SALICYLATE AND SALICYL CONJUGATES: FLUORIMETRIC ESTIMATION, BIOSYNTHESIS AND RENAL EXCRETION IN MAN[1]

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Three urinary salicyl conjugates account for over 80 per cent of salicylate ingested by a normal subject excreting acid urine (1–4). The metabolites, which are estimated by specific methods, are salicylurate, salicyl *acyl* glucuronide, and salicyl *phenolic* glucuronide (1). Alkaline urines also contain the same conjugates plus variable amounts of salicylate (5–8). With the existing methods, only salicylate has been detected in human plasma (9). It has been suggested, therefore, that the salicyl conjugates are formed within the kidney in man (7).

The present report describes sensitive, fluorimetric methods for the specific estimation of salicylate and each of its principal metabolites in plasma and urine. With these methods, the salicyl conjugates have been detected and estimated in the plasma of normal subjects given a single oral dose of salicylate and values for the renal clearance of each compound obtained. The clearances do not appear to exceed the renal plasma flow, suggesting that salicylate conjugation is mainly extrarenal in man. In addition, the renal clearance values yield information concerning the mechanisms for the urinary excretion of conjugated and unconjugated salicylate.

METHODS AND MATERIALS

Drug experiments. The experiments were conducted in 21 normal male subjects, aged 14 to 63, including 17 medical students, resident physicians, and technicians, aged 22 to 35. The group included 17 white subjects, 3 Negroes, and 1 Chinese. During the experiments the subjects drank sufficient water to maintain urine flows greater than 1 ml. per minute; otherwise their diets and activities were unrestricted.

Sodium salicylate powder, 0.4 millimole per kilogram body weight to a maximal dose of 4.0 grams, was given in one oral dose, either in solution or in gelatin capsules. Samples of blood and urine were collected for as long as 30 hours after the drug. Voided urine samples were collected for periods of 90 to 120 minutes, with a minimum of two successive collection periods. Venous blood samples, drawn with oxalate at the mid-points of the collection periods, were centrifuged at once at 5° C. for 15 minutes. All plasma and urine samples were treated immediately with neutral hydroxylamine to convert the unstable salicyl acyl glucuronide (SAG) to its stable hydroxamate derivative (1). Plasma and urine samples obtained prior to the drug were found to contain minimal quantities of fluorescent materials, as estimated by the following methods.

Fluorimetric estimation of plasma salicyl compounds. The fluorescence of sodium salicylate and salicylurate was measured with the Aminco-Bowman spectrophotofluorometer (10). In neutral aqueous solutions, salicylate fluorescence exhibited its peak intensity at 420 mμ, when activated at the optimal wave length, 310 mμ. As little as 1.0 μM per liter (10^{-6} M) salicylate can be detected, and the intensity of fluorescence is proportional to the salicylate concentration. The salicylate fluorescence remains constant from pH 5 to pH 12. In contrast, the fluorescence of salicylurate solutions increases fortyfold as the pH increases from 6 to 10, and remains maximal thereafter (11). This increment in fluorescent light (peak intensity 420 mμ, activated at 340 mμ) is proportional to the salicylurate concentration, and independent of the salicylate concentration. Each of the salicyl conjugates can be estimated in plasma in a concentration as little as 1.0, μM per liter, and in the presence of a one thousandfold excess of salicylate.

Salicyl acyl glucuronide. Five ml. of plasma is incubated with 4.0 ml. of 2 M hydroxylamine at pH 7.0 for two hours at room temperature to convert SAG to salicyl hydroxamate, and the latter is extracted into 30.0 ml. of ether by shaking for two minutes. A 20 ml. aliquot of ether is washed successively with 4.0 and 2.0 ml. of 0.4 M sodium phosphate of pH 7 to remove traces of salicylate. Salicyl hydroxamate solutions exhibit very little fluorescence, but on acid hydrolysis they liberate salicylic acid, which can be estimated fluorimetrically. Accordingly, duplicate aliquots of the washed ether are evaporated to dryness at 30 to 40° C. One of the samples is hydrolyzed in 6 N H_2SO_4 at 100° C. for 30 minutes. Subsequently, both samples are adjusted to pH 7.4 with 0.2 M sodium phosphate buffer. The increment in fluorescence (activating wave length 310 mμ, fluorescence wave length

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420 mμ) following hydrolysis is proportional to the quantity of salicyl hydroxamate added. Because pure SAG was not available, a urine sample containing the glucuronide in high concentration, and standardized against crystalline salicyl hydroxamate, was the reference standard. Recoveries of the urinary SAG varied from 86 to 114 per cent, and were determined with each series of determinations.

