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## Increased ileal absorption of salicylic acid induced by carbonic anhydrase inhibition

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PRETREATMENT with the carbonic anhydrase inhibitor, acetazolamide, has been shown to enhance the uptake of certain drugs by the brain.[1,2] Recently it has been observed that acetazolamide treatment may affect the absorption of drugs through other body membranes.[3] Therefore, we have studied the absorption of $^{14}$C-salicylic acid *in vivo* from intestinal sacs located in the ileum of rats after treatment with acetazolamide and CL-13,850, an inactive analogue of acetazolamide.[4]

Male, Holtzman albino rats (170–200 g), fasted for 24 hr, were pretreated with acetazolamide (50 mg/kg, s.c.), CL-13,850 (50 mg/kg, s.c.) or an equivalent volume of saline (pH adjusted to 8·5, the same as the solvent for acetazolamide). The animals were anesthetized with urethane (1·25 g/kg i.p.) and intestinal sacs 5–7 cm in length were formed by the method of Levine and Pelikan[5] in the ileum approximately 2 cm from the ileocecal junction. The ligatures were carefully placed so they did not interfere with the blood supply to the ileal sac.

The intestinal segment was carefully washed with warm saline, and then 1·0 ml of warmed (37°) saline containing 1 mM $^{14}$C-salicylic acid (0·25 $\mu$c/ml) was introduced into the sac via a polyethylene cannula 30 min after the appropriate pretreatment. The cannula was then withdrawn and the ligature tightened. The sac was replaced into the abdominal cavity and the incision was closed.

After an appropriate interval, a blood sample was obtained by cardiac puncture. The ileal sac was removed and the pH of the contents was determined by using a Beckman hypodermic electrode assembly (No. 39022) which prevents exposure of the sample to air. The ileal contents were emptied into a beaker and the sac was washed several times with saline. The ileal contents and washings were quantitatively transferred to a 25 ml volumetric flask and brought to volume with distilled water. A 0·5 ml aliquot was mixed with 16 ml of TC scintillation fluid (6 g of 2,5-diphenyloxazole dissolved in a mixture of 600 ml ethyl cellosolve and 1000 ml toluene) and counted in a Beckman LS 100 scintillation spectrometer. Each sample was corrected for quenching. The extent of drug absorption was calculated from the difference between the amount of drug placed into the ileal sac and the amount recovered. Blood samples were prepared for liquid scintillation analysis by the method of Mahin and Lofberg[6] and counted for total radioactivity by the same method used for the ileal contents. Statistical analyses were performed using Student's *t*-test.

The results are presented in Fig. 1. There was a significant ($P < 0·05$) decrease in the pH of the ileal contents at all time intervals after treatment with acetazolamide when compared to either the saline control or CL-13,850 treatment. The pH values obtained from the CL-13,850-treated animals

did not differ from the corresponding values obtained from the saline controls (Fig. 1a). A significant ($P < 0.05$) increase in the percentage of $^{14}$C-salicylic acid absorbed after the acetazolamide treatment compared to either saline controls or CL-13,850 treatment was observed. No differences were found between corresponding values obtained from the latter two groups (Fig. 1b). The observed increase in the absorption of $^{14}$C-salicylic acid from the ileal sac after acetazolamide treatment was reflected in increased ($P < 0.05$) blood levels of the labeled drug (Fig. 1c). Blood levels of labeled drug obtained from CL-13,850-treated animals and saline controls did not differ.

