SECRETION OF LIPOPROTEIN COMPLEX IN HEPATIC BILE

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Among the various substances secreted in the bile, particular importance has recently been attached to the phospholipids [1, 2, 4, 6, 11, 12, 15], which are again absorbed in the intestine and play an important role in general metabolism.

The problem of what chemical mechanism conditions the separation of phospholipids and cholesterol from the bile in a soluble form is of great interest, all the more because the concentration of phospholipids in the bile is approximately ten times greater than their plasma concentration.

In the present study the results of our experiments are given and show that hepatic tissue forms and secretes a lipoprotein complex compound into the bile. This compound, evidently, plays an important role in the mechanism of removal of lipids from the liver tissue.

It was suggested long ago that certain components of bile are found in close interrelationships with one another [9, 10]. Using human gallbladder bile, Isaksson [10] found, based on experiments with extraction, micelles of cholesterol, lecithin and cholic acid present in the bile. This question was studied in greater detail by Verschure [14]. The method used was paper electrophoresis; he directly demonstrated the presence of lipoprotein complex in human gallbladder bile. After this study, widespread doubt was expressed in the literature that the given complex represented the product formed in the gallbladder by the participation of several substances secreted by the gallbladder mucosa. However, as it was elucidated, this complex is secreted by the hepatic tissue.

METHODS

The investigation was carried out with chronic and acute experiments.

To obtain hepatic bile in the chronic experiment we used an operative method, comprising the removal of the gall bladder and the bringing of the cystic duct to the abdominal wall [5]. At the time of the operation a plastic tube was inserted in the cystic duct. Outside of the operation these animals lost almost no bile, since the cystic duct was compressed by the muscles of the abdominal wall.

In the acute experiment the bile was obtained from the ducts with preservation of the gallbladder and compression of the cystic duct.

To determine the lipoprotein complex in fresh bile we used paper electrophoresis. On each strip of paper (five cm wide), 0.1 ml of bile was placed, stained with sudan black as done by several authors to measure blood lipoproteins [7]. The electrophoretic conditions were as following: gradient field of 15 V/cm, veronal-medinal buffer with pH 8.6, time period one hour (30 min with closed chamber and 30 min with open chamber). We carried out a series of preliminary measurements with different dilutions of bile. It was established that there is a direct proportionality between the quantity of lipoprotein complex and the results of the determination which holds for hepatic bile in the range of dilutions 1 :4 to 1 :10. The electrophoresis paper used was type "B" from the Leningrad factory.

After drying, the lipoprotein complex was eluted from the stained zone with a mixture of alcohol and acetic acid (3 :1) and the solution was measured in a photometer. The amount of lipoprotein complex was expressed in

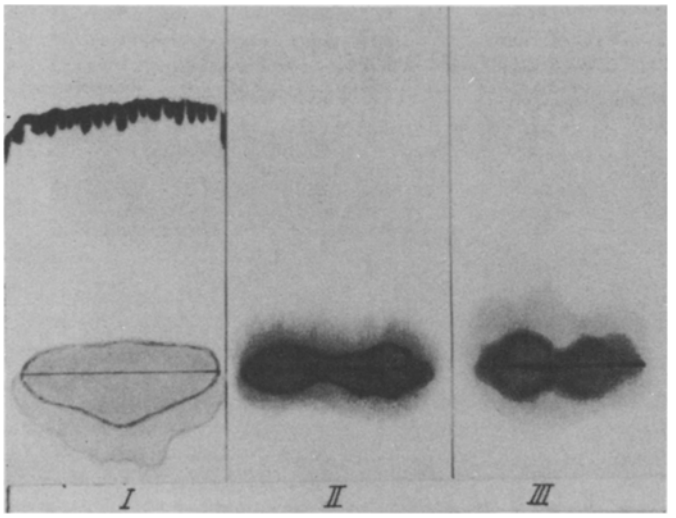

Fig. 1. Electrophoregram of different digestive secretions. Lipid stain. I) bile; II) pancreaticjuice; III) intestinal juice.