Salicylate and salicylurate. After extraction of salicyl hydroxamate, the residual plasma is diluted 1:2 and the protein is precipitated by the addition of perchloric acid to a final concentration of 7 per cent. After centrifugation, 10.0 ml. of the supernatant is shaken for two minutes with 15.0 ml. of ether to extract salicylic and salicyluric acids. One ml. of the ether is evaporated to dryness, the dried residue dissolved in 0.2 M sodium phosphate pH 7.4, and the salicylate, present in great excess relative to salicylurate, estimated fluorimetrically. Ten ml. of ether is evaporated to dryness, the residue dissolved in 4.0 ml. of 0.05 N H_2SO_4, and the resulting mixture of salicyluric and salicylic acids washed twice with nine volumes of water-saturated CCl_4 to remove salicylic acid (5). Salicyluric acid is extracted from the CCl_4-washed solution with two volumes of ether, and duplicate portions of the ether evaporated to dryness. One dried residue is dissolved in 0.2 M sodium phosphate (pH 6.0), the other in 0.5 M Na_2CO_3 (pH > 10.5), and the salicylurate concentration estimated by the increment in fluorescence at the alkaline pH (activating wave length 340 mμ, fluorescence wave length 420 mμ). The recoveries from plasma of added salicylate and salicylurate varied from 80 to 90 per cent, and were determined with each series of estimations.

Salicyl phenolic glucuronide. Residual traces of salicylic acid are removed from the protein-free supernatant by washing twice more with one and one-half volumes of ether. When a portion of the washed solution is subsequently hydrolyzed in 6 N H_2SO_4 at 100° C. for 90 minutes, salicyl phenolic glucuronide (SPG) liberates an equimolar quantity of salicylic acid. The latter is extracted with an equal volume of ether and estimated fluorimetrically as described. A duplicate, unhydrolyzed aliquot serves as the control. When a sample of urine containing SPG in high concentration was added to plasma, the recoveries varied from 60 to 65 per cent, and were determined with each series of estimations.

Fluorimetric estimation of urinary salicyl compounds. The methods for urine are modifications of those for plasma. After incubation of 1.0 ml. of urine with 1.0 ml. of neutral hydroxylamine, the salicyl hydroxamate formed is extracted with 10.0 ml. of ether. Portions of the ether are evaporated to dryness and the salicylic acid released on acid hydrolysis is determined fluorimetrically. When the urine is alkaline and contains excess salicylate, 8.0 ml. of ether is washed with 1.0 ml. of 0.2 M sodium phosphate, pH 7.4, before evaporation and hydrolysis. The SAG-free urine is acidified to pH 2 with H_2SO_4 and extracted with two volumes of ether to remove salicylic and salicyluric acids. A portion of the ether is evaporated to dryness, the residue is dissolved in 1.5 ml. of 0.05 N H_2SO_4, and equilibrated with 10.0 ml. of water-saturated CCl_4. One portion of the aqueous layer containing the salicyluric acid is adjusted to pH 6 with 0.2 M sodium phosphate and another to pH > 10.5 with 0.5 M Na_2CO_3. Salicylurate is estimated by the increment in fluorescence observed at the alkaline pH. Salicylate is extracted from an aliquot of the CCl_4 layer with five volumes of 0.2 M sodium phosphate, pH 7.4, and estimated fluorimetrically. Finally, the ether-extracted urine is washed twice more with equal volumes of ether, and SPG is estimated by the salicylic acid liberated on acid hydrolysis. Crystalline salicyl hydroxamate, salicylate and salicylurate were the reference standards for these determinations.

