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In the recent literature considerable attention has been paid to the protein component of bile [2-6, 8, 9]. This question is of interest because with gallbladder disease the content and ratio of the protein fractions are altered and, according to some authors, may create conditions favoring the formation of calculi in the gallbladder [4, 8, 9]. Upon investigation of the gallbladder of patients using electrophoresis the appearance of a non-characteristic protein resembling albumin in it is noted. This suggests that during inflammatory processes in the gallbladder this protein enters the bile from the blood vessels in the mucous membrane of the gallbladder [4].

To understand the nature of alterations in the qualitative composition of gallbladder proteins it is necessary first of all to ascertain the protein components of bile in the healthy organism. It is important to know the composition both of the gall bladder bile and of the bile that enters the bladder.

Data in the literature concerning the qualitative composition of gallbladder and hepatic bile are related mainly to the experimental conditions, indicating that gallbladder bile does not differ from hepatic bile in the composition of the protein fractions as determined electrophoretically [6]. It must be noted that in all studies the gallbladder bile, concentrated by dialysis, and obtained at operation by gall bladder puncture, was subjected to electrophoresis.

However, the similar method for obtaining gallbladder bile, which has been the only method up to the present time, in our opinion, has certain deficiencies since we cannot be confident that the preoperative preparation of the animals and the operative process itself are not reflected in the composition of the protein fraction of gallbladder bile.

In the present work we studied the protein components of pure gallbladder and hepatic bile obtained in a chronic experiments from healthy non-anesthetized dogs.

METHODS

The method we have earlier elaborated [1] was employed, enabling us to obtain bile from a gallbladder which retained the integrity of all layers of the wall, the normal position, blood supply and innervation. The principle of the operation included the suturing of one end of an isolated section of small bowel (of length 10 cm) to the serosal coat of the gallbladder and the other into the abdominal wall. After the incision had healed, the gallbladder was punctured with a fine needle and pure gallbladder bile was obtained. This method permitted us to obtain and study bile in a simple animal over the course of many months. Hepatic bile was collected in the chronic experiment from bile ducts previously isolated in the

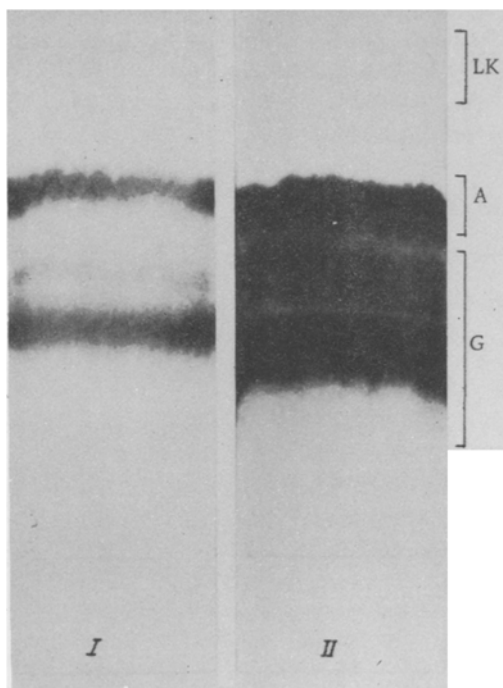


Fig. 1. Protein fractions of hepatic bile (I) and serum albumin (II); LK) lipoprotein complex; A) albumin; G) globulins.

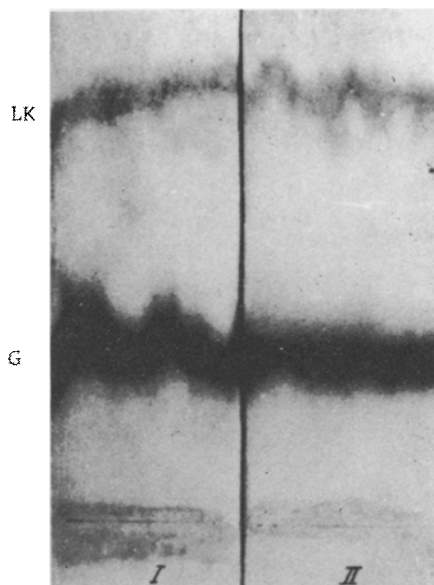


Fig. 2. Protein fractions of gallbladder bile.
Designations same as in Fig. 1.

