UREA EXCRETION IN HUMAN SWEAT AS A TRACER FOR MOVEMENT OF WATER WITHIN THE SECRETING GLAND

By IRVING L. SCHWARTZ*, M.D., JØRN HESS THAYSEN, M.D., AND VINCENT P. DOLE, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

(Received for publication, September 19, 1952)

Previous investigators have shown that the concentration of urea in human sweat is greater than the concentration of urea in plasma (1–7). Almost nothing is known, however, about the processes which produce this difference in concentration. As a first step toward understanding these processes, systematic data on the excretion of urea in sweat have been obtained under varied conditions of glandular activity and concentration of urea in plasma. The results indicate that urea probably is brought into the gland in some precursor solution, and then raised to a higher concentration by the reabsorption of water from this solution.

Procedure

Thirteen adult human beings served as subjects. One of them was normal, ten had benign hypertension, and two had severe hypertension with terminal uremia. The concentration of urea in the plasma of the non-uremic patients was varied in the range of 10 to 40 mg. per 100 cc. by variation of dietary protein from 0.12 to 1.5 gm. per kg. day. Higher concentrations of urea in the plasma were produced transiently by the oral administration of 50 to 75 gm. of urea. Sustained elevations of the concentration of urea in plasma were present in the two uremic patients, one of whom was studied both before and after the appearance of uremia. Together, diet, orally administered urea, and kidney disease caused the concentration of urea in plasma to vary over a range of 12 to 300 mg. per 100 cc.; the distribution of these values can be seen in Fig. 2.

One hour before a test the subject was given either 250 cc. of water or from 50 to 75 gm. of urea in 250 cc. of fruit juice; thereafter, water was available *ad libitum*. The test areas, either both inner surfaces of the forearms or anterior surfaces of the thighs, were washed with distilled water and dried with filter paper. Two or more aluminum rings of the collection unit (Fig. 1) were fixed on the skin with Duco cement, and held in place for 15 minutes to insure a firm, leak-free seal. After this was done, sweating was induced within each ring by an intradermal injection of mecholyl (β-acetylmethylcholine-HCl, 2 mg. in isotonic saline) through a needle (25 gauge, length 1.5 inches) introduced into the skin outside the ring and passed intradermally until its tip lay in the epidermis at the center of the encircled area. This procedure was considered standard and used in 38 experiments. Alternatively, in four

* Research Fellow of the American Heart Association; supported in part by the Rosenstock Memorial Foundation.

experiments, sweating was induced by use of a hot room (air temperature 40°C.). This method was much less convenient than local stimulation and was used only to learn whether the concentration of urea in the sweat was the same after local stimulation as in the normal response to heat. In either case sweat was allowed to flow for about 5 minutes before the collections were started, in order to establish a relatively steady state of production. Thereafter, sweat was collected during three to six accurately timed periods of about 20 minutes each. As described previously (8), the sweat was absorbed onto discs of filter paper; its quantity determined by weight, and its content of urea by microadaptation of the colorimetric method of Archibald (9). In the earlier work a plastic ring and cover were used to prevent evaporation during the collection period, but with continued use these became unreliable because of warping. At present an aluminum unit with a rubber compression gasket (Fig. 1) is in use and has proven quite satisfactory. Samples of venous blood were drawn at intervals

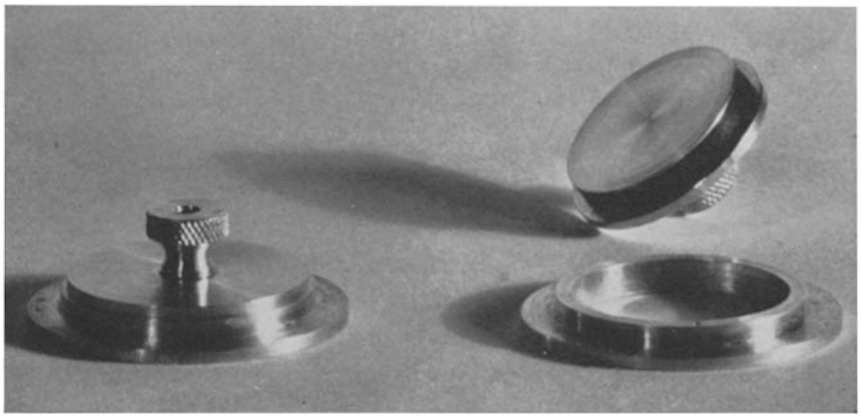

FIG. 1. Aluminum unit for the collection of sweat. The chamber formed when the lid is fixed into the ring has an internal diameter of 25 mm. and a height of 1 mm.

