Effect of Ethanol on the Conjugation of Benzoate and Salicylate with Glycine in Man[1] (35150)

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It has been reported by Childs and Lieberman (1) that the urinary excretion of hippuric acid after oral or intravenous administration of sodium benzoate to human subjects was significantly decreased by ethanol. It was suggested that this effect is due to the inhibition of hippurate formation, but the possibility that ethanol decreased the renal clearance of hippuric acid or interfered with its release from the liver could not be excluded. The purpose of the study to be described here was to determine the mechanism of the ethanol effect and to establish if ethanol influences the conjugation of salicylic acid with glycine. This latter process is of considerable interest since it represents the major route of elimination of salicylate in man (2). A comparison of the effect of ethanol on the formation and excretion of hippurate and salicylurate may help to elucidate differences or similarities in the conjugation of benzoate and salicylate with glycine. Also, the common use of aspirin by the public for alleviating the after-effects of excessive ethanol consumption makes it desirable to establish if ethanol can affect the biotransformation of salicylate in man.

Methods. The test subjects were healthy young male volunteers. Two to 5 g of benzoic acid as sodium benzoate were administered orally in 200 ml water after a standardized light breakfast. Fifty ml of ethanol diluted with an equal volume of orange juice were given orally 15 min before or 90 min after benzoate. Control experiments were carried out without ethanol. Urine was collected at intervals and assayed for benzoate, hippurate, and benzoic glucuronide as described previously (3).

Two g of salicylic acid, as sodium salicylate, were administered orally in aqueous solution. Fifty ml of ethanol were taken over a 15 min period 2 hr later. In another experiment, 1 g of salicylic acid orally was followed 3 hr later by 3.2 g of benzoic acid (as sodium benzoate). Fifty ml of ethanol were taken over 10 min starting 25 min before benzoate. An additional 10 ml of ethanol (suitably diluted) were taken with the dose of benzoate. Salicylate and its metabolites in the urine were determined by previously described methods (4).

Results and Discussion. The terminal exponential phase of hippurate excretion after benzoate administration reflects the renal excretion rate constant of hippuric acid (3). Ethanol had no effect on this phase (Fig. 1) and therefore does not affect the renal clearance of hippurate. Benzoate rather than hippurate was administered in this experiment because the latter is too slowly absorbed to permit accurate characterization of the exponential excretion phase.

Administration of 3.2 or 5 g of benzoic acid yields essentially identical maximum urinary excretion rates of hippurate which differ only in duration (Fig. 2). These rates represent the maximum formation rates of hippuric acid and are indicative of the limited capacity of man to synthesize this metabolite (3). In less than 1 hr after oral administration, ethanol decreases appreciably the output of hippurate in the urine (Fig. 2). The time lag may reflect the utilization of a readily avail-

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able glycine pool, with the subsequent mobilization of glycine being inhibited by ethanol.

Since benzoate is eliminated primarily by hippurate formation but to a minor degree also by glucuronide conjugation, any inhibition of hippurate formation should result in an increase in the benzoic glucuronide fraction. Such an increase is shown in Figs. 3 and 4 after ethanol administration. An inhibition of the release of hippuric acid from the liver (rather than an effect on the formation of this metabolite) would have no effect on the terminal composition of urinary metabolites.

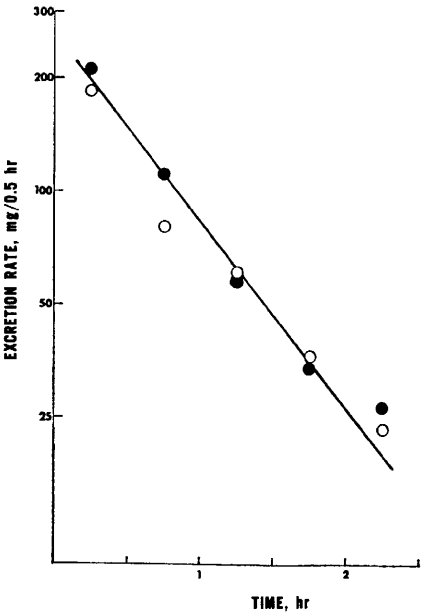

FIG. 1. Effect of ethanol on urinary excretion of hippuric acid in man: Shown are the urinary excretion rates of hippuric acid (as benzoate equivalent) as a function of time after benzoate (2 g) administration with 50 ml of ethanol (●) and after 2 g of benzoate alone (○). Zero time is the time of ethanol administration which occurred 1.5 hr after the administration of benzoate. Subject A. The excretion rate scale in Figs. 1-5 is logarithmic.

Figures 3 and 4 also illustrate the decreased output of hippurate due to ethanol. Ethanol was administered in these experiments 90 min after benzoate to exclude any possible effect on benzoate absorption. The results of these experiments establish that the decreased excretion of hippurate after ethanol administration is due to the inhibition of hippurate synthesis. The consequent delay in the elimination of benzoate is reflected by the lateral displacement of hippurate excretion curves relative to the control curves in Figs. 2 to 4.

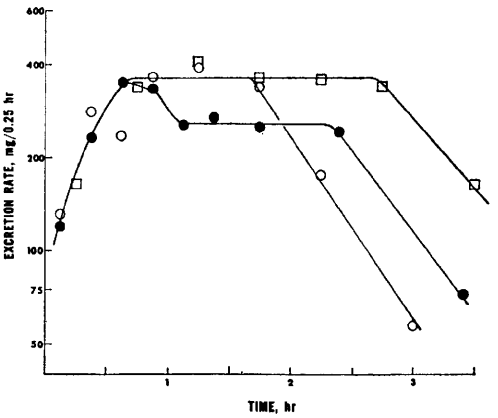

FIG. 2. Effect of ethanol on the elimination of benzoate in man: Shown are the urinary excretion rates of hippurate (as benzoic acid equivalent) as a function of time after oral administration of 5 g (□); and 3.2 g (○) of benzoic acid and after 3.2 g of benzoic acid (●) with ethanol (50 ml 15 min before and 10 ml together with benzoic acid) (Subject A).

Administration of 1 or more g of salicylate

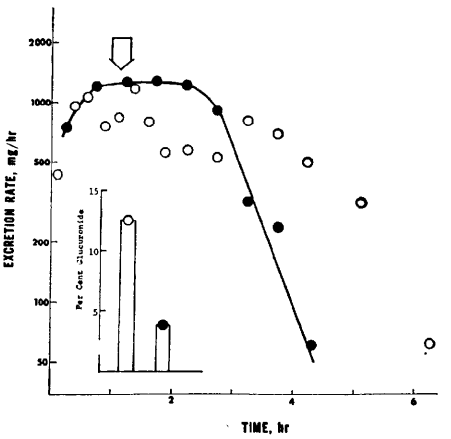

FIG. 3. Effect of ethanol on hippurate formation in man: Shown are the urinary excretion rates of total benzoate metabolites (in terms of benzoic acid) as a function of time after the administration of 5 g of benzoate with (○); and without ethanol (●). Arrow indicates time of ingestion of 50 ml of ethanol. The inset indicates percentage of dose excreted as glucuronide (Subject B).

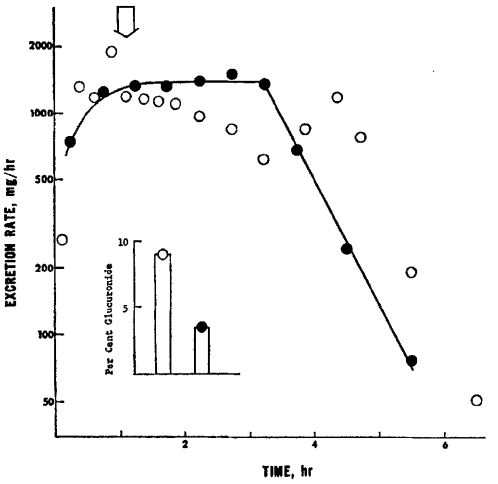

FIG. 4. Effect of ethanol on hippurate formation in man: Shown are the urinary excretion rates of total benzoate metabolites (in terms of benzoic acid) as a function of time after the administration of 3.6 g of benzoic acid with (○), and without (●) ethanol. Arrow indicates time of ingestion of 50 ml of ethanol; inset depicts percentage of dose excreted as glucuronide (Subject C).

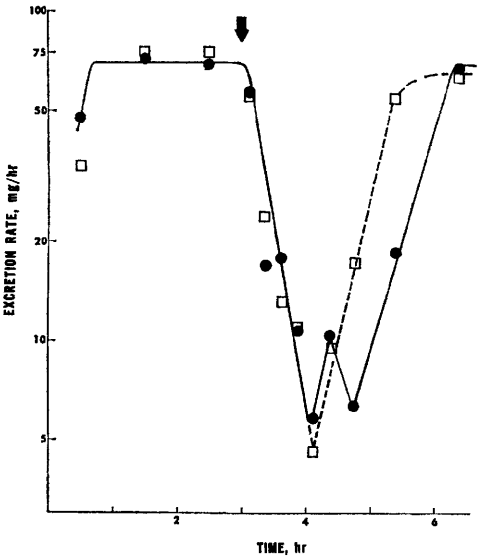

FIG. 5. Effect of ethanol on the excretion kinetics of salicyluric acid in man: Shown are the urinary excretion rates of salicyluric acid as a function of time after the administration of 1 g of salicylic acid followed 3 hr later by 3.2 g of benzoic acid with (●); and without (□) ethanol. The arrow indicates the time of ingestion of 3.2 g of benzoate and 10 ml of ethanol; 50 ml of ethanol were ingested 15 min before the benzoate (Subject A).

to adult human subjects results in the urinary excretion of salicylurate at an essentially constant rate for some hours, due to the limited capacity of man to conjugate salicylate with glycine (2). The formation of salicylurate can be blocked abruptly by administering benzoate, and the slope of the log excretion rate versus time curve following such a block can be used to determine the renal excretion rate constant of salicylurate

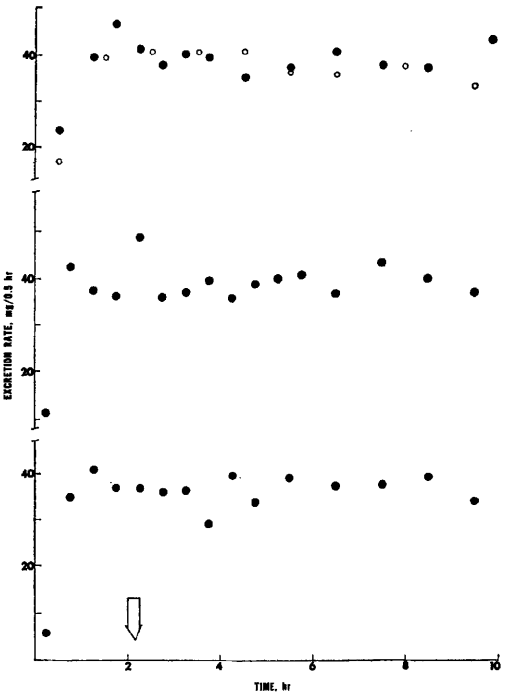

FIG. 6. Effect of ethanol on salicylurate formation in 3 subjects: Shown are the urinary excretion rates of salicyluric acid as a function of time after oral administration of 2 g of salicylic acid to each subject. Arrow indicates time of ingestion of 50 ml of ethanol; (○) results obtained in Subject A after administration of 1.7 g of salicylic acid without ethanol; (middle and lower curves) Subjects B and C, respectively.

(5). By this method it was found that 50 ml of ethanol has no effect on the renal excretion kinetics of salicylurate (Fig. 5). However, the blocking effect of benzoate on salicylurate formation is prolonged by ethanol. This is a consequence of the slower elimination of benzoate due to the inhibition of hippurate formation by ethanol.

Fifty ml ethanol had no effect on the urinary excretion of salicylurate following 2-g doses of salicylate. This is shown by the constancy of the urinary excretion rates of salicylurate before and after ethanol administration (Fig. 6). An inhibition of salicylurate formation would have resulted in an immediate decrease in the excretion rate of this metabolite. Thus, a dose of ethanol which inhibits the maximum formation rate of hippurate has no apparent effect on the maximum formation rate of salicylurate in man.

The maximum formation rate of hippurate from benzoate is limited by the availability of glycine (3) while the maximum formation rate of salicylurate from salicylate in man is apparently controlled by the rate of activation of the substrate (3, 6). The fact that ethanol inhibits hippurate formation but not salicylurate formation suggests that ethanol depresses the activity of glycine *N*-acylase (the enzyme which catalyzes the transfer of the activated drug to glycine) or decreases the availability of glycine to the conjugation system.

Summary. Ethanol (50 ml) inhibits the formation of hippurate from benzoate but has no effect on the maximum rate of formation of salicylurate from salicylate in healthy human subjects. This is consistent with other evidence showing that the conjugation of benzoate and salicylate with glycine in man involves different rate-limiting steps.

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