Binding of salicyl compounds to plasma proteins. Information concerning the binding of salicylate and its principal conjugates on the plasma proteins was obtained for use in the interpretation of the clearance data. The protein binding of each salicyl compound in whole plasma was studied by the dialysis-equilibrium technique, using cellophane (Visking) membranes.[2] The data obtained with all four compounds conformed to the Freundlich isotherm ($x/m = Kc^{1/n}$), with the range of concentrations examined and a plasma albumin of 2.8 grams per cent. The binding constants obtained were as follows: for salicylate (at plasma levels of 580 to 1,810 μM per liter) K equals 8.03, 1/n equals 0.35; for salicylurate (at plasma levels of 11 to 223 μM per liter) K equals 0.69, 1/n equals 0.77; for SPG (at plasma levels of 6 to 95 μM per liter) K equals 0.16, 1/n equals 0.72; and for SAG (at plasma levels of 4 to 64 μM per liter) K equals 0.03, 1/n equals 0.76. The filtrable fraction (F) of each compound was obtained

[2] We are grateful to Dr. J. V. Taggart for details of the following unpublished method. Two ml. plasma is introduced into a bag of 1.5 inch cellophane (Visking) sealed at the bottom by glass rods held together with rubber bands. The upper end is similarly sealed, the bag placed flat in a Petri dish, and covered with 20.0 ml. of 0.067 M sodium phosphate, pH 7.4, containing 0.05 M NaCl. The covered Petri dish is rotated 80 times per minute on a moving platform. Dialysis equilibrium is achieved within four hours, when the compound studied is added either to the plasma or to the dialyzing medium. A specimen of blood bank plasma was used for studies with the salicyl compounds. The sources of the salicyl glucuronides were the urine standards for plasma estimations. Varying amounts of the salicyl compound were added to the plasma and the dialyzing medium to approximate the equilibrium concentrations. After rotation at 5° C. (to minimize hydrolysis of the unstable SAG) for 6 hours, 85 to 95 per cent of the added salicyl compounds were recovered. The protein binding conformed in each instance to the Freundlich isotherm: $x/m = Kc^{1/n}$, where x is the number of μM of salicyl compound bound by m grams of albumin, c is the equilibrium concentration of free salicyl compound in plasma water (μM per liter), and k and 1/n are constants.

TABLE I

Plasma concentrations of salicyl compounds after one oral dose of salicylate

Hours after ingestion	Number of subjects	Plasma concentration*			
		Salicylate	Salicylurate	SAG†	SPG‡
2	18	1,782§ ± 245	9.8 ± 1.9	6.8 ± 2.1	7.7 ± 3.5
4	19	1,628 ± 179	10.1 ± 2.3	6.2 ± 1.7	12.5 ± 4.1
8	7	1,400 ± 110	9.5 ± 2.0	4.3 ± 0.7	16.4 ± 2.3
24	9	467 ± 200	10.5 ± 2.2	2.2 ± 1.1	14.8 ± 4.6

* Concentration in μM per liter plasma, expressed as the mean plus or minus one standard deviation.
† Salicyl acyl glucuronide.
‡ Salicyl phenolic glucuronide.
§ Corresponds to 24.6 mg. per cent.

from the isotherm.[3] In three experiments (Figure 3) the filtrable fraction of salicylate was determined directly by dialysis of plasma samples at 38° C. The values observed were 0.08 to 0.12, and corresponded to the values obtained from the isotherm.

The renal clearance (ml. per minute) of "free," or nonprotein bound, salicylate (C_{Frs}) was calculated as $C_{Frs} = UV/PF$, where U and P are the salicylate concentrations (μM per liter) in urine and plasma, V is the urine flow (ml. per minute), and F is the filtrable fraction. A similar expression was used to calculate the clearance of "free" SPG.

Other methods and materials.[4] Urine pH values were determined with a Beckman glass electrode pH meter immediately after voiding. The endogenous creatinine clearance served as an estimate of the glomerular filtration rate (15). Total plasma protein concentration was estimated by the biuret method (16), and the plasma albumin level with the Spinco Model R paper electrophoresis apparatus. Optical density measurements were made in the Beckman DU spectrophotometer. The preparation of crystalline salicyluric and salicyl hydroxamic acids has been described (1).

[3] F is defined as the ratio c/P, where c is the concentration of the freely diffusible compound in plasma water, and P is the plasma concentration (12). From the Freundlich isotherm c, and hence F, may be calculated when P, the constants for the isotherm, and the plasma albumin (estimated electrophoretically) are known.

