THE PERMEABILITY OF HUMAN SWEAT GLANDS TO A SERIES OF SULFONAMIDE COMPOUNDS

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In a previous paper (1) it was suggested that the formation of sweat, like the formation of urine (2), involves the elaboration of a precursor solution from which water is reabsorbed. This concept of an internal circulation of water in the gland was based on evidence that urea is concentrated in sweat by the indirect process of water reabsorption and not by a specific secretion of urea molecules into tubular water. The purpose of the present work has been to study the permeability of the epithelium of the sweat gland. The rates of excretion in the sweat of a series of sulfonamide compounds, para-aminohippurate, and inulin were measured and related to the corresponding concentrations in the plasma. The results indicate that the sweat gland, unlike the glomerular nephron, presents a barrier to the free diffusion of solute molecules from plasma to glandular lumen. A quantitative relationship was shown to exist between the rate of excretion in the sweat and the degree of ionization of each of the sulfonamide compounds at the physiological pH.

Analytical Methods

Determination of diffusible sulfonamide and para-aminohippurate in the plasma. 1 ml. of plasma was used for analysis of total sulfonamides and para-aminohippurate according to the method of Bratton and Marshall (3).

The concentration of diffusible sulfonamide and para-aminohippurate in the plasma was determined by equilibration of plasma and buffer in the dialysis unit of Hamilton and Archibald (4); 3 ml. of plasma was placed in the inside chamber and 10 ml. of phosphate buffer (pH 7.8, μ 0.1) in the outside flask. Samples of plasma with various concentrations of sulfanilamide were equilibrated with buffer, and other samples of plasma, initially free from sulfanilamide, were dialyzed against buffer with various concentrations of sulfanilamide. Complete equilibration was obtained within 4 hours, and quantitative recovery showed that binding to the cellophane membrane was negligible. It was found that the fraction of sulfanilamide in plasma which is freely diffusible is not constant, but increases as the concentration of total sulfanilamide increases. Therefore, the concentration of diffusible sulfanilamide in a plasma sample must be examined under such circumstances that the concentration of total sulfanilamide in the plasma is kept essentially unchanged. To this end, sulfanilamide was added to the buffer solutions of two dialysis units in amounts 1 mg. per 100 ml. higher and 1 mg. per 100 ml. lower, respectively, than the estimated concentration of diffusible sulfanilamide in the plasma sample. The concentration of diffusible sulfanilamide was determined by interpolation from the results obtained. The concentrations of diffusible sulfapyridine, sulfathiazole, sulfadiazine, and para-aminophippurate were determined in a similar manner.

Determination of diffusible sulfonamides and para-aminohippuric acid in sweat.—3 to 5 ml. of 3.5 per cent fresh trichloracetic acid was added to the weighing bottles containing the filter paper discs on which the sweat had been absorbed. The bottles were shaken gently for 2 hours. The samples were filtered and 1 ml. of the filtrate was used for analysis by the method of Bratton and Marshall (3). With this procedure no sulfonamide blank was found in any sweat sample.

The degree of binding in the sweat of the various sulfonamides and of para-aminohippurate was tested. The compounds were added in known amounts to large samples of sweat obtained from the forearms in a hot room. Dialysis was carried out according to the procedure described for the plasma samples. It was found that sulfonamides and para-aminohippuric acid in sweat were all diffusible.

The precision of the determinations was estimated in a recovery experiment. A solution containing 10 mg. of sulfanilamide per 100 ml. was added to filter paper discs in 15 weighing bottles. In groups of three the discs received approximately 20, 15, 10, 5, and 2.5 μg. of sulfanilamide. The concentration of sulfanilamide in each bottle was estimated by weight increment of the disc, extraction and analysis—just as in a measurement of sweat. The coefficient of variation was 4 per cent.

Determination of urea in sweat and in plasma was carried out by a micromodification (1) of Archibald's colorimetric method (5).

Determination of inulin in sweat and plasma was carried out by Harrison's modification (6) of the method of Alving, Rubin, and Miller (7).

