

## DATA CONCERNING ABSORPTIVE PROPERTIES OF THE GALL BLADDER

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Much of the work carried out in the laboratories directed by I. P. Razenkov has been concerned with elucidation of the role of the digestive tract in intermediate metabolism [8]. To this group belong also our studies on the circulation of bile phosphorus compounds in the organism.

In a series of works published earlier we reported the results of studies on the rate of formation of organic phosphorus compounds in the liver and their secretion in the bile. Isotope technique has revealed that 2 hours after administration of radioactive sodium phosphate to a dog per os up to 30% of  $P^{32}$  found in the bile is associated with organic compounds, the percentage rising to 85 after 6 hours and to 99% of all the bile  $P^{32}$  after 24 hours. Chemical methods and radiochromatography of bile and products of hydrolysis of the organic phosphorus compound extracted from bile, and other methods have shown that almost all the bile phosphorus (up to 99%) is secreted as phospholipid of the type of lecithin.

The possibility of obtaining labeled bile phosphorus compounds in the body by means of biosynthesis allowed us to study the circulation of organic phosphorus compounds of the bile on introduction of whole bile or phospholipids extracted from it into the digestive tract.

Our experiments furnished an answer to two questions: 1) whether organic phosphorus compounds were absorbed in the digestive tract as such or whether inorganic phosphorus was split off from them and 2) whether there was direct passage of absorbed phospholipids into the composition of newly secreted bile.

It was found that phospholipids secreted with bile into the intestine could be absorbed without splitting off inorganic phosphorus.

When dogs are given strