ROLE OF THE BILE-FORMING FUNCTION OF THE LIVER IN METHIONINE METABOLISM

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The object of this investigation was to study the absorption of methionine-S^{35} from the digestive tract, the rate of its disappearance from the blood, and the rate of its incorporation in the serum proteins in normal conditions and during the development of a pathological process in the liver.

In the same experimental conditions a simulataneous study was made of the role of the bile-forming function of the liver in methionine metabolism in vivo. This investigation is part of a project to examine the role of the digestive tract in intermediate metabolism in the body.

EXPERIMENTAL METHOD

The model of a pathological state of the liver was represented by dogs with a fistula of the gall bladder formed by the methods of Schiff (without ligation of the common bile duct) or of Schwann (with ligation of the bile duct). As described previously, soon after operation such dogs developed hepatitis [1]. The present experiment was carried out on three dogs (Ryzhik, Keri and Liza) on which Schiff's operation was performed, and three other dogs (Mumu, Tikhii, and Fok), undergoing Schwann's operation. Control experiments were performed on the same dogs before the formation of the gall bladder fistula or on a group of healthy, intact dogs.

Experiments on the same dog were carried out at various times after operation, and the duration of the investigation was between 6 and 18 months. The fasting dogs were given methionine-S^{35} mixed with milk (50 ml) and water (100 ml) in a dose of 200-300 pulses/g body weight. The residual activity of the blood and bile were studied in repeated investigations. Blood and bile samples were taken at a strictly determined time—15, 30, 60, 90, 120, 180, 240, and 300 min and also 24 h after the administration of methionine-S^{35}.

To study the rate of incorporation of methionine-S^{35} into the blood and bile proteins, the proteins were precipitated by a 10% solution of trichloroacetic acid (TCA) and then washed with 3-5% TCA. Lipids were extracted with a mixture of alcohol and ether. The protein residue was dissolved in 5% caustic soda and transferred quantitatively to a Plexiglas target. At the same time a standard preparation of methionine-S^{35} was prepared, and to it was added 0.2 ml of blood serum, bile, or dissolved protein, obtained from the animal before administration of methionine-S^{35}. The radioactivity of the samples was measured on a type B apparatus with an end-type counter. The results of the measurements were expressed as the radioactivity of 1 ml of blood serum or bile, as a percentage of the radioactivity of the methionine administered per gram body weight.

The concentration of free methionine-S^{35} excreted with the bile was also determined by dialysis of various bile samples in cellophane bags, immersed in physiological saline.

EXPERIMENTAL RESULTS

In the control experiments the maximal increase in radioactivity of the blood occurred 90-180 min after the animals had received methionine-S^{35}, and it amounted to 140-160% of the administered activity per gram body weight. Later in the experiment the level of the blood radioactivity remained unchanged or fell slightly. The radioactivity of the blood serum 20-24 h after administration of methionine-S^{35} was 90-130%.

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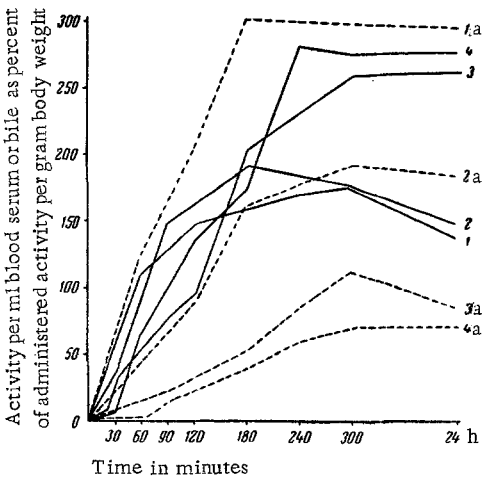

Fig. 1. Level of radioactivity of the blood serum (1-4) and bile (1a-4a) of the dog Ryzhik (operation by Schiff's method) on the day of administration of methionine-S^{35} and 24 h later. 1, 1a) 2 month; 2, 2a) 6 months 10 days; 3, 3a) 10 month; 4, 4a) 14 months 10 days after operation.

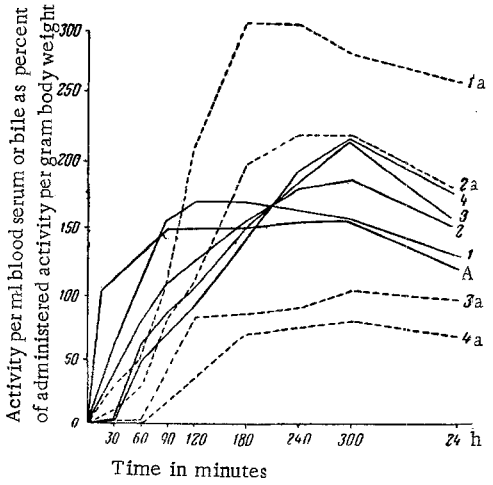

Fig. 2. Level of radioactivity of the blood serum (1-4) and bile (1a-4a) of the dog Mumu (operation by Schwann's method) on the day of administration of methionine-S^{35} and 24 h later. A – Control data (before operation); 1, 1a) 1 month; 2, 2a) 3 months; 3, 3a) 8 months; 4, 4a) 13 months after operation.

