THE EFFECT OF SOME ENZYME POISONS ON THE SECRETION OF BILE

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Recently published investigations [7] into the mechanism of bile formation indicate that active secretion plays the major role in choleresis. For the secretion of bile to take place, energy is required, and this may be provided only by the chemical reactions taking place with the aid of appropriate enzymes. Enzyme poisons may therefore change the volume of bile secreted, and also its composition. In 1957, working with the isolated cat's liver, we discovered a decrease in bile formation after perfusion with sodium cyanide, sodium fluoride, monoiodoacetic acid, and 2,4-dinitrophenol [1]. Similar results were later obtained by Vanlerenberghe and co-workers [3, 6, 7], working with the isolated rat's liver.

The object of the present study was to investigate the effect of certain enzyme poisons on choleresis in the intact animal, and to compare the results thus obtained with the results of experiments on the isolated liver.

EXPERIMENTAL METHOD

Experiments to determine the choleresis were carried out on albino rats weighing 170-230 g by la Barre's method [5], in which an intestinal pouch 3 cm long was exteriorized. Before the experiment, the animals fasted for 12 to 18 h. Bile was collected for a period of 2 h. Estimations were made of the volume of bile (with an accuracy of 0.1 ml), and the concentrations of bilirubin [2] and bile acids [5].

Rats possess a stable biliary secretion. The absence of a gall bladder in these animals facilitates the investigation of bile formation. By the use of this technique it is possible to carry out acute experiments on rats without prolonged anesthesia. A high degree of correlation has been established in rats between bile formation, the body weight, and the weight of the liver.

To determine the influence of enzyme poisons on the functional state of the liver cells, in experiments on guinea pigs with a fistula of the bile duct (the cystic duct was ligated) we determined the rate of excretion of bromsulfalein (BSF) injected into the blood stream, in the bile. Experiments were conducted on animals weighing 400 to 500 g. BSF was injected into the heart in a dose of 2 ml. Bile was collected for a period of $2\frac{1}{2}$ h every 30 min, and the BSF concentration in each sample was measured colorimetrically. Since the volume of bile was measured accurately, it was easy to determine the excretion of BSF during every 30 min. The criterion of the rate of excretion of the dye was the time required to excrete 50% of the amount injected, i.e., 1 ml of BSF.

Sodium fluoride was injected in a dose of 20-40 mg/kg, monoiodoacetic acid (MIA) in a dose of 50-100 mg per kg, sodium arsenate in a dose of 50 mg/kg, and 2,4-dinitrophenol in a dose of 20 mg/kg. All the solutions were injected intraperitoneally.

EXPERIMENTAL RESULTS

The mean values of the bile flow and of the concentrations of bilirubin and bile acids in the bile were determined in 34 control experiments in which physiological saline was injected (Table 1).

As glycolytic poisons we used sodium fluoride and MIA. Fluorides are inhibitors of acid and alkaline phosphomonoesterases, phosphoglucomutase, adenosinetriphosphatase, glucose-1-phosphatase, and hexose-1,6-diphosphatase. MIA inhibits hexokinase and triose phosphate dehydrogenase; in its presence all oxidation-reductionsrequiring the participation of codehydrogenase 1 are inactivated.

The effect of sodium fluoride was shown by a decrease in the volume of bile secreted and in the concentration of bilirubin in the bile. The intensity of its action rose with an increase of the dose, and it was most marked when

TABLE 1. Action of Enzyme Poisons on Bile Secretion

Poison	Volume of bile (ml)	Bilirubin (mg%)	Bile acids (mg%)	No. of expts.
Sodium fluoride (40 mg/kg)	0.25 ± 0.029	5.1 ± 0.523	2.150 ± 364.2	19
MIA (100 mg/kg)	0.26 ± 0.024	6.3 ± 0.560	1.100 ± 237.1	20
Sodium arsenate (50 mg/kg)	0.51 ± 0.038	6.7 ± 0.437	1.760 ± 279.6	10
Dinitrophenol (20 mg/kg)	0.34 ± 0.026	8.2 ± 1.03	1.530 ± 257.4	10
Control	0.57 ± 0.046	8.0 ± 0.808	2.420 ± 343.5	34

TABLE 2. Effect of Enzyme Poisons on the Elimination of BSF

Poison	Time of excretion of 50% of BSF (in min)	Mean conc. of BSF in bile during excretion of 50% of dose (in %)	No. of expts.
Sodium fluoride	56.5	0.35	9
MIA	85.6	0.28	10
Dinitrophenol	48.7	0.45	16
Sodium arsenate	74	0.40	14
Control	50.3	0.47	22

the dose was 40 mg/kg. A similar picture was obtained in the experiments with MIA. Both substances, especially MIA, inhibited the excretion of BSF in the bile: the time of excretion was prolonged and the concentration of BSF in the bile was lowered (Table 2).