[4] A conjugate of salicylurate appears in the plasma and urine of normal subjects after ingestion of salicylate. This material, which may be identical with "uraminosalicylsaüre" (3), cannot be extracted with ether at pH 2. After hydrolysis of an ether-washed solution in 6 N H_2SO_4 for 90 minutes at 100° C., salicyluric acid is liberated and estimated fluorimetrically (plasma) or with a paper chromatographic method (urine), using the colorimetric reaction of Gaffney, Schreier, DiFerrante, and Altman (13) to estimate the salicylurate areas on the chromatogram (1, 14). The plasma levels and excretion rates of this metabolite increased for 24 hours following oral salicylate, and reached values of 3.5 to 5.5 μM per liter and 0.2 to 0.6 μM per minute, respectively.

RESULTS

Salicyl compounds in plasma

After a single oral dose of salicylate, detectable quantities of salicylurate, SAG and SPG appeared in the plasma of each subject. The mean concentrations observed at various times following the administration of salicylate are listed in Table I. The time course of each salicyl compound in plasma is illustrated in Figure 1. The patterns of the curves were similar for all the subjects. Maximal plasma concentrations of salicylate, salicylurate and SAG were observed within two hours after salicylate, when mean levels of 1,780, 9.8 and 6.8 μM per liter, respectively, were attained. The maximal concentrations of SPG were observed only after eight hours and reached 16.4 μM per liter. The levels of salicylate and SAG fell progressively, while those of salicylurate and SPG remained fairly constant for 24 hours

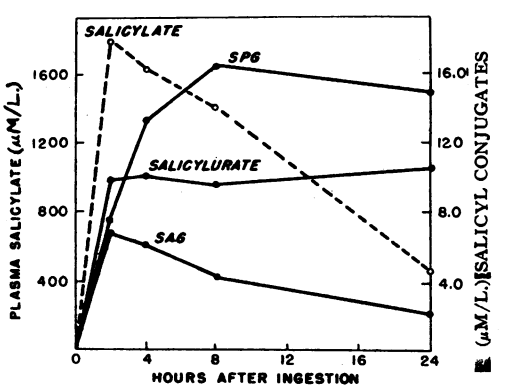

FIG. 1. MEAN PLASMA CONCENTRATIONS OF SALICYLATE (--○--) AND SALICYL CONJUGATES (—●—) AFTER ONE ORAL DOSE OF SALICYLATE

The right- and left-hand vertical scales refer to the salicyl conjugates and to salicylate, respectively.

Salicylate was the principal salicyl compound in plasma, with a concentration 70 times that of the combined conjugates 2 hours after the drug, and 15 times that of the conjugates after 24 hours.

Salicyl compounds in urine

The maximal urinary excretion rates of the salicyl conjugates, listed in Table II, reflect the capacity of normal subjects to form and excrete these compounds. Salicylate is conjugated slowly in man. For example, the maximal rate of excretion of salicylurate in the present studies was approximately 5 μM per minute. After a comparable dose of benzoate, the hippurate excretion can exceed 100 μM per minute (1, 17). After ingestion of *p*-methoxybenzoate, the rate of acyl glucuronide excretion can be six times that of SAG; and after salicylamide the phenolic glucuronide excretion can be 20 times that of SPG (18).

When the excretion of total salicyl conjugates was maximal, two to six hours after the drug, salicylurate accounted for 64 per cent, SAG for 23 per cent, and SPG for 13 per cent of the urinary conjugates. These values agree with previous observations (1–3). The rate of excretion of each conjugate remained approximately proportional to its plasma concentration. As previously reported (1), SAG was the principal glucuronide in the early hours following the drug, and SPG predominated in the later hours.

Water diuresis, with increases in urine flow to 12 ml. per minute, failed to alter significantly the excretion rates of the conjugates. Moreover, the rates were unchanged in three subjects when the oral administration of 10 grams of sodium bicarbonate increased the urine pH from an original level of 5.0 to 6.2 to a final value of 7.6 to 8.0. In contrast, bicarbonate administration increased the excretion of salicylate from approximately 1.5 μM per minute to 12 μM per minute. Thus, the total salicyl excreted at any given time includes a relatively predictable quantity of salicylate conjugates and a variable, pH-dependent quantity of salicylate.