during the tests, precipitated by the Somogyi zinc method (10), and the filtrate analyzed in the same manner as the sweat.

Whereas the majority of experiments were planned to provide a constant concentration of urea in the plasma during the periods of sweat collection, in two a sharp rise in the plasma urea concentration was produced in order to test the time relation between the removal of urea from plasma and its delivery onto the skin. In these experiments six periods of sweat collection preceded the oral administration of urea (50 gm. in 250 cc. of fruit juice taken within 1 minute). This was followed immediately by six more periods of sweat collection. Samples of blood were taken during each of the collection periods preceding the administration of urea, and thereafter at 2 to 10 minute intervals in order to determine the rate of increase of urea concentration in the plasma following the oral dose. Some 10 minutes of collection were required to provide enough sweat for accurate analysis; for this reason any delay in the appearance of urea would have to be several minutes in length to be measured. This limit of resolution, although barring a study of the finer dynamics of sweat production, allowed a critical test of the possibility that urea might have accumulated in the sweat glands during a long period of inactivity on their part and might have merely been washed out when the flow of water through them commenced.

The precision of the measurements was estimated in a recovery test. A solution contain-

ing 60 mg. of urea per 100 cc. was delivered onto discs placed in 18 weighing vessels, six of the vessels receiving about 10 μg. of urea, six about 20 μg., and the remaining six about 40 μg. The size of each sample was measured by weight and the quantity of urea by colorimetric microanalysis just as in the routine procedure. A statistical analysis of the results yielded a coefficient of variation of 6 per cent, and showed the variance to be independent of differences in the absolute quantities brought to chemical analysis. The determination of urea in the plasma was subject to a 4 per cent variation, this value being lower than the preceding

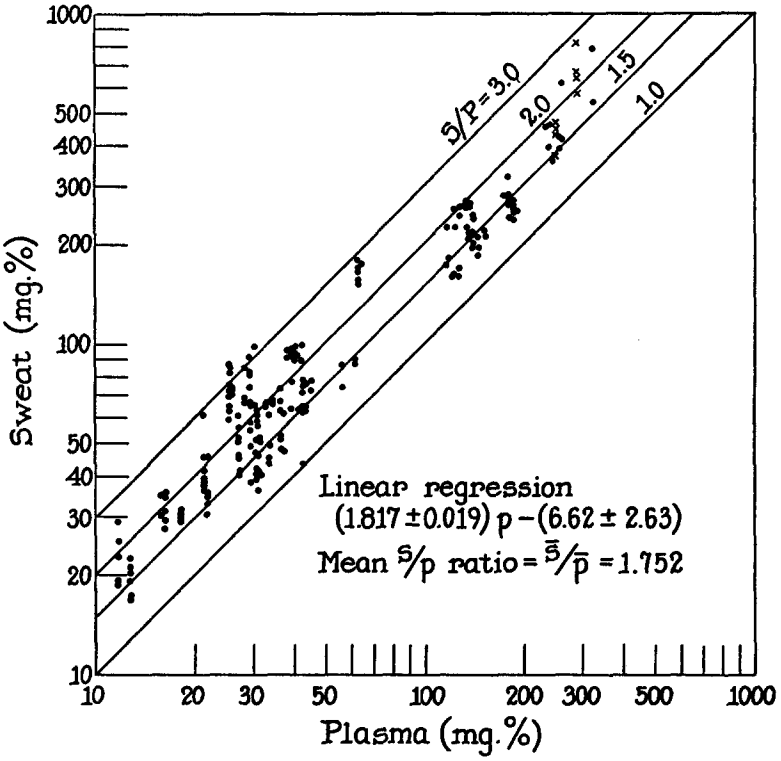

FIG. 2. Relation between urea concentrations in sweat and plasma. The data are plotted logarithmically so that proportional variations follow parallel straight lines. Data from the uremic patients are indicated by the symbol, ×.

because the volume of samples could be measured without appreciable error. When these values were combined, the variation of the ratio of the concentration of urea in the sweat to the concentration of urea in the plasma attributable to technical errors was estimated to be about 7 per cent.

Analysis of the Findings

The primary data are set forth in Figs. 2 to 5. Fig. 2 shows the concentrations of urea in the samples of sweat in relation to the concentrations of urea in the corresponding samples of plasma. Fig. 3 shows the ratio of the concentration of

urea in sweat to the corresponding concentration of urea in plasma, S/P, in relation to the flux of urea through a unit area of skin. Fig. 4 shows the ratio, S/P, in relation to the flux of water through a unit area of skin. Fig. 5 shows the

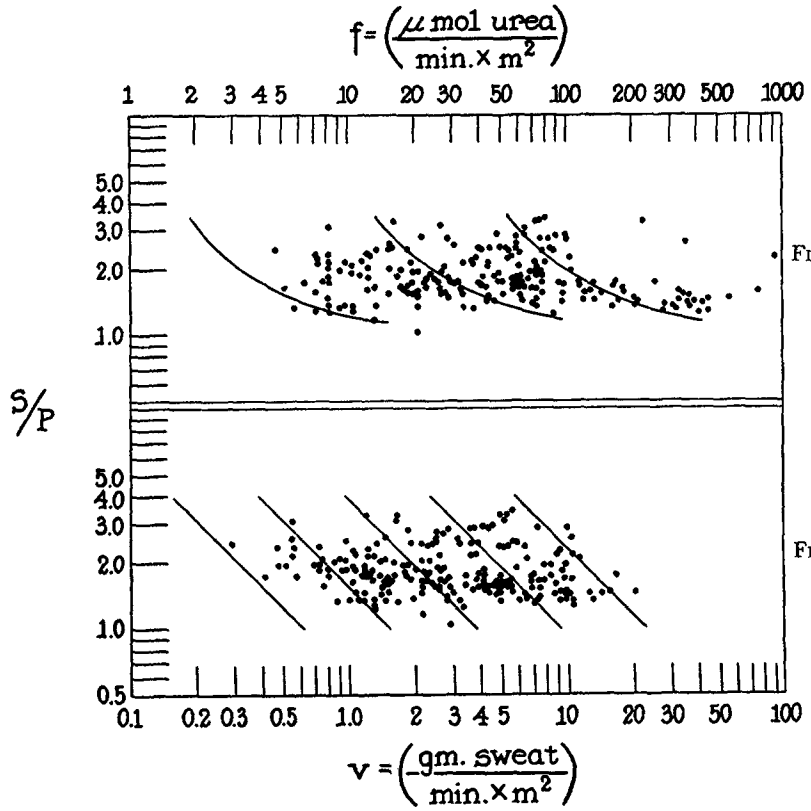

FIG. 3.

FIG. 4.

FIG. 3. Relation between the ratio of the concentration of urea in sweat to the concentration of urea in plasma, S/P, and the flux of urea through the sweat glands. The parallel curves in the background represent constant values of thermodynamic work.

FIG. 4. Relation between the ratio of the concentration of urea in sweat to the concentration of urea in plasma, S/P, and the flux of water through the sweat glands. The parallel lines in the background represent constant values of the clearance, SV/P.

change with time in the concentrations of urea in sweat and plasma during an experiment in which urea was administered orally.