Experimental Procedure

Five adult males served as subjects for the tests, which were conducted during the autumn and winter months.

One of the subjects was normal and four had benign essential hypertension, uncomplicated by cardiac or renal failure. 1½ to 2 hours before a test the subject was given 250 ml. of water (or fruit juice) and tablets of sulfonamide in sufficient quantity to bring the plasma concentration to the desired level, which was reached 1½ to 3 hours later. The subject remained at rest in bed, room temperature 20–24°C, until the experiment was completed. Following the initial water intake no fluids were permitted until after the test, when ample water was given to enhance the excretion of the drug.

Sweating was stimulated by intradermal injection of 2 mg. beta-methylacetylcholine-hydrochloride ("mecholyl") in 0.5 ml. of isotonic saline in the center of each site of collection. Sweat was collected from the ventral surfaces of both forearms, three sweat collection chambers (1) being glued to the skin of each forearm. Thirty sweat samples (5 consecutive periods of 20 minutes duration at each of the 6 sites) were obtained in each test, with samples of venous blood drawn at the beginning, at the middle, and at the end. The average concentration of diffusible sulfonamide in the three plasma samples was assumed to be equal to the average sulfonamide level in the extracellular fluid during the test and was used for the calculations in the analysis of the findings.

The excretions in sweat of inulin and para-aminohippurate were studied in individual experiments during which the concentration in plasma was established by a priming dose of the test substance and sustained by a constant intravenous infusion. Collections of sweat were made from individual skin sites following stimulation with mecholyl as described above. In the experiment with inulin an additional 50 cc. of sweat was obtained from the forearm and back of a subject freely sweating in a hot room; the large amount allowed the use of a sensitive method (6) which would detect inulin in the sweat at a concentration as low as 0.1 mg. per 100 cc.

RESULTS

Excretion of sulfanilamide. In five experiments the concentration of sulfanilamide in the sweat was determined at various rates of sweating and at various concentrations of sulfanilamide in the plasma. In all 150 sweat samples were obtained from 4 subjects, with rates of sweating ranging from 0.15 to 2.73 mg./cm.^{2} minutes and concentrations of diffusible sulfanilamide in the plasma ranging from 1.39 to 7.58 mg. per 100 ml.

The concentration of sulfanilamide in the sweat, S, was directly proportional to the concentration of freely diffusible sulfanilamide in the plasma, P, over a

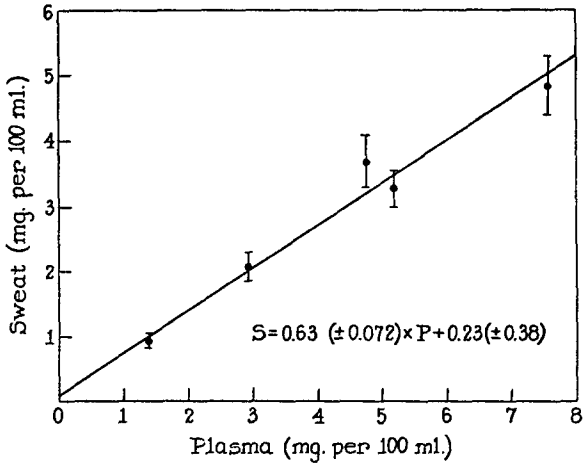

FIG. 1. Relation between the concentration of diffusible sulfanilamide in sweat and plasma. The points represent average values of thirty analyses, the vertical lines the corresponding standard deviations.

5-fold range of variation in P (Fig. 1). The ratio of the concentration of sulfanilamide in the sweat to the concentration of diffusible sulfanilamide in the plasma, S/P, was independent of an 18-fold variation in the rate of sweat production (Fig. 3) and independent of a 70-fold variation in the amount of sulfanilamide molecules transported (Fig. 2). The mean ratio of S to P from all experiments, $\overline{S/P}$ was 0.69 $\pm$ 0.08.