TABLE II

Maximal rates of urinary excretion of salicyl conjugates after one oral dose of salicylate

Metabolite	Number of subjects	Hours after ingestion*	Maximal rate of urinary excretion†
Salicylurate	19	2–6	5.2 ± 1.1
SAG‡	19	2–6	1.9 ± 0.4
SPG‡	6	6–10	1.3 ± 0.2
Total salicyl glucuronides	19	2–6	3.0 ± 0.6
Total salicyl conjugates	19	2–6	8.1 ± 1.1

* The period during which the maximal rate of excretion was observed.
† Micromoles per minute; mean plus or minus one standard deviation.
‡ SAG, salicyl acyl glucuronide; SPG, salicyl phenolic glucuronide.

Renal clearances of salicyl conjugates

The renal clearances of salicylurate, SAG and "free" SPG considerably exceeded the endogenous creatinine clearances, as summarized in Table III. Thus glomerular filtration and tubular secretion appear to be the principal renal mechanisms for the excretion of these compounds. The clearances of the salicyl conjugates did not exceed the approximate renal plasma flows, when the latter were calculated from the endogenous creatinine clearances and a normal value (0.19) for the filtration fraction in man (12). Therefore, no evidence for renal synthesis of the conjugates was obtained.

TABLE III

Renal clearances of endogenously formed salicyl conjugates

Metabolite	Plasma concentration	Number of clearance periods	Number of subjects	Renal clearance*	Renal clearance/ endogenous creatinine clearance
	μM/L.			*ml./min.*	
Salicylurate	5.7–14.9	70	19	444 ± 112	4.2 ± 0.9
SAG†	3.0– 6.0	29	13	331 ± 70	3.3 ± 0.8
SPG†	10.0–15.0	22	12	73 ± 22	0.7 ± 0.2
"Free" SPG‡	2.0– 3.0	22	12	384 ± 145	3.0 ± 1.0

* Each clearance value has been adjusted to 1.73 square meters body surface, and is expressed as the mean plus or minus one standard deviation.
† SAG, salicyl acyl glucuronide; SPG, salicyl phenolic glucuronide.
‡ Nonprotein bound SPG.

Clearance studies with salicylurate by Smith, Finkelstein, Aliminosa, Crawford, and Graber (19) in two male subjects yielded salicylurate to inulin clearance ratios of 2.9 and 4.3. These figures agree with the clearance data obtained in the present study with endogenously formed salicylurate, and suggest that the renal synthesis of salicylurate is relatively unimportant.

The effect of probenecid on the salicyl conjugate clearances was studied in six subjects. After two control periods, 2.0 grams of probenecid was given by mouth and the effects observed for five hours. Probenecid, an inhibitor of certain tubular secretory mechanisms (20), uniformly reduced the salicylurate clearances by 29 to 53 per cent (mean, 40), and the SAG clearances by 42 to 71 per cent (mean, 57). There was a concomitant rise in the plasma levels of these metabolites. The SPG clearances were altered less consistently, with a fall of 42, 43 and 46 per cent in three subjects, and no significant change in three others.

The plasma concentration of SAG appeared to influence its renal clearance. With plasma levels of 1 to 3, 3 to 6, and 6 to 12 μM per liter, the corresponding clearance rates (ml. per minute) were 502 ± 84 (16 periods, 11 subjects),[5] 331 ± 70 (29 periods, 13 subjects), and 254 ± 27 (18 periods, 10 subjects). A similar but less marked trend was noted for the SPG clearances. The loads of SAG and SPG presented to the renal tubules (μM per minute), and the rates of secretion of the glucuronides were calculated by conventional methods[6] (12), and plotted in Figure 2. At comparable loads, the SAG secretion considerably exceeded that of SPG, and with loads less than 1.0 μM per minute SAG secretion was essentially complete. Secretion of both glucuronides became progressively less complete at higher loads, suggesting a limitation in the capacity of the tubules to transport these compounds.