In experiments on the isolated cat's liver, we found [1] that sodium fluoride in a concentration of 0.01 M and MIA in a concentration of 0.0005 M depress the level of biliary secretion on the average by 40%. Vanlerenberghe [6] found no changes in bile formation by the isolated rat's liver when perfused with sodium fluoride in concentrations of $(4\text{-}7.5) \cdot 10^{-5}$. In our opinion, the explanation of this difference between the results is as follows. Vanlerenberghe perfused the rat's liver with a mixture of blood and physiological saline containing calcium ions. On the addition of sodium fluoride to the perfusate, calcium fluoride was undoubtedly precipitated, being only sparingly soluble. As a result, the active fluoride ion was bound, and the vessels of the liver were occluded. So far as MIA is concerned, this considerably reduced the secretion of bile by the isolated rat's liver; the concentration of bilirubin in the bile was lowered and the excretion of BSF in the bile was sharply depressed.

Hence, there was a complete analogy between the action of the glycolytic poisons (MIA and fluoride) on the choleresis of the isolated liver and of the liver in vivo. Depression of the glycolytic processes in the liver depressed bile formation and the excretion of BSF.

Enzyme poisons influence the process of bile formation either by depressing energy metabolism or by acting on the specific reactions of bile secretion. During the formation of the fluid phase of the bile, the principal source of energy is the tissue respiration and the associated oxidative phosphorylation [1].

We have previously suggested that glycolytic processes are important in the secretion of bile, but that their role has nothing to do with the energy producing aspect of bile formation. We may support this hypothesis by citing the results of our experiments with sodium arsenate (Table 1). In the presence of arsenate, the oxidation of triose phosphate can take place without the simultaneous phosphorylation of ADP, i.e., the connection between glycolytic oxidation-reduction and the phosphorylation of ADP is broken [6].

Injection of arsenate into rats in a dose of 50 mg/kg, like perfusion of arsenate through the isolated liver [1], did not change the rate of bile formation and slightly lowered the concentration of bilirubin and bile acids in the bile. A considerable delay in the elimination of BSF was observed (see Table 2).

Experiments showed that 2,4-dinitrophenol, as in the experiments on the isolated liver, reduced the volume of bile secreted but had no effect on the concentration of bilirubin in the bile or on the rate of elimination of BSF. The concentration of bile acids was lowered.

The difference between the action of MIA and sodium fluoride on the excretion of bile acids is of interest. MIA did not change the concentration of bile acids in the secreted bile, but sodium fluoride lowered it considerably (to 1400 mg%). It is possible to interpret these observations by recalling that MIA paralyzes triose-phosphate dehydrogenase and halts the formation of phosphoglyceric acids. Sodium fluoride, on the other hand, stimulates the formation of phosphoglyceric acids, while paralyzing the enzyme enolase and preventing the formation of phosphoenol-pyruvic and phosphoglyceric acids. Hence, it may be postulated that the formation of phosphoglyceric acids is very important in the synthesis of bile acids.

The results of the experiments in which the character of the action of certain enzyme poisons on bile formation was investigated in vivo agreed with the results of our previous investigations and with Vanlerenberghe's findings, obtained on the isolated liver; it may thus be concluded that enzyme poisons have different effects on the quantitative and qualitative aspects of bile secretion. For instance, while 2,4-dinitrophenol and cyanides, like MIA and sodium fluoride, decrease the volume of bile secreted in experiments on the isolated liver, they do not affect the elimination of bilirubin and BSF in the vile, whereas MIA and sodium fluoride inhibit these processes.

Depression of the secretion of bile by enzyme poisons is in favor of a secretory mechanism of bile formation. It is evident that different mechanisms exist for the formation of the fluid phase of the bile and for the concentration of the substances dissolved in the bile.

SUMMARY

Experiments were staged on albino rats and guinea pigs with a common bile duct fistula. The effect of enzymatic poisons on bile secretion, its qualitative composition and bromsulfalein (BSF) excretion were studied. Sodium fluoride monoiodacetic acid (MIA) and 2,4-dinitrophenol decrease bile secretion; 2,4-dinitrophenol has no effect on bilirubin and BSF bile excretion, whereas MIA and sodium fluoride inhibit these processes. MIA considerably reduces the bile concentration of bile acids.

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