe. Local diaphoresis is rapid and copious. In 5 minutes an excess of 20 λ has usually been secreted.

When it is desired to collect the sweat, the arm is placed so the air bubble is near the center of the disc. The tip λ pipette is engaged with the vinyl plastic bottom and gentle downward pressure exerted on the disc. This causes the skin to bulge into the sweat collecting chamber and expresses the sweat into the pipette (Fig. 1, 3). It is best to slightly over fill the pipette and to remove the excess fluid from the tip by capillary action. If this is done, the tip should be wiped with a silk rag rather than filter paper or cotton which may be too highly absorbent and remove the excess too rapidly. Automatically self-filling pipettes are also available from most laboratory supply houses. The sweat is then transferred to the dilution fluid and prepared for flame photometry, or other chemical analysis. We have used the conductivity method to determine sodium. It is described in the preceding paper.

Summary and conclusion. A simplified method of collecting and measuring small quantities of rapidly secreted sweat is described. This method employs local chemical induction of sweating and the collection of this sweat in a hollowed out plastic chamber. The sweat can be transferred to a λ pipette by expressing the sweat through a capillary orifice in the chamber.

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An Antimalarial Effect *in vitro* of Parapyruvic Acid. (22093)

WILLIAM TRAGER AND IRA SINGER.
Laboratories of the Rockefeller Institute for Medical Research, New York City.

When preparations of the bird malaria parasite *Plasmodium lophurae* were removed from their host duck erythrocytes and maintained extracellularly *in vitro,* their survival and development were favored by the addition to the culture medium of both adenosinetriphosphate (ATP) and sodium pyruvate (1,2). It was noted that a commercial preparation of sodium pyruvate did not have the favorable effect obtained with sodium pyruvate prepared in the laboratory(3). This fact, combined with the recent isolation by Montgomery and Webb(4) of an impurity present in some samples of sodium pyruvate, which inhibited the tricarboxylic acid cycle in isolated mitochondria, suggested an investigation of the possible antimalarial effects of this impurity. Two samples of this material, identified as a lactone of parapyruvic acid (the dimer of pyruvic acid), have been obtained through the kindness of Dr. Carmel Montgomery. They have been tested against *P. lophurae* maintained *in vitro* both extracellularly and intracellularly, as well as against the infection *in vivo.*

Materials and methods. The lactone of parapyruvic acid was dissolved in water (redistilled in a Pyrex glass still), and the solu-