The coefficient of variation of S, after correction for the regression on P, was 5 per cent, only slightly larger than that to have been expected from technical errors. A more detailed analysis of the findings, however, showed significantly greater variance ($p < 0.01$) among mean values of different experiments than among consecutive values of a single experiment. From these results it can be concluded that the variance of S/P was due in small part to physiological differences, and that the main source of fluctuation was simply technical.

One experiment was designed to obtain an estimate of the "delay time" for the excretion of sulfanilamide; *i.e.*, the time elapsing from the removal of sulfanilamide from the plasma until its appearance in the sweat.

Samples of venous blood were drawn at 20 minute intervals after the intake of sulfanilamide in order to follow the rise in plasma concentration. Sweat was collected from six sites on the forearm, stimulated consecutively at 20 minute intervals by an intradermal injection of mecholyl. Three 10 minute periods of sweat collection were obtained at each site. Since the

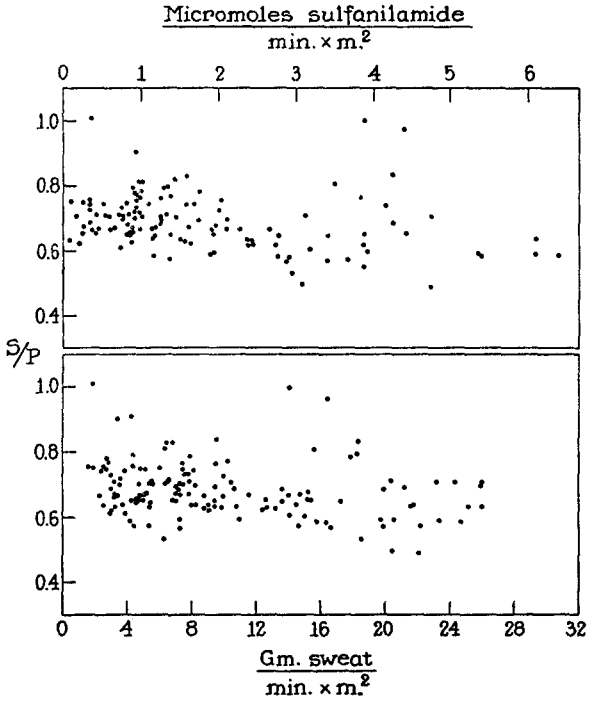

FIG. 2

FIG. 3

FIG. 2. The S/P ratio for sulfanilamide is independent of the flux of sulfanilamide molecules in micromoles per m.² of skin × minutes.

FIG. 3. The S/P ratio for sulfanilamide is independent of the flux of water in gm. per m.² of skin × minutes.

interval between successive injections of mecholyl was 20 minutes and the total period of sampling from each site was extended over 30 minutes, duplicate sweat samples were obtained alternating with single sweat samples. The duplicate samples represented sweat formed during the 5 minutes prior to and the 5 minutes following each bleeding. In this way the rise in the concentration of sulfanilamide following an ingestion of 6.5 gm. of sulfanilamide was determined in 7 samples of plasma and in 19 samples of sweat.

The results of the experiment are shown in Fig. 4. The delay time for the excretion of sulfanilamide was 7 minutes. It was calculated from the formula: $t = \frac{rP - S}{a}$, where r is the S/P ratio at equilibrium, P and S are concurrent

concentrations of plasma and sweat, and a is the average rate of increase with time of the sulfanilamide concentration in sweat.

Excretion of urea and sulfanilamide simultaneously was studied in order to see whether the two substances competed in any process of active transport. Altogether, 30 sweat samples were obtained from three different subjects, with concentrations of diffusible sulfanilamide in the plasma varying from 1.39 to 7.58 mg. per 100 ml. The mean ratio of sweat to plasma concentrations for urea, S/P, was 1.84 $\pm$ 0.23, the same that had been found previously (1) in the absence of sulfanilamide. The urea/sulfanilamide clearance ratio was 2.93 $\pm$ 0.47, independent of the sweating rate (range of variation 0.22 to 2.75 mg./cm.^{2}

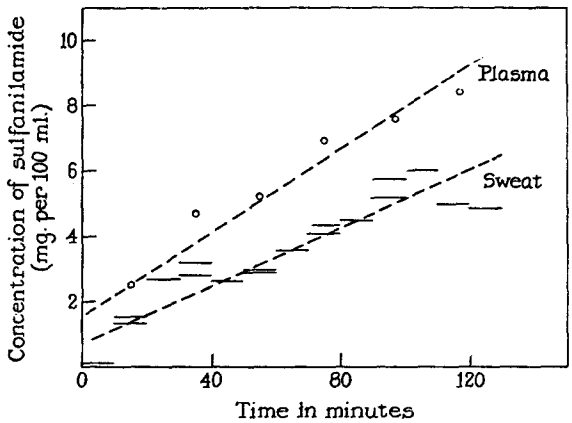

FIG. 4. The rise in the concentration of diffusible sulfanilamide in the sweat and in the plasma following oral ingestion of 6.5 gm. of sulfanilamide. The plasma concentrations are shown as solid circles, the sweat concentrations as solid lines in order to illustrate the duration of the period of sweat collection (10 minutes). Dashed lines have been fitted by inspection for approximate estimation of the delay time (see text).

minutes) and of the number of sulfanilamide molecules transported (range of variation: 0.13 to 4.74 micromoles/m.^{2} minutes). It follows that urea and sulfanilamide were excreted independently within the range examined.

Excretion of Sulfapyridine, Sulfathiazole, Sulfadiazine, and para-Aminohippurate was tested in 4 individual experiments with rates of sweat flow ranging from 0.15 to 2.66 mg./cm.^{2} minutes. The concentration in plasma of total and diffusible compound with the corresponding S/P ratio is shown in Table I. All S/P ratios were independent of the sweating rate.

Excretion of inulin was tested in one experiment. Neither small samples of sweat obtained on filter paper discs following local stimulation with mecholyl nor gross samples of thermal sweat, collected simultaneously from the forearm and the back, contained demonstrable inulin, despite the fact that the inulin concentration in the plasma was maintained at 54.0 to 56.8 mg. per 100 ml. throughout the entire experiment.

DISCUSSION

Like urea (1), the sulfonamide compounds showed constant sweat to plasma concentration ratios, unaffected by large variation in the plasma concentration or in the rate of sweating. Each substance emerged in the sweat at a concentration that was proportional to its diffusible amount in the plasma, the constant of proportionality proving different for each compound. It seems probable that all these compounds entered the glandular lumen by a process of simple diffusion, and that the epithelium of the gland was unequally permeable to them.

In view of these findings it is of interest to observe the distribution of these compounds into other compartments of body water. A comparison between the S/P ratios, the rates of entry into the cerebrospinal fluid, and the volumes of distribution shows that the compounds that most readily pass into spinal fluid

TABLE I

Concentration in Plasma of Total and Diffusible Sulfapyridine, Sulfathiazole, Sulfadiazine, and para-Aminohippurate and the Corresponding S/P Ratios

Compound	Concentration in plasma		S/P ratio
	Total	Dialysable	
	mg./100 ml.	*mg./100 ml.*	
Sulfapyridine	7.95	5.20	0.58 (±0.05)
Sulfathiazole	15.10	4.23	0.13 (±0.02)
Sulfadiazine	2.30	1.35	0.11 (±0.03)
para-Aminohippurate	16.70	15.10	0.02 (±0.01)

or enter into cellular water of the body as a whole are those that are excreted in highest concentrations in the sweat (Table II). Inulin, which does not enter into cellular water (9), could not be detected in the sweat. Thus it appears that a substance, not actively transferred into the sweat, must diffuse through a cellular barrier. Anatomically this would be expected, since the tubules are formed by a continuous sheet of epithelial cells. The results indicate that the permability of these glandular cells is similar to that of most other cells throughout the body.