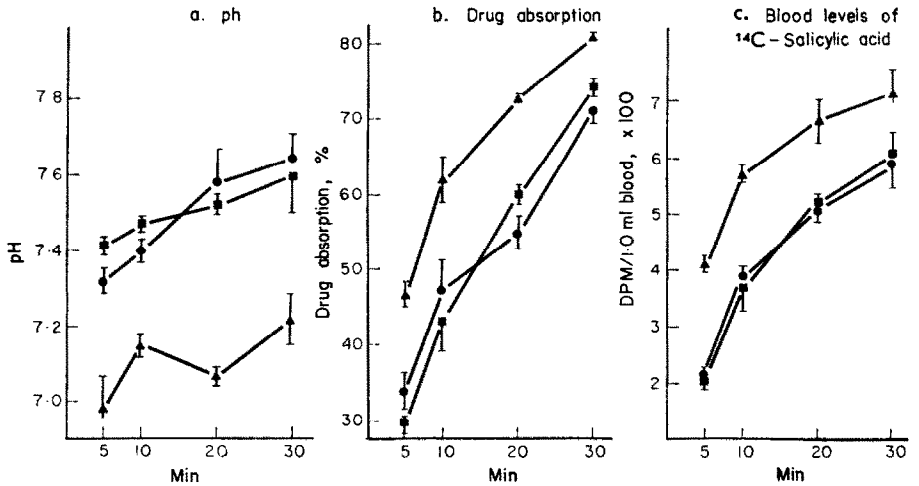


FIG. 1. Effect of acetazolamide (50 mg/kg, s.c.), CL-13,850 (50 mg/kg, s.c.) and saline (pH 8·5) on: (a) the pH of the ileal contents; (b) per cent of drug absorbed; and (c) blood levels of labeled drug. Each value represents a mean of three to four animals ± standard error. Triangles, acetazolamide; squares, CL-13,850; circles, saline controls.

The decrease in the pH of the ileal contents after acetazolamide treatment has been previously reported by Parsons.[7] Because of the specific nature of the inhibition of carbonic anhydrase by acetazolamide, this would infer that carbonic anhydrase found in the ileum[8] may be involved in the acid–base balance of the organ.[9] Because salicylic acid is an acidic drug ($pK_a$ 3·0), a decrease in pH would result in an increase in the rate of absorption.[10, 11]

In this study, alterations in the pH of the ileal contents and in the percentage of salicylic acid absorbed were observed after acetazolamide treatment, but not after CL-13,850 treatment. This suggests that carbonic anhydrase inhibition produces physiological alterations (e.g. pH changes) which may influence the rate of absorption of drugs.

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## Effect of clofibrate (ethyl-chlorophenoxyisobutyrate) feeding on glycolytic and lipogenic enzymes and hepatic glycogen synthesis in the rat*

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CLOFIBRATE (ethyl-chlorophenoxyisobutyrate) decreases the plasma triglyceride and cholesterol concentrations in some patients with hyperlipidemia.[1–4] This effect is predominantly on the plasma triglycerides, but the mechanism of this action is understood poorly. Since the plasma triglyceride concentration must be dependent, in part, on the rate of hepatic fatty acid synthesis,[5] clofibrate could decrease the plasma triglyceride concentration by decreasing the activity of liver enzymes which are involved in the conversion of carbohydrates to fatty acids. The activity of both the lipogenic and glycolytic enzymes are important determinants of the rate of hepatic fatty acid synthesis.[6–8] In the rat, clofibrate also decreases the hepatic glycogen concentration.[9] In the present experiments we report data on the effect of clofibrate on the activity of several glycolytic and lipogenic enzymes and on glycogen synthesis in the rat liver.

### METHODS

In the studies on glycolytic and lipogenic enzymes, male Holtzman rats weighing 350–500 g were pair-fed diets containing per 100 g: 70 g fructose, 15·5 g casein, 7 g corn oil, 5 g vitamin mixture (Nutritional Biochemical Corp.) and 2·5 g salt mixture (Nutritional Biochemical Corp. No. XIV). In the glycogen synthesis experiments, the diet contained per 100 g: 70 g fructose or glucose, 20 g casein, 4 g corn oil, 3 g vitamin mixture (NBC), 2·5 g salt mixture (NBC XIV), and cystine, 0·3 g. Clofibrate, when added, was present at a level of 1·0 g per 100 g of diet.

In the enzyme studies, all animals had a net loss of a small amount of weight on this diet, but the weight losses were nearly the same in the control and clofibrate-fed rats. Animals were fed these diets for a period of 23–26 days. Two studies were done in the glycogen synthesis experiments. The animals in experiment I were fed *ad lib.*, and in experiment II were pair-fed. The animals were housed in individual cages and had free access to water. Body weight and food consumption were determined weekly in experiment I and daily in experiment II.

The rats were killed by decapitation and the livers were quickly removed. In some animals the entire liver was removed in order to determine the ratio of total liver weight to body weight. In all

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