Hydrolysis of Aspirin by Rat Small Intestine

Sir:

A recent study of the effect of certain antirheumatic drugs on active amino acid transport across the small intestine revealed that the hamster small intestine is able to hydrolyze aspirin at an appreciable rate (1). This was considered to be due to the presence of esterase(s) in the intestinal epithelium which are known to occur also in all regions of the human gastrointestinal tract (2). It was suggested on this basis that appreciable hydrolysis of aspirin may take place during the absorption of this drug from the gastrointestinal tract (1). A subsequent report by Rowland and co-workers on the kinetics of aspirin disposition in man provided further evidence suggesting aspirin hydrolysis in the gut wall (3). In view of the apparent enzymic nature of the hydrolytic process it was of interest to determine its dependence on aspirin concentration.

Five-centimeter segments of everted small intestine were obtained from male Sprague-Dawley rats as described previously (1). Individual segments were placed in 50-ml. capacity conical flasks containing 15 ml. of 0–120 m*M* aspirin in Krebs-Ringer bicarbonate solution (final pH 7.4). Other segments of small intestine were first immersed in boiling water for 5 min. and then placed in aspirin solutions (controls). Flasks with drug solution but without an intestine segment were also prepared. All flasks were flushed with 95% oxygen–5% carbon dioxide mixture, sealed, and agitated mildly at 37° in a water bath. An aliquot of solution was removed after 1-hr., placed in a chilled test tube containing 1 *N* hydrochloric acid, diluted if necessary, and then extracted immediately with carbon tetrachloride and assayed colorimetrically for salicylic acid by the method of Brodie and co-workers (4).

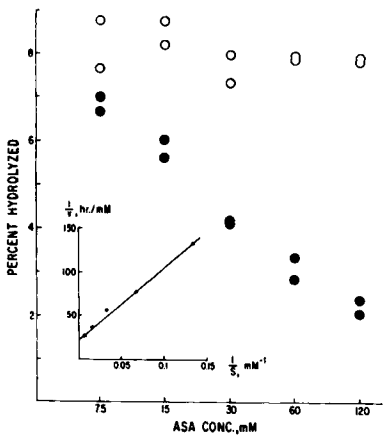

Fig. 1—Hydrolysis of aspirin by rat small intestine as a function of aspirin concentration. Key: ○, *percent hydrolyzed in 1 hr. in the presence of intestine segments inactivated by 5-min. immersion in boiling water (controls);* ●, *percent hydrolyzed in excess of control values by active intestine segments. Inset: Lineweaver-Burk-type plot of the average data.*

The hydrolysis of aspirin by everted intestine segments of rats as a function of aspirin concentration is shown in Fig. 1. The percent hydrolysis decreased markedly with increasing aspirin concentration. A double reciprocal plot of the Lineweaver-Burk-type yielded a linear relationship between the reciprocal of hydrolysis rate and the reciprocal of the initial aspirin concentration (inset of Fig. 1). The percent hydrolysis of aspirin in solutions containing boiled intestine segments was concentration independent and did not differ measurably from that in solutions without intestine.[1] These properties (saturability and heat inactivation) are characteristic of enzymic processes and suggest that aspirin hydrolysis by intestinal tissue is due to esterase(s). The presence of esterases in the human gastrointestinal tract is well established (2) and it is likely that the activity of nonspecific esterase differs in different regions of the gastrointestinal tract. The saturability of aspirin-hydrolyzing activity in the rat intestine is readily apparent at reasonable concentrations of aspirin under the conditions of the present study. It can be expected that this limited capacity of esterase activity will be reflected by a concentration and dose dependence in the extent of aspirin hydrolysis during absorption. Rapid absorption, with high concentrations of aspirin at sites of hydrolysis in the intestinal wall, is likely to result in less extensive hydrolysis of the drug than would be encountered when the same amount of drug is absorbed more slowly. Dosage-form characteristics, inasmuch as they affect the site and rate of release of aspirin in the gastrointestinal tract, may therefore be expected to influence the extent of hydrolysis of aspirin during absorption. The

[1] These control data reflect the extent of hydrolysis of aspirin during the preparation and incubation of the solutions.

magnitude of this effect in man must remain speculative pending appropriately designed clinical trials.

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A Cardioactive Steroidal Iodoacetate

Sir:

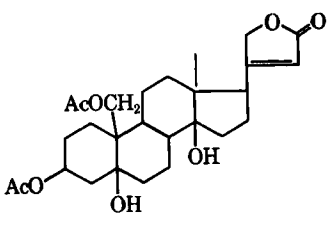
Empirical relationships between the chemical constitution and biological activity of cardiac glycosides and aglycones, *e.g.*, I, have been tabulated (1), and the unsaturated lactone at C-17 appears essential for activity. Moreover, it seems likely that these cardiotonic substances act through inhibition of enzyme systems involved in ion transport (2).

The chemical basis for the essentiality of the lactone is unknown. Portius and Repke (3) have proposed that the α,β-unsaturated carbonyl *Wirkguppierung* is a proton acceptor in hydrogen bonding, whereas Glynn (4) has postulated that addition of essential sulfhydryl groups to the unsaturated center may be involved. We have evaluated the last suggestion by the preparation of analogs.

The reaction of II with chloroacetic anhydride at 55° produced the corresponding 21-chloroacetate ester m.p. 109–111°, $[\alpha]_D$ + 51°[1] which on treatment with sodium iodide in acetone solution gave the iodoacetate III, m.p. 150–155°, $[\alpha]^{20}$ + 49°.

Biological evaluation was performed in the usual way (6) in cats under chloralose-urethan anesthesia. A volume of 1 ml. of 47.5% alcohol containing 0.4 mg. of drug was injected at 3-min. intervals into the femoral vein. The effect of the drug was observed by EKG and blood-pressure readings recorded on a Grass polygraph. The following lethal doses were determined (LD ± SE in mg./kg.): III (1.37 ± 0.26), IV (7) (15.59 ± 2.28). The high activity of III indicates that the lactone ring is not an essential feature for a cardioactive compound.

Iodoacetates are known to react with nucleophiles, such as sulfhydryl groups. Thus a reasonable explanation for the great difference in potency of the iodoacetate III relative to the acetate IV is that alkylation of an essential nucleophilic



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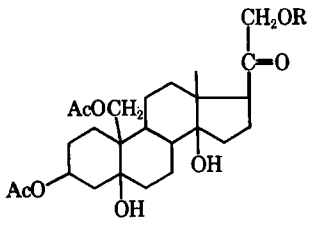
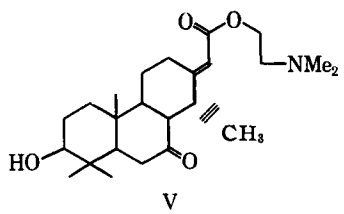


II, R = H
III, R = $COCH_2I$
IV, R = $COCH_3$



V

Strophanthidiol 3, 19-diacetate (I) (5) in ethyl acetate solution was allowed to react with ozone at −70°, and the resulting ozonide was decomposed with zinc dust in acetic acid. Isolation of the product afforded 3β, 5β, 14β, 19, 21-pentahydroxypregnan-20-one 3,19-diacetate (II).

group on the receptor is required for drug action. This is in harmony with the concept that the unsaturated lactone performs a similar function by addition of the nucleophile to the double bond. Cavallito and Haskell (8) have described such

[1] Satisfactory analyses have been obtained for all new compounds in this paper.