remained at the starting point, and also a fraction which migrated in the electric field with a speed exceeding that of serum albumin. The latter fraction corresponds to the lipoprotein complex which stains well with lipid stains and contains phospholipids, cholesterol, bilirubin and cholic acid. The same picture of distribution of protein fractions was observed in electrophoresis of hepatic bile withdrawn by needle in the acute experiment directly from the ducts (Fig. 1).

On the electrophoretograms of gallbladder bile obtained from dogs in the chronic experiment by gallbladder puncture, the protein fraction which corresponds to albumin was absent. In gallbladder bile the lipoprotein, globulin and non-migrating fractions remain at the point of origin (Fig. 2). Only when gallbladder bile was obtained at the time of operation was the albumin fraction (as traces) observed in a number of cases by electrophoresis. Its presence in negligible quantities may be explained as the entrance of fresh hepatic bile in the gallbladder, the probability of which must be admitted during the operation. In such cases when the gallbladder bile was concentrated it was possible to see a rise in the albumin fraction; its protein composition was not different from hepatic bile.

Thus, in healthy dogs hepatic bile differs from gallbladder bile in that an albumin fraction, absent in gallbladder bile, is present in addition to other proteins. Upon study of certain other characteristics of hepatic and gallbladder bile the following was noted in particular. When a small amount of hydrochloric acid was added, imitating the acidity of gastric juice, a heavy precipitate formed in hepatic bile, whereas gallbladder bile only became turbid. If a small amount of serum protein (1/15 volume) containing albumin was added beforehand to gallbladder bile, the latter then reacted to hydrochloric acid as did hepatic bile (formation of a heavy precipitate). Consequently, the presence of an albumin fraction gives bile less stability to the action of certain unfavorable factors.

The disappearance of the albumin fraction from gallbladder bile may be explained by the presence of proteolytic enzymes which may split albumin. In order to verify this hypothesis in relation to the different dogs we collected hepatic bile and subjected it to incubation in the incubator at 37° for 24 h. As the results of these experiments show, such a procedure did not result in change in the initial composition of the protein fractions of hepatic bile as determined electrophoretically and, consequently, the process of albumin disappearance in the gallbladder is not related to the breakdown of albumin.

It follows from the data in the experiments that the albumin fraction of hepatic bile in healthy dogs is absorbed by the mucous membrane of the gallbladder. Upon alteration of the conditions for normal functioning of the gallbladder this process is disturbed and a protein composition is preserved in the gallbladder which is characteristic for hepatic bile. Thus, under the conditions of the acute experiment in anesthetized animals the albumin fraction

abdominal wall using a fine plastic drain inserted in it. The gallbladder was removed for this.

Bile was collected for study on fasted animals at 15-18 h after feeding. Electrophoresis of native bile without preliminary concentration was performed in a horizontal chamber with veronal-medinal buffer at pH 8.6. Duration of electrophoresis was 3 1/2 h in a gradient field of 15 V/cm. After the electrophoregram was dried it was stained with a blue-black stain (0.2 g stain, 100 ml glacial acetic acid and 900 ml methanol) and was washed with a mixture of phenol and acetic acid (40 ml phenol, 100 ml glacial acetic acid and 860 ml of water).

A total of six dogs in the chronic and seven in the acute experiment were observed.