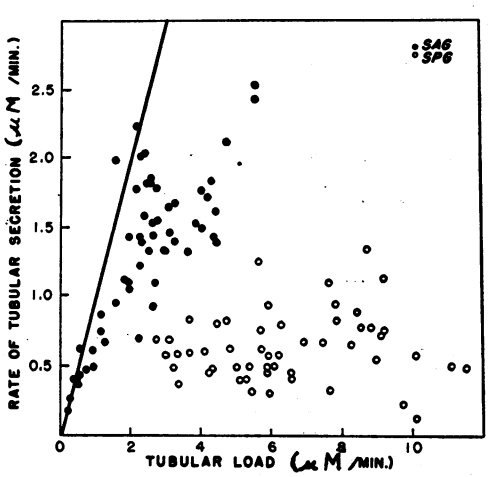

FIG. 2. RATE OF TUBULAR SECRETION OF SALICYL GLUCURONIDES AT VARIOUS TUBULAR LOADS

The straight line connects the points at which the tubular load is completely secreted.

[5] Mean plus or minus one standard deviation.

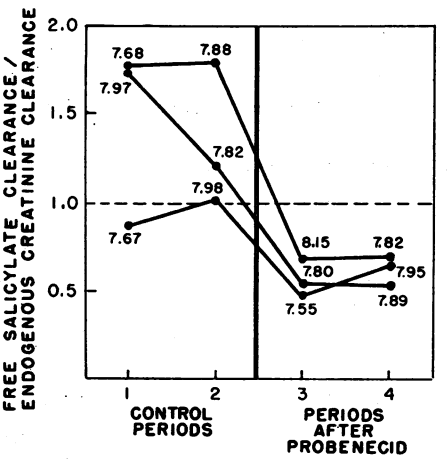

FIG. 3. EFFECT OF PROBENECID ON THE "FREE" SALICYLATE TO ENDOGENOUS CREATININE CLEARANCE RATIO

The lines connect the values for an individual subject. The urine pH value for each period is indicated.

Variations in urine flow from 1 to 12 ml. per minute, or in urine pH from 6 to 8, did not significantly affect the clearances of the salicyl conjugates.

Renal clearances of salicylate

Alkalinization of the urine causes a marked increase in the clearance of "free" salicylate (C_{FrS}), as described previously (5–8), and confirmed in the present studies with methods which clearly distinguish between conjugated and unconjugated salicylate. When urine pH was less than 6, C_{FrS} was 5 to 15 per cent of the endogenous creatinine clearance, indicating that glomerular filtration and

[6] Tubular load of $x = P_xRPF - P_xC_FFW$, where P_x is the plasma concentration, RPF is the renal plasma flow, C_F is the glomerular filtration rate, F is the filtrable fraction, and W is the plasma water content expressed as a fraction. Tubular secretion rate of $x = U_xV - P_xC_FFW$, where U_xV is the total rate of excretion of x. C_F was estimated by the endogenous creatinine clearance, and RPF was calculated at $C_F/0.19$.

tubular reabsorption were the renal mechanisms involved. With urine pH greater than 7.4, C_{FrS} to endogenous creatinine clearance ratios exceeded 1.0 in 4 of 7 subjects (9 of 15 clearance periods), and the highest value attained was 1.8 (Figure 3). Previous investigators have reported values greater than 1.0 for the salicylate clearance to filtration rate ratio in man (5, 7, 8), the dog (21), and the rabbit (22). These observations suggest that tubular secretion is a third mechanism involved in salicylate excretion.

It has been proposed that tubular transport of salicylate, either reabsorptive or secretory, could result from a passive transfer process, dependent on a pH gradient between the tubular urine and the peritubular fluid (23). To investigate this possibility, the following experiment was performed in three subjects. The urine pH was maintained consistently greater than 7.5 for the experimental period of nine hours by the oral administration of 18 grams of sodium bicarbonate in divided doses. C_{FrS} in two subjects exceeded the endogenous creatinine clearances, as shown in Figure 3. The subjects then ingested 2.0 grams of probenecid. A fall in C_{FrS} was observed in each instance, with no significant change in urine pH, endogenous creatinine clearance, or the fraction of salicylate bound to plasma proteins. Gutman, Yü, and Sirota (8) have also observed a reduction in C_{FrS} after probenecid. These results provide supportive evidence for the tubular secretion of salicylate, for it seems highly improbable that probenecid would enhance salicylate reabsorption. Inasmuch as probenecid can inhibit tubular transfer processes, presumably by a direct action on the cells, the secretion of salicylate appears to require cellular activity. The C_{FrS} to endogenous creatinine clearance ratios following probenecid varied from 0.5 to 0.7 (Figure 3), with urine pH values close to 8. Thus the tubules can reabsorb salicylate at a time when a highly alkaline urine is being elaborated. These findings are not consistent with the view that only pH gradients across the tubule are responsible for the transport of salicylate.

