Effect of Alkalis on Conjugation of Sulfonamides with Glucuronic and Acetic Acid.* (18678)

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The glucuronide of sulfonamides is more soluble(1) and bacteriostatic(2) than the acetylated derivative. These considerations make it worthwhile to determine the effect of various substances given to increase the solubility of sulfonamides in urine upon their tendency to form conjugates of acetic or glucuronic acid.

The present study was undertaken to obtain comparative data on the effects of sodium bicarbonate, sodium citrate, sodium lactate, and urea on the blood concentration, urinary excretion, and the extent of conjugation with glucuronic and acetic acid, of ingested sulfadiazine and sulfamerazine in human beings.

Methods. These studies were carried out on 2 hospitalized patients with well controlled diabetes mellitus. One patient was a 24-year-old white female requiring 64 units of insulin daily. Moderate glycosuria† was present part of the time. The other was a 70-year-old white female receiving 20 units of insulin daily who had no glycosuria. Both were on a constant, quantitative diet throughout the experimental period. After several days without sulfonamide medication, in order to establish control glucuronic acid values, the sulfonamide was given orally every 6 hours for 3 days. The adjuvant drug was given with the sulfonamide and continued for several days after the sulfonamide was stopped so that it could be determined whether the changes in glucuronic acid values were due to the sulfonamide or the adjuvant. Sulfonamide was not given again until no sulfonamide or only traces were present in the urine and blood, and another control glucuronic acid value was obtained before giving another adjuvant. Sodium bicarbonate was given as the powder in water, sodium citrate as effervescent tablets,‡ sodium lactate as an effervescent mixture of sodium bicarbonate and lactic acid, and urea as the U.S.P. drug. Urea was given in 15 g doses every 6 hours in fruit juice which was part of the constant, quantitative diet. Alkali dosages were equivalent to 24 g of sodium bicarbonate daily. Venous blood samples were drawn each morning just prior to medications. Twenty-four urines were collected under toluene. Sulfonamide determinations were made by the method of Bratton and Marshall(3) using 1:40 blood filtrates and the Evelyn photolometer. Urea nitrogen was determined by the method of Gentzkow(4). Glucuronic acid was estimated by the method of Maughn, Evelyn, and Browne(5) with slight modification. The acetylated sulfonamide fraction is calculated as the difference between "free" and total sulfonamide as determined by the method of Bratton and Marshall(3). The concurrent increase over control levels of urinary glucuronic acid when sulfonamide is excreted in the urine is assumed to be due to glucuronic acid conjugated in equimolar combinations with sulfonamide. The sulfonamide conjugated with glucuronic acid is included in the free fraction since sulfapyridine glucuronic acid(2) was diazotized without hydrolysis. The sulfonamide excreted in an uncombined or unconjugated form is calculated as the difference between the "free" form and that combined with glucuronic acid.

Results. There was no general correlation

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2. Weber, C. J., Lalich, J. J., and Major, R. H., PROC. SOC. EXP. BIOL. AND MED., 1943, v53, 190.

† Concentrations of glucose present in urine did not interfere with the method used.

‡ Alka-Vess tablets, Ames Company, Elkhart, Ind. One effervescent tablet contains 2 g sodium citrate.

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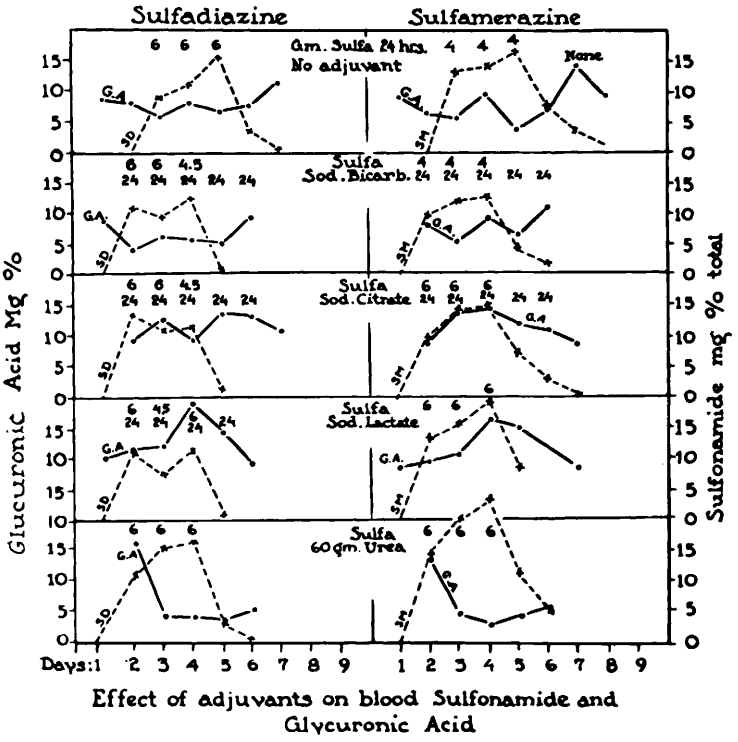


FIG. 1.

Effect of adjuvants on blood concentration of glucuronic acid and total sulfonamide.

of glucuronic acid and sulfonamide blood levels (Fig. 1). Blood glucuronic acid concentrations were above control levels when sulfadiazine or sulfamerazine was given with sodium citrate or sodium lactate adjuvants, and unchanged or below control levels when given with urea, sodium bicarbonate, or with no adjuvant. Average blood glucuronic acid concentrations are shown in Table I.

The average acetylated fraction in the blood was 5% with sulfadiazine and 7.7% with sulfamerazine. No significant differences due to the various adjuvants were demonstrated.

TABLE I. Average Blood Glucuronic Acid Concentrations.

	Patient A mg %	Patient B mg %
Controls*	8.9	8.1
No adjuvant	7.0	9.4
Sodium bicarbonate	5.6	8.1
" citrate	11.3	11.7
" lactate	14.4	13.0
Urea	7.0	6.9

* There were 18 controls for sulfadiazine (Patient A) and 5 controls for sulfamerazine (Patient B).

Fig. 2 shows 24-hour urinary contents of free and total sulfonamide and glucuronic acid with the administration of various adjuvants. An increase of glucuronic acid is shown in urines containing either sulfadiazine or sulfamerazine both with and without adjuvants. All values are of single experiments except the curves with sodium citrate and urea given with sulfadiazine which are averages of 2 curves each.

Fig. 3 shows the percentage of total urinary sulfonamide conjugated with glucuronic acid, acetic acid, and uncombined. Total conjugation (acetylated plus glucuronates), was not altered by any of the adjuvants, with the exception of sodium bicarbonate on the patient given sulfadiazine. No explanation can be offered for this exception. Conjugation with acetic acid was less after the administration of adjuvant alkalis than after urea or no ad-

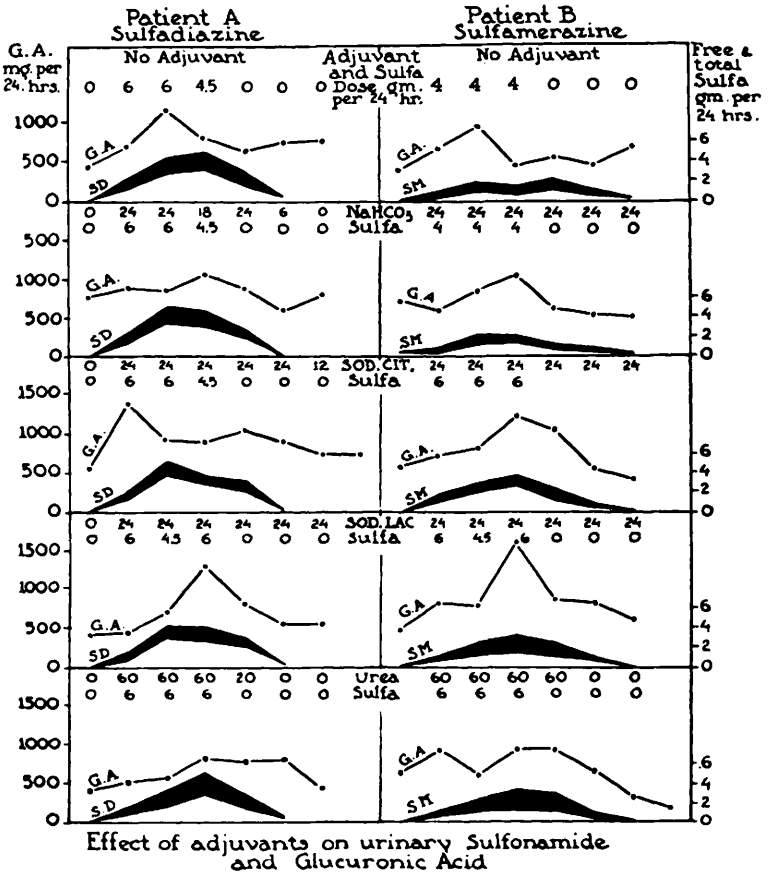


FIG. 2.

Effect of adjuvants on urinary excretion of glucuronic acid and sulfonamides. Top border of black band represents total sulfonamide and bottom border represents free sulfonamide.