RESULTS

As the observations showed, on stained electrophoregrams of different forms of bile obtained in the fasting state, a significant difference between the composition of gallbladder and hepatic bile is noted. In hepatic bile certain protein fractions were constantly present, one of which migrated with the mobility of blood serum albumin and two other with the mobility of globulins. In addition, in a number of instances a non-migrating fraction was isolated which

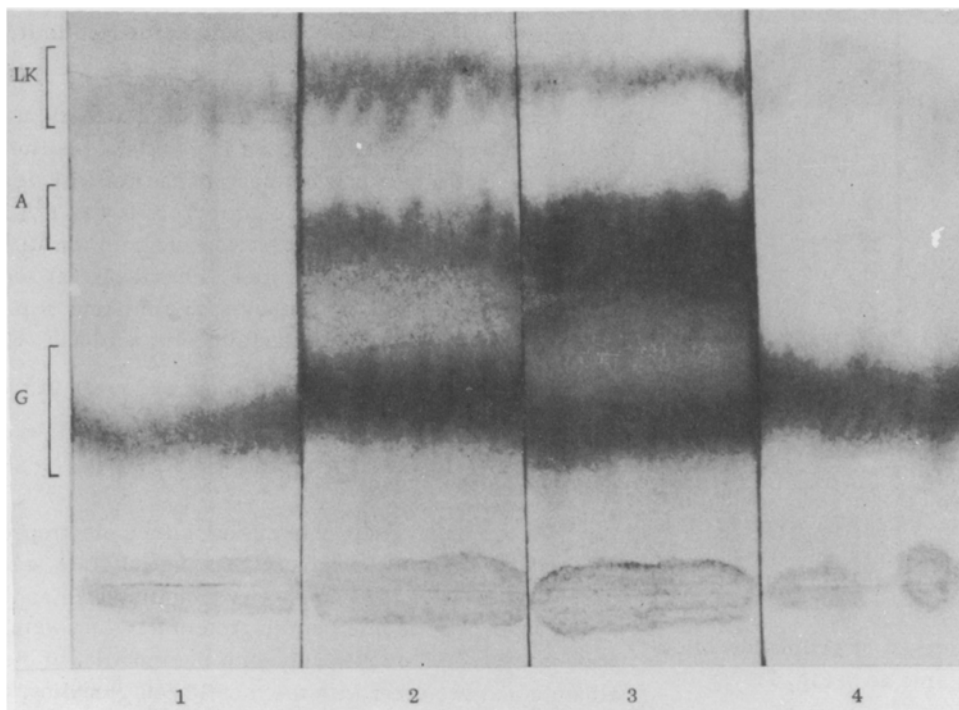


Fig. 3. Protein fractions of bile, obtained from the gallbladder in a chronic experiment after different time intervals. 1) Puncture; 2) at four hours after first puncture; 3) at four hours after second puncture; 4) at 18 h after third puncture. Remaining designations same as in Fig. 1.

of hepatic bile does not disappear from hepatic bile after 24 h of residing there, whereas in healthy animals under ordinary conditions of existence, after approximately the same period, hepatic bile entering the gallbladder takes on the character of gallbladder bile.

In particular, this is illustrated by the following experiment. In a single animal prepared as described above repeated puncture of the gallbladder was performed. In the first portion of the gallbladder bile, during fasting, the albumin fraction was absent. In the second portion of the bile obtained from the gallbladder after an interval of four hours, an albumin fraction was present as is characteristic for hepatic bile. This may be explained as the accumulation of newly arrived hepatic bile in the gallbladder and the absence of its absorption during the four h interval. In the portion of gallbladder bile obtained in this dog after 18 h, the albumin fraction had again disappeared (Fig. 3).

Indirect confirmation of the absorption of protein in the gallbladder may be offered by data indicating the different degree of concentration of bile components [7]. According to these data, the proteins in gallbladder bile of man are concentrated two to two and a half times, whereas bile acids, choline and lecithin are concentrated nine to 12 times.

The results of the experiments we carried out indicate that several protein fractions enter into the composition of hepatic bile in the dog. One of these has the electrophoretic mobility of albumin. When the bile enters the gallbladder the albumin fraction disappears—this is the basis of the consideration that it is reabsorbed.

It may be suggested that as a result of this process agents accumulate in the gallbladder which are necessary for the digestion of fats as a complex which permits greater stability of the bile. During the development of pathological processes albumin appears, the origin of which has been shown may be the disruption of the capacity for the gallbladder mucosa to reabsorb this protein.

We are performing further observations on the effect of different conditions on the nature of the protein fractions in bile in chronic experiments.

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