DISCUSSION

Metabolism of salicylate

The methods developed for the estimation of salicyl conjugates in the plasma, as well as in the urine, have permitted more detailed observations on the fate of salicylate in normal men. Following an oral dose, the drug is rapidly absorbed and slowly conjugated with glycine or glucuronic acid, mainly at extrarenal sites. The conjugates are excreted in part by tubular secretion, and the renal clearance of these metabolites considerably exceeds that of salicylate. Consequently, the bulk of the urinary salicyl may be in the form of conjugates, while the principal compound in the plasma is salicylate. The conjugation of other compounds has also been observed to result in products which are cleared by the kidneys more rapidly than is the parent compound (24–26).

The rates of urinary excretion of the salicyl conjugates are related to their rates of net synthesis in the body. SAG is formed relatively rapidly for two hours after oral salicylate. In this time the urinary excretion of SAG exceeds that of SPG by as much as fourfold in some subjects. Although the plasma levels of the two glucuronides are approximately equal at two hours (Figure 1), SAG excretion exceeds that of SPG because of its greater rate of clearance. A progressive reduction in net synthesis of SAG is noted as the plasma salicylate concentration falls. In contrast, a fairly constant synthesis of SPG and salicylurate, or a slow release of the compounds from the tissues, is maintained for as long as 24 hours after the drug is administered.

The organs which conjugate salicylate in man are unknown. By analogy with the probable formation of hippurate by human liver (27), and the synthesis of bilirubin glucuronide by human liver homogenates (28, 29), it seems probable that this organ is a major site for similar conjugations. Recently, evidence has been obtained in this laboratory for the *in vitro* synthesis of salicyl glucuronides by slices of intestinal mucosa, kidney, liver, spleen, lung and urinary bladder from various animal species.[7] It is possible, therefore, that the glucuronides may be formed by many human tissues.

[7] The formation of salicyl glucuronides *in vitro* was observed with the following tissues: intestinal mucosa (guinea pig, rabbit, hamster, rat, cat); liver (guinea pig, rat, hamster, dog); kidney (guinea pig, rabbit, rat, hamster, dog); urinary bladder (guinea pig, rabbit); spleen (guinea pig, hamster); and lung (guinea pig).

Renal clearance of salicylate

In the present studies salicylate was estimated with a relatively specific method. The clearance data obtained confirm and extend the observations of previous investigators (5, 7, 30). Salicylate, like thiosulfate (31) and potassium ion (32, 33), can be reabsorbed and secreted by the renal tubules, as well as filtered at the glomerulus. Tubular reabsorption predominates when the urine is acid. Tubular secretion is evident after alkalinization of the urine, which appears to partially inhibit the reabsorptive process. Probenecid causes a reduction in salicylate clearance, which is not dependent on a change in urine pH, and is probably due to inhibition of active tubular secretion.

It is of interest that low doses of salicylate, probenecid and other uricosuric drugs may depress the renal clearance of urate in man, as demonstrated by Yü and Gutman (34). The evidence for active tubular secretion of salicylate, which can be inhibited by probenecid, lends support to a suggestion by Yü and Gutman that urate, salicylate and other substances may compete for a common tubular secretory mechanism.

SUMMARY

1. Fluorimetric methods are described for the detection and estimation of salicylate, salicylurate, salicyl acyl glucuronide, and salicyl phenolic glucuronide in plasma and urine.

2. Salicylate conjugation is mainly extrarenal in man. The conjugates formed are secreted by the renal tubules, and their clearance exceeds that of salicylate.

3. The net synthesis of salicyl acyl glucuronide generally exceeds that of salicyl phenolic glucuronide immediately after the oral administration of salicylate, and the converse is observed 8 to 24 hours later. The renal tubules secrete the acyl glucuronide more rapidly than the phenolic glucuronide.

4. The renal excretion of unconjugated salicylate involves glomerular filtration, tubular reabsorption, and active tubular secretion.

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