TABLE 1. Content of Lipoprotein Complex (in mg %) in Hepatic Bile in Different Dogs

Number of dog	Lipoprotein complex
5	1,320-2,900
6	1,000-1,900
9	1,650-2,600
10	1,300-2,450
11	1,380-2,160

milligram per cent of fat, for which the calibration curve was obtained with a mixture of cholesterol, triolein and tributyrin (150 : 200 : 150 mV per 100 ml of absolute alcohol).

At the same time the bilirubin, cholic acid [13], total phosphorus [3] and lipid phosphorus were measured on the bile by extraction with a mixture of methanol and chloroform according to Folch [8].

RESULTS

Investigation of hepatic bile obtained in fasted dogs showed that the lipoprotein complex of the bile on the stained electrophoregram in the zone of proportionality has a characteristic picture similar to a comb. The mobility of this complex in the electric field exceeds that of blood serum albumin. With electrophoresis of gallbladder bile a similar picture is obtained. When other pure digestive secretions are analyzed—gastric and intestinal juices—the stained fluid remains at the starting line, indicating the absence of a similar lipoprotein component (Fig. 1).

Bile acids, phospholipids, cholesterol, proteins and bilirubin all enter into the composition of the liporpotein complex of hepatic bile. The presence of several of these was established by direct measurement. All pigments are displaced from the zone of movement of the complex and are present in the same band as the stained lipids. As the studies showed, up to 50% of the bile acids and all the lecithin enter into the composition of the lipoprotein complex zone. This is confirmed by the following experiment. A solution of sodium phosphate containing radioactive phosphorus (total of 400 microcuries of P^{32}) was injected intravenously into a dog. After the injection hepatic bile was withdrawn at various intervals and subjected to electrophoresis. Autoradiograms were obtained from the electrogram by exposure to x-ray film The site of darkening and its configuration on the film completely corresponded to the localization of the lipoprotein complex (Fig. 2).

The concentration of the lipoprotein complex in hepatic bile in different dogs was the same. In a single animal over the course of several months of observation it varied from a minimal value, more or less, by $1^1/_2$ to 2 times.

During bile secretion in the fasting state the lipoprotein complex concentration in hourly portions did not vary significantly. We attempted to trace how the complex is secreted during the period of feeding. It appeared that after feeding the dog with meat (100 g) or butter (40 g) the content of the complex decreased three to five times or more beginning with the second hour. The inhibitory action continued for some hours, and after 7-8 h the initial level had not been regained. At the same time with the decreased concentration of lipoprotein complex

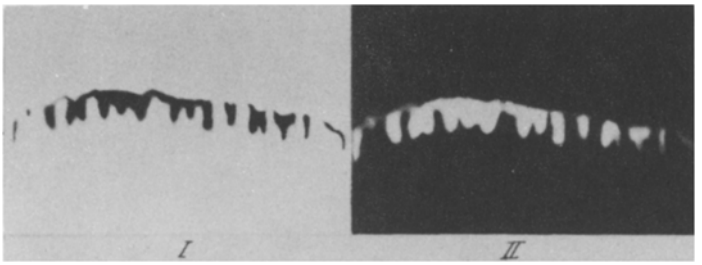

Fig. 2. Autoradiogram (II) obtained from electrophoregram (I). Bile was collected and subjected to electrophoresis at 24 h after the intravenous administration of P^{32} to the dog.

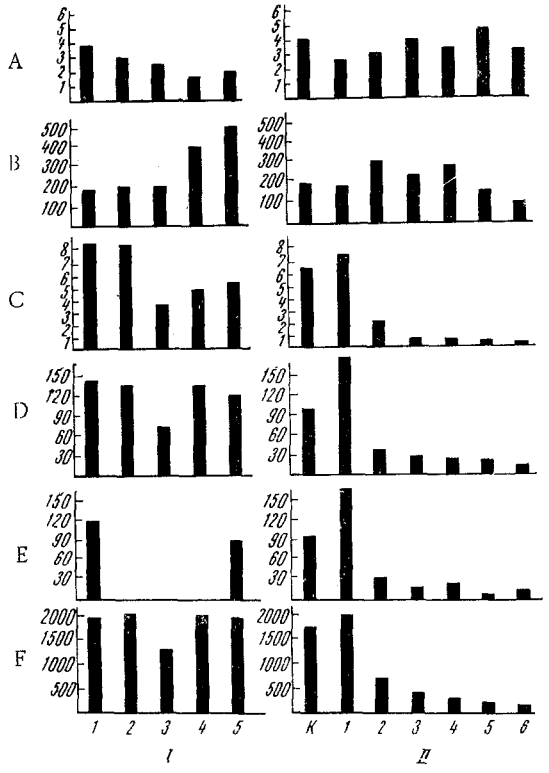

Fig. 3. Secretion of component fractions of bile taken from dog No. 5 while fasting (I) and after feeding of 100 g of meat (II). a) Bile (in ml); b) bilirubin in mg %; c) cholic acid (in %); d) phosphorus (total) (in mg %); e) lipid phosphorus (in mg %); f) complex (in mg %). Numbers on abscissa—hours. K) Control h before feeding. 1-5 h after feeding.

the concentration of bile acids, total and lipid phosphorus also declined. The bilirubin level in hepatic bile during the feeding period did not change significantly from the initial value (Fig. 3).

In the connection that in the operated dogs not all the bile secreted by the liver was drained through the externalized bile duct, the lower concentration of most bile components might be related to the increased volume of secretion in response to the feeding stimulation. To evaluate this point we performed experiments in which the cystic duct was externalized together with tieing of the common bile duct.

After such an operation we observed a decrease in the concentration of all bile fractions under study. At 10-12 days after ligation of the common bile duct experiments were performed with feeding the dogs meat or butter and these showed also, that as the result of feeding the concentration of all components of the bile except for the bilurubin was depressed. The degree of decrease was not as marked as in the experiments using dogs without ligated common bile ducts. The secreted quantity of bile after eating did not increase markedly; in the majority of cases it stayed at the same level and sometimes it decreased (Table 2).

The results of studies also indicate that changes in the secretion of the lipoprotein complex is not related to the dilution of the bile and does not depend on the presence or absence of bile in the intestine. Subsequent experiments showed that parenteral administration of insulin also provokes a lowering of the lipoprotein complex concentration, which begins within two hours and continues for several hours more. When glucose is administered this is not observed.

It may be hypothesized that the decrease in level of the complex after feeding is related to the changes in direction of synthetic processes in the liver. It is possible that these processes, in the absence of feeding, are directed mainly at the maintenance of the external secretions of the liver and after feeding—at the handling of substances which are absorbed and transferred to the bloodstream.

The factual material here presented gives us a basis for suggesting that the lipoprotein complex formed in the liver is a transport form, in which a large amount of phospholipid together with bile acids are transferred to the intestinal surface.

TABLE 2. Secretion of Component Parts of Hepatic Bile Taken Fasting and after Feeding in Dog No. 19 (with ligation of common bile duct)

	Bile (in ml)	Bilirubin (in mg %)	Cholic acid (in %)	Total phosphorus (in mg %)	Lipid phosphorus (in mg %)	Lipoprotein complex (in mg %)
Fasting						
in 1st h	7	48	1.64	40	–	600
in 2nd h	7	48	1.62	45	–	570
in 3rd h	6	62	1.64	48	–	650
in 4th	5	61	1.41	40	–	570
in 5th h	5	78	1.53	45	–	650
Before feeding	5.3	60	1.62	48	42	825
After feeding of 100 g of meet						
in 1st h	5	52	0.97	36	27	620
in 2nd h	5	55	1.0	34	25	620
in 3rd h	5	55	0.8	29	20	480
in 4th h	5.2	56	0.95	29	23	480
in 5th h	5	61	0.96	30	24	550

This fact again underlines the great importance of the secretion of phospholipids from the bile which are reabsorbed with nutrient materials and participate in metabolic processes [1, 3, 4, 6, 11, 15].

In addition to this function, the lipoprotein complex, evidently, plays an essential role in the digestion of fat in the intestine, as it includes the most important necessary elements: bile acids, phospholipids and protein. These elements contained in a single complex are the effective factor in the formation of fat emulsion and the creation of the conditions for the action of pancreatic lipase.

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