The different S/P ratios of the sulfonamide compounds seem to be related to ionization differences (Table II). Assuming that only the non-ionized molecules pass the lipid barrier of cellular membranes, the S/P ratios should be determined by the ionization of the compounds at pH 7.4. This is indeed the case, as is shown in Fig. 5. Compounds, which are mainly non-ionized at the physiological pH, are excreted with high S/P ratios, while the ionized compounds appear with low ratios. Fig. 5 also explains why the largest differences

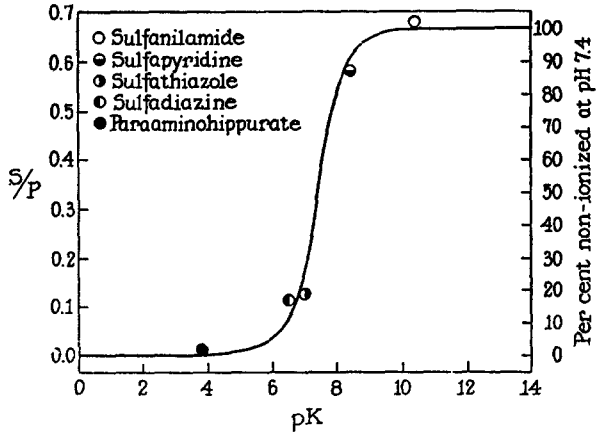

FIG. 5. Relation between pK, S/P ratio (circles) and the percentage of non-ionized compound at pH 7.4 (solid line).

TABLE II

Relation between S/P Ratios, Other Biological Properties, and Physicochemical Characteristics of the Various Compounds Examined

The volumes of distribution of the sulfonamides and the ratios of the concentrations in cerebrospinal fluid to the concentrations in plasma were obtained on cats (8); the volumes of distribution of para-aminohippurate and inulin were obtained on man (9, 10). The pK values for the sulfonamides were determined by Bell and Roblin (11).

Compound	S/P ratio	Volume of distribution (per cent of body weight)	$\frac{\text{CSF}}{\text{Plasma}}$ ratio	Molar weight	pK	Degree of protein binding
						per cent
Urea	1.84 (±0.23)			60	13.8	0
Sulfanilamide	0.69 (±0.08)	98.2	0.68	172	10.4	10
Sulfapyridine	0.58 (±0.05)	82.5	0.62	249	8.4	35
Sulfathiazole	0.13 (±0.02)	58.5	0.10	255	7.1	75
Sulfadiazine	0.11 (±0.03)	46.0	0.31	250	6.5	40
para-Aminohippuric acid	0.02 (±0.01)	27.4	—	196	3.8	10
Inulin	0.00	16.2	0.00	5100	—	0

in S/P ratio are seen between compounds with pK values just above and just below pH 7.4. The assumption that only non-ionized compounds diffuse readily into cells is supported by the studies of Osterhout (12), who showed that the weak acid, H_2S penetrated into the cell sap of *Valonia* only when in the non-ionized form.

SUMMARY

A series of sulfonamide compounds, para-aminohippurate, and inulin were used to study the permeability of the epithelium of human sweat glands.

Inulin was not excreted in the sweat. The ratios of the concentrations in sweat to the concentrations in plasma, S/P, of sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, and para-aminohippurate were found to be 0.69, 0.58, 0.13, 0.11, and 0.02 respectively, independent of the plasma concentrations and the sweating rates. The fact that the S/P ratios are thus unaffected by the absolute number of molecules transported suggests that these compounds enter into the sweat by simple diffusion and not *via* a specific secretory mechanism which could become saturated by increasing load. If this is so, the difference in the S/P ratios must be explained by an unequal permeability of the epithelium of the sweat gland to the various compounds and some explanation for these differences in the rate of excretion must exist in terms of physicochemical properties of the compounds.

A comparison between the S/P ratios and the pK values of the various sulfonamides indicates that the differences in their rates of excretion in the sweat depend upon the degree of ionization of the various compounds at the physiological pH. Compounds which are mainly non-ionized are excreted with high S/P ratios, whereas ionized compounds appear with low ratios. A quantitative relationship was shown to exist between the S/P ratio for each compound and the percentage of the compound which is non-ionized at pH 7.4.

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