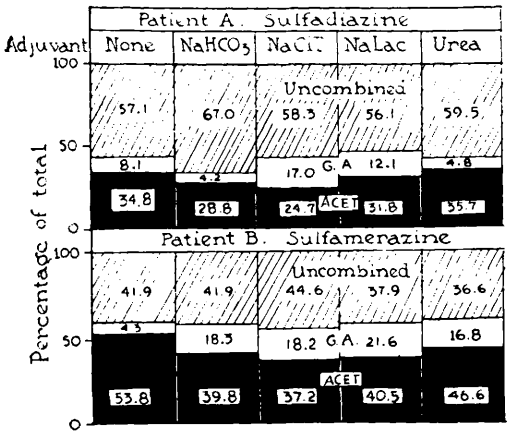


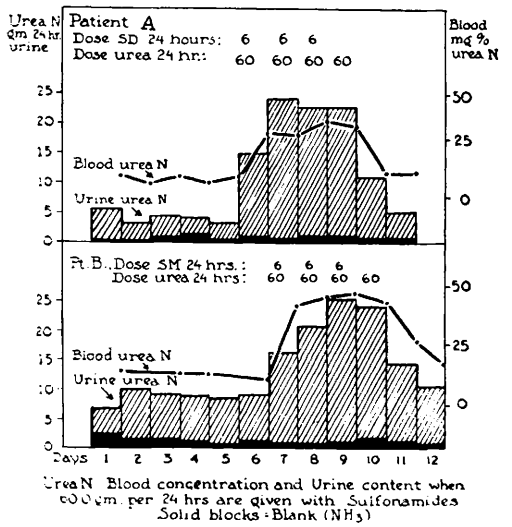
FIG. 3.

Effect of adjuvants on percentage of urinary sulfonamide uncombined or conjugated with glucuronic acid or acetic acid.

juvant was given. Conjugation with glucuronic acid was greater after alkalis were administered as adjuvants with sulfamerazine, and after sodium citrate and sodium lactate were given with sulfadiazine. Urea had no significant effect on the conjugation of either sulfonamide with acetic or glucuronic acid.

Fig. 4 shows the blood urea nitrogen concentration and urine urea nitrogen content after the administration of 60 g of urea daily with sulfonamides. Seventy-two per cent of the ingested urea was recovered in the urine of patient A and 55% in patient B. Blood and urine urea nitrogen values had returned almost to control levels 24 hours after stopping the administration of urea.

Discussion. Slightly lower blood sulfona-



Urea N Blood concentration and Urine content when 60 Gm. per 24 hrs are given with Sulfonamides Solid blocks = Blank (NH_3)

FIG. 4.

Urea nitrogen blood concentration and urine content when 60 g of urea is given daily with sulfonamides.

mide concentrations after alkali administration have been reported(6,7) and may be explained on the basis of accelerated urinary excretion due to decreased tubular reabsorption of the glucuronide derivative. Increased blood glucuronic acid after sulfadiazine therapy has been reported(8). It has been reported(9) that sulfamerazine, but not sulfadiazine, increases glucuronic acid in human urine. Fig. 2 and 3 show increased urinary glucuronic acid after both sulfamerazine and sulfadiazine. The effect of sodium salts on glucuronide formation may be related to the extra base available for excretion, to alkalosis or the effect of the anion. The fact that sodium bicarbonate had less effect on the blood and urine glucuronic acid content than sodium citrate or lactate suggests that these compounds may have a specific effect on glucuronide formation. Martin(10) reported that lactic acid increased urinary glucuronic acid in the rat. Lipschitz and Bueding found lactate to increase conjugated glucuronides *in vitro*(11). Quick(12) reported that in the dog decreased urinary excretion of benzoic acid conjugated with glucuronic acid when lactic acid was given with benzoic acid and no effect when sodium bicarbonate was substituted for lactic acid. Reduction in the percentage of acetylated sulfadiazine in the urine of human beings receiving sodium lactate has been reported(13). Sodium bicarbonate as well as citrate and lactate salts reduced the percentage of acetylated sulfonamide in urine.

Urea apparently has no effect on the blood concentration, excretion or conjugation of sulfonamides. Clinically, urea reduces the incidence of crystalluria but not as effectively as 24 g of sodium bicarbonate per day(6). However, alkalis may be contraindicated under certain circumstances.

Summary. These studies indicate that alkalis, particularly sodium citrate and sodium lactate, may increase the conjugation of sulfamerazine and sulfadiazine with glucuronic acid and decrease conjugation with acetic acid in human subjects.

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