ON THE BIOENERGETICS OF BILE SECRETION

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According to recent communications [6-8] on the mechanism of bile formation, it appears that this process consists of the active secretion of the elements of bile by the liver cells, followed by filtration from the blood stream via capillary membranes and a slight resorption of water and some other bile ingredients form the bile ducts. Bile formation through secretion is obviously an energetic process. These considerations prompted the present study of the bioenergetics of bile formation and of the action of some cholagogues, widely applied in the treatment of liver disease with demonstrable dysfunction of bile secretion.

The present communication deals with some problems of the chemical dynamics of bile formation.

EXPERIMENTAL METHODS

Isolated cat liver preparations were made according to accepted procedure. The afferent cannula was placed into the portal vein; the bile was collected through a cannula placed in the main bile duct. The liver was perfused with physiological saline, previously aerated and warmed to 39°. Perfusion was carried out under a hydrostatic pressure of a 20 cm column of water. The rate of bile secretion was determined over a two hour period, and was expressed in ml per 30 min. Inhibitors were employed at the following concentrations: Sodium cyanide — $M/500$, monoiodoacetic acid — $M/2000$, sodium fluoride — $M/100$, 2,4-dinitrophenol — $M/700$ and sodium arsenate — $M/100$.

In situ experiments were carried out either with rabbits or with dogs prepared with either a gall-bladder fistula or a main bile duct fistula (in the latter case the bladder duct was ligated). Sodium fluoride was given intravenously in doses 30 mg/kg.
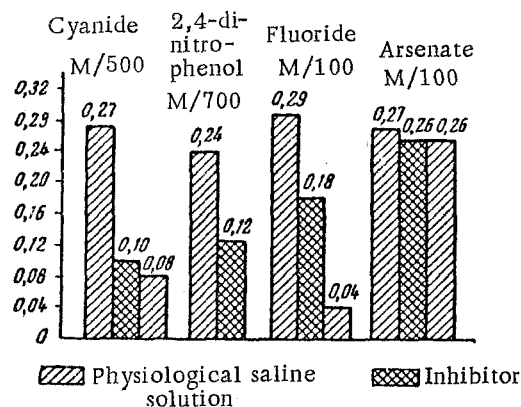
In experiments dealing with liver oxidation and phosphorylation, aliquots of rabbit liver homogenate (400 mg) were incubated for 40 min at 26° in Warburg vessels containing phosphate-salt mixture (pH 8.1-8.2), 15 mg of glucose, and 6.5 mg of sodium fluoride in a total volume of 3 ml. Incubations were carried out under both aerobic (oxygen) and anaerobic (hydrogen) conditions. Intensity of phosphorylation was determined by the uptake of inorganic phosphate, the remainder of which was estimated in the trichloroacetic acid extract of the incubation mixture using the molybdate reagent and eikonogen.

Adenosinetriphosphate and inorganic orthophosphate were estimated in liver tissue frozen in liquid air. Inorganic phosphate (P_0) in the trichloroacetic acid extract was determined after precipitation with magnesia mixture, and the labile phosphate of ATP etc. — as the difference between P_7 mineral phosphate after 7 min at 100° in 1 N HCl) and P_0. Using a specific enzymatic method and the preparative isolation of ATP from liver, G. P. Toropova [5] showed that the "pyrophosphate fraction" of liver, as determined by acid hydrolysis, represented the true ATP content of this tissue. The high glycogen content of liver did not interfere with ATP determinations.

Isolated livers were perfused with physiological saline for two hours in each of six independent experiments. The rate of bile secretion was found to be fairly stable throughout; only in one case there occurred a spontaneous decrease of secretion rate toward the end of the second hour.

Whenever inhibitors were employed, the normal secretion rate was first determined over a 30-min period, and the inhibitor was then introduced with the perfusate. Tissue respiration was depressed by sodium cyanide. The completeness of the respiratory block was confirmed directly gasometrically in the Warburg apparatus. Perfusion with sodium cyanide (12 experiments) led to an inhibition of bile secretion to the extent of 32-80%, mean value — 63% (see figure). Further perfusion with fresh saline did not reverse the inhibition: bile secretion remained on the low level or, sometimes, decreased further. Similar observations were made by S. Ia. Rapoport and K. A. Gerchikova [3], who found that asphyxia in situ caused a sharp drop, or even cessation, of the bile flow. Interference with tissue respiration was thus shown to depress bile secretion severely.

The effect of enzyme inhibitors on bile secretion by the isolated cat liver (in ml per 30 min).

Glycolysis in livers was depressed by perfusion of the isolated organs with monoiodoacetic acid or with sodium fluoride (20 experiments). Sodium fluoride inhibited bile formation in the isolated liver to the extent of 16-60%, mean 38% (see figure). Washing with physiological saline caused further inhibition of the bile flow. Similar results were obtained with monoiodoacetic acid. In in situ experiments, using rabbits, it was found that monoiodoacetic acid caused a mean inhibition of 42% of the bile secretion, when administered in amounts 30 mg/kg. Thus, in isolated livers, and in experiments in situ, the bile secretion was found to be inhibited when either tissue respiration or glycolysis were interfered with.

According to established concepts, the energy of glycolysis and respiration is not utilized directly, but is channelled for the resynthesis of ATP; the splitting of this compound then provides the necessary energy in the various biosynthetic reactions. ATP resynthesis in the liver may take place through the coupling of phosphorylation with either respiration or glycolysis. In order to distinguish between the respective contribution of respiration and glycolysis toward the energetic balance in the synthesis of the high transfer potential groups of ATP, the respective values of respiratory and glycolytic phosphorylation were determined using rabbit liver homogenates (see table).

The P-uptake values found were, under aerobic conditions, 0.66 mg P_0 per test, under anaerobic conditions — 0.11 mg. The P/O ratios were 1.57. The present data are thus in agreement with existing opinion [2, 4], that oxidative reactions in the liver provide the main source of energy, through the formation of high transfer potential phosphate groups of ATP. This gains further support from the present experiments dealing with the levels of ATP after the introduction of cyanide and fluoride. As a rule, liver transfusion with cyanide caused considerable fall in ATP concentration (mean, from 12.3 to 5.1 mg%) while fluoride had virtually no effect on the liver ATP levels.

In another series, involving 38 experiments, attempts were made, with the use of 2,4-dinitrophenol and sodium arsenate, to elucidate the energetic role of aerobic and anaerobic phosphorylation in the process of bile formation and its mechanism. As is known, 2,4-dinitrophenol causes the uncoupling of oxidation from the resynthesis of ATP, thus rendering the former process energetically useless, while in the presence of arsenate, phosphotriose oxidation may take place without ADP phosphorylation, i.e., there takes place the uncoupling between the glycolytic oxidation—reduction processes and ADP rephosphorylation [1].

If anaerobic phosphorylation be energetically essential for the process of bile secretion, then arsenate poisoning should cause the depression of the phenomenon under investigation, in the same way as did monoiodoacetic acid and fluoride. However, experimental evidence (involving perfusion of the isolated liver with arsenate) showed that arsenate did not affect the rate of bile secretion. An entirely different result was obtained with dinitrophenol: bile secretion fell by 24-78% (mean — 50%; see figure). The observation thus showed that bile formation depended energetically on oxidative phosphorylation.

Expt. No.	Inorganic phosphorus esterified mg		Oxygen consumption in mm^3 per 40 min	P/O ratio (anaerobic phosphorylation excluded)
	N_2	O_2		
39	—	0,68	200,3	1,23
40	—	0,73	142,4	1,86
41	0,12	0,67	137,5	1,76
42	0,11	0,65	157,5	1,49
43	0,10	0,72	177,1	1,47
44	—	0,78	169,5	1,67
45	—	0,52	111,0	1,70
46	0,13	0,70	166,9	1,52
47	0,10	0,57	144,2	1,43
48	0,09	0,65	156,9	1,50
49	0,10	0,67	141,4	1,71
50	0,11	0,55	138,1	1,45
Mean	0,11	0,66	153,6	1,57

The evidence presented above allows the conclusion that tissue respiration, together with the coupled phosphorylation process, provide the main energy source for bile secretion. Glycolytic processes affect some specific reactions of bile formation, rather than its energetics. The latter aspect requires further investigation.

SUMMARY

Experiments were performed on animals with fistula of the common bile duct and on isolated liver with fistula of the gall bladder. It was established with the aid of enzyme poisons (sodium cyanide, sodium fluoride, monoiodacetic acid, 2,4-dinitrophenol, sodium arsenate) that tissue respiration of liver and oxidative phosphorylation connected with it are the main origins of energy in bile secretion.

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