The concentration of urea in the sweat remained proportional to the concentration in plasma, although in the latter it varied from 12 to 300 mg. per 100 cc. (Fig. 2). Therefore the process responsible for the greater concentration of urea in sweat as compared to plasma was unaffected by a 25-fold variation in the number of molecules transported. Exposure of the sweat gland tubules to the chronic load presented by uremic blood did not result in ac-

commodation in the physiological sense of the term, the relation of the concentration of urea in the sweat to the concentration of urea in the plasma in the uremic patients (crosses in Fig. 2) remaining the same as that in the non-uremic subjects.

Numerical analysis of the data from 200 periods yielded the linear regression $S = (1.817 \pm 0.019)P - (6.62 \pm 2.63)$. After correction for the regression the

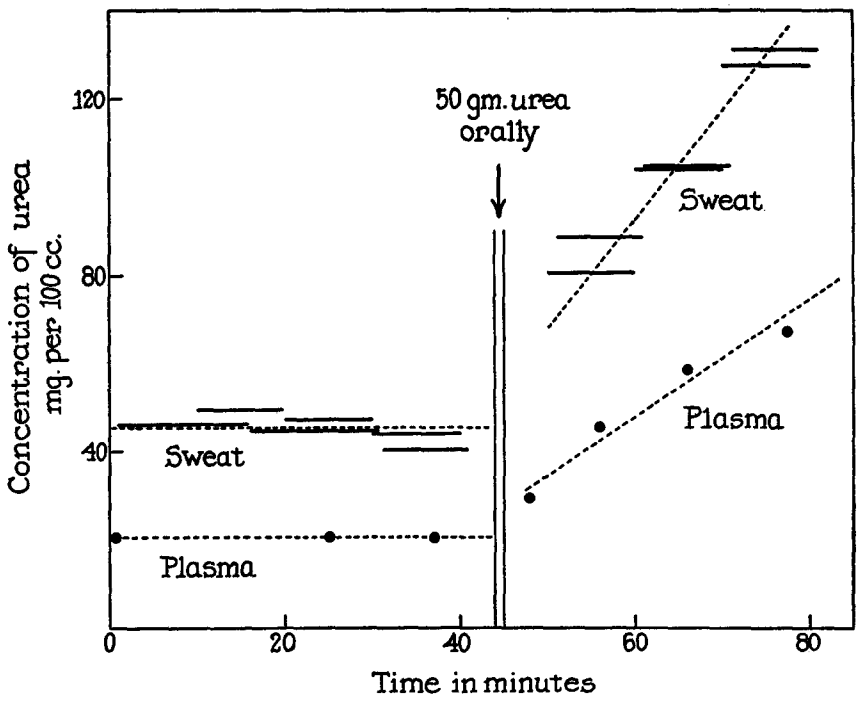

FIG. 5. Urea concentrations in sweat and plasma during a period of rapid increase in plasma concentration. The horizontal lines indicate the concentrations of urea in sweat, and the time relationships and lengths of the collection periods. Dashed lines have been fitted by inspection for approximate estimation of the delay time, t, from the relationship, $t = \frac{rP - S}{a}$. Solid circles represent urea concentrations in plasma. $r = 2.19$, $a = 2.48$ mg. (100 cc.)$^{-1}$ (min.)$^{-1}$, $t = 5.1$ min.

coefficient of variation of S was 4 per cent, approximately of the magnitude to be expected from technical errors, since the values of S were determined from an average replication of fourfold. However, a more detailed analysis of variance showed significantly ($p < 0.01$) greater variance among the mean values of different experiments, even on the same person, than among the consecutive values of single experiments. From these results it can be concluded that the residual variation of S, after allowance for the dominant influence of P, was due in small part to physiological differences and in major part to errors of

technique. The fact that physiological effects entered into the residual variance to such a small extent can be explained by the standardized conditions of the study. Preliminary observations on the initial discharge of the glands after stimulation and the terminal discharge of glands near exhaustion suggest that greater physiological variance of the ratio, S/P, might be encountered under both circumstances, but these extremes were excluded from the present study.