With an increase in the time after formation of the gall bladder fistula, the rate of absorption of methionine-S^{35} and of its utilization from the blood stream diminished. The maximal increase of radioactivity of the blood serum took place later and was higher than in the control experiments (200-250%). Twenty-four hours after administration of methionine-S^{35} the level of the blood radioactivity was higher than in the control experiments on the same dogs or on other intact dogs.

The results of investigations carried out at different times after the operation on the dogs Ryzhik and Mumu are shown in Figs. 1 and 2. The results obtained with the other dogs were similar.

Methionine-S^{35} was intensively excreted in the bile of the dogs. Soon after the operation the radioactivity of the bile after administration of methionine-S^{35} was much higher than the radioactivity of the blood. In these circumstances methionine-S^{35} was incorporated into the bile proteins in the liver. Radioactivity in the bile proteins was found in samples taken 60-90 min after administration of methionine-S^{35}. The results were similar to those of measurement of the radioactivity of a dialysate of bowel samples obtained at different time intervals after administration of the labeled amino acid to the animals. During dialysis of the first samples the radioactivity in the outer liquid was higher than during dialysis of subsequent samples.

With an increase in the period after operation, during development of the pathological process in the liver, the excretion of methionine-S^{35} with the bile was reduced. The level of radioactivity of the bile fell below the radioactivity of the blood serum. In the dogs Ryshik and Mumu, for example, in the early periods after the operation (1-2 months) the maximal level of radioactivity of the bile after administration of methionine-S^{35} reached 200-300% of the administered activity per gram body weight. In the later periods after the operation (6-10 months) the maximum was only 50-70%. Consequently, compared with the first experiments, the excretion of methionine-S^{35} with the bile was lowered by 75-80% (Figs. 1 and 2).

The rate of incorporation of methionine-S^{35} into the blood serum proteins also fell sharply in this period. Instead of the normal 100%, it reached only 30-40% (Fig. 3). In the dog with a fistula of the gall bladder and ligation of the bile duct, these changes took place sooner after the operation, presumably because of changes in the normal circulation of bile in the body and the more rapid development of the pathological process in the liver.

The activity was calculated not only per milliliter of bile, but also relative to its total volume excreted during that particular time interval. The volume of bile secreted varied in the course of an experiment and from one experiment to another. When the second method of calculation was used, the results

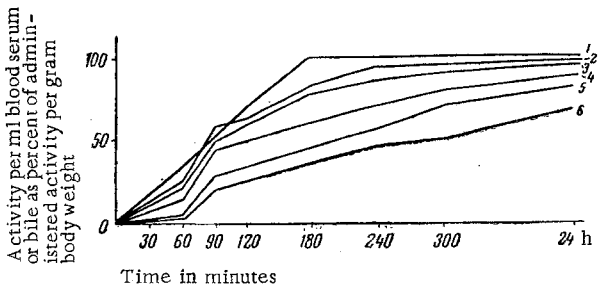

Fig. 3. Rate of incorporation of methionine-S^{35} into the serum proteins of the dog Ryzhik. 1 and 2) Control data; 3) 2 months, 4) 6 months 24 days, 5) 10 months, 6) 14 months 10 days after operation.

obtained were similar. Soon after the operation the ability of the liver to excrete methionine was reduced.

Histological investigations of the liver carried out on some of the experimental dogs revealed hyperemia of the blood vessels and capillaries of the liver, vacuolation of the liver cells, infiltration of the liver stroma with leukocytes, and droplets of fat in the epithelium of the bile ducts. At autopsy on some dogs, cirrhotic changes were found in the liver. The pathological changes in the liver were indistinguishable from those described by the authors previously [1].

Hence, during the development of a pathological process in the liver of dogs, the rate of absorption of methionine-S^{35} from the digestive tract was lowered. The utilization of the amino acid from the blood was slowed; the level of radioactivity of the blood after administration methionine remained higher than in the control animals. Meanwhile, the incorporation of methionine-S^{35} into the serum proteins was depressed.

A similar phenomenon—a low level of incorporation of methionine-S^{35} into the serum proteins and its slow disappearance from the blood—has been observed in other experimental conditions: after partial resection of the stomach by Finsterer's modification of the Billroth II method or after resection of the proximal portion of the small intestine. This finding was interpreted as the result of disturbance of the functional state of the liver [2, 3].

Changes in absorption of methionine-S^{35} from the degestive tract and in its utilization in liver disease (steatosis and cirrhosis) have also been reported by other authors [4, 5].

It follows from the results of the experiments described above that the incorporation in the label was depressed not only into the serum proteins, but also into the proteins excreted in the bile. The excretion of methionine, like that, evidently, of other amino acids, in the bile is evidently one link in their circulation in the organism. Diseases of the liver, when reflected in its bile-forming function, lead to a disturbance of this circulation.

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