The minimum rate of energy expenditure required for the excretion of urea was approximated by the relation $E = KF \log S/P$, in which S and P are the concentrations of urea in sweat and plasma, respectively, F the outward flux of urea, and K a constant which depends on the choice of units. If the accumulation of urea in sweat were determined by a limitation of energy, it would be expected that the data would be distributed along a trajectory paralleling one of the curves in the background of Fig. 3. It is evident that the points are not so constrained; the accumulation is limited neither by energy nor by a maximum value of the flux (Fig. 3).

Statistical analysis of all the individual experiments showed that the ratio of the concentration of urea in the sweat to the concentration of urea in the plasma, S/P, was independent of the rate of sweat production. Fig. 4 is a composite of all of the data showing that there is no regression of the ratio, S/P, on the volume flow of sweat, V. If the flux of urea at any given level of plasma concentration were constant, the ratio, S/P, would vary inversely with V and the points would be distributed along one of the lines of constant clearance in the background of the figure. The points, in fact, do not follow any of these lines (Fig. 4).

A delay in the transfer of urea from plasma to sweat was demonstrated in the experiments in which urea was fed orally. This delay was manifested by a depression of the ratio, S/P, since the sweat that emerged at any instant had been formed from an earlier plasma of lower concentration. The delay time, t, may be estimated from the relation, $t = \frac{rP - S}{a}$, in which r is the ratio, S/P, during the preceding period of equilibrium, P and S are concurrent concentrations of plasma and sweat, and a is the rate of increase with time of concentration in sweat. The delay in transfer of urea from plasma to sweat, t, was calculated to be 4.5 minutes in one experiment and 5.1 minutes in a similar experiment on another individual (Fig. 5). It is clear from this result that the accumulation of urea was a continuous process, practically concurrent with the discharge of sweat.

DISCUSSION

It is convenient to divide accumulative processes into two classes, direct and indirect. A direct process may be defined as one whereby a specific cellular apparatus removes molecules from solution, propels them during an intracellular transit and discharges them into another solution at a higher thermo-

dynamic potential. These features imply that a direct process must be subject to a definite upper limit which may be determined by a maximum rate of exchange at the finite number of receptor sites or by the delivery of usable energy to the active loci. An indirect process, on the other hand, is incidental to the transport of another component, most commonly water. In this case part of the solution is segregated and then concentrated by the reabsorption of water. The output of solutes in an indirect process does not depend upon receptor sites and may not increase the net requirement for energy. Of course, the presence of the solute is reflected in the net energy requirement, but there is no necessary relation between the net energy change of a total process and the change in potential of a single component. The sweat, in particular, is markedly hypotonic to the plasma because of the lower concentration of electrolyte; consequently the water in sweat is at a higher thermodynamic potential than the water in plasma and the accumulation of urea actually decreases rather than increases the net osmotic work.

The data show that the excretion of urea in sweat remains simply proportional to the amount in plasma despite large variations in the excretion rate. This lack of saturation of the transfer mechanism suggests that the urea in sweat is concentrated by an indirect process. It remains possible, although unlikely, that the delivery of urea molecules from the blood to the gland cells is so limited by a meager flow of blood that a direct process remains unsaturated even when the concentration in plasma is elevated 25-fold. If we reject this possibility, we are led to the conclusions that at least half of the water entering the sweat gland is reabsorbed into the blood, and that the fraction of water reabsorbed is constant over a wide range of flow rates.

A comparison of the sweat gland and the human kidney shows both a similarity and a difference. Both appear to raise the concentration of urea by reabsorption of water from a precursor solution (11, 12). They differ, however, in the effect of flow rate on the concentration of urea: when the output of water is varied the sweat gland maintains a fixed concentration of urea, whereas the kidney shows a reciprocal variation of concentration and volume flow. The constant flux of urea from the kidney may be explained by the constancy of glomerular filtration, which is maintained despite large variations in the outflow of urine (13). No such constant production of precursor solution could account for the fixed concentration of urea in sweat. On the contrary, the result is best explained by the assumption that a constant fraction of water is reabsorbed from the precursor solution, and therefore that variations in the outflow of sweat simply reflect variations in the formation of precursor. To illustrate such a process, it may be recalled that the damaged kidney of chronic nephritis excretes a urine of fixed composition, and, having lost the power to vary the reabsorption of water from the tubules, alters output of water only by variation of the filtration rate (14).

It is tempting to speculate on the anatomical basis for an internal circulation

of water in the sweat gland. The most obvious way in which a solute can be accumulated by a circulation of water in such a system is to bring the water into a tubule through some wall that is permeable to the solute and to take part of the water back through a region of lower permeability. There exists in the sweat gland an anatomical differentiation which might serve this purpose. At the base the coiled tubule is lined with a single layer of epithelial cells, while the duct has a double layer of epithelial cells with a hyaline layer along the luminal border of the cells (15). If it be assumed that these are regions of greater and lesser permeability to urea, respectively, and that a reabsorption of water takes place through the wall of the duct, the phenomenon would be explained. Some additional support for this idea is given by the separation of blood supply to the basal coil and to the duct; the arterial supply to the coil is from a deeper plexus whereas that to the duct comes from a distinct and more superficial system. Naturally, these speculations should not be taken too seriously until tested, but it is at least clear that there is a morphological differentiation which accords with the conception that water moves both into and out of the sweat gland, the amount that emerges as manifest sweat being determined by the difference between the opposing fluxes. Urea, carried passively in these currents, enters the gland more readily than it departs, and thus remains to signify a circulation of water.

SUMMARY

Previous workers have found that the concentration of urea in human sweat exceeds the concentration of urea in plasma. This fact has been confirmed in the present work and extended by measurements of the concentrations of urea in the sweat and the corresponding concentrations of urea in the plasma over a range of 25- fold variation in the latter.

An analysis of the data showed that the ratio of the concentration of urea in sweat to the concentration of urea in plasma, S/P, was independent of the plasma concentration, independent of the absolute flux of urea or water, and independent of the calculated energy expenditure. Two experiments showed that the concentration of urea in sweat followed a rising concentration in plasma with a time lag of approximately 5 minutes; this fact indicates that the accumulation of urea in sweat is a continuous process which is in operation during the observed discharge.

These observations suggest that urea is carried into the gland in some precursor solution and subsequently raised to a higher concentration by the reabsorption from this solution of a constant fraction of the water.

BIBLIOGRAPHY

1. Plaggemeyer, H. W., and Marshall, E. K., Jr., *Arch. Int. Med.*, 1914, **13,** 159.
2. Barney, R. E., *J. Am. Med. Assn.*, 1925, **85,** 1373.

3. Talbert, G. A., Finkle, J. R., and Katsuki, S. S., *Am. J. Physiol.*, 1927, **82,** 153.
4. Dill, D. B., Jones, B. F., Edwards, H. T., and Oberg, S. A., *J. Biol. Chem.*, 1933, **100,** 755.
5. McSwiney, B. A., *Proc. Roy. Soc. Med.*, 1934, **27,** 839.
6. Mickelsen, O., and Keys, A., *J. Biol. Chem.*, 1943, **149,** 479.
7. Lobitz, W. C., Jr., and Osterberg, A. E., *Arch. Dermatol. and Syphilol.*, 1947, **56,** 827.
8. Dole, V. P., Stall, B. G., and Schwartz, I. L., *Proc. Soc. Exp. Biol. and Med.*, 1951, **77,** 412.
9. Archibald, R. M., *J. Biol. Chem.*, 1945, **157,** 507.
10. Somogyi, M., *J. Biol. Chem.*, 1930, **86,** 655.
11. Smith, H. W., The Kidney, Structure and Function in Health and Disease, New York, Oxford University Press, 1951.
12. Dole, V. P., *Am. J. Physiol.*, 1943, **139,** 504.
13. Van Slyke, D. D., Rhoads, C. P., Hiller, A., and Alving, A. A., *Am. J. Physiol.*, 1934, **109,** 336.
14. Hayman, J. M., Longley, L. P., and Bobey, M., *Tr. Assn. Am. Physn.*, 1941, **56,** 291.
15. Lobitz, W. C., and Mason, H. L., *Arch. Dermatol. and Syphilol.*, 1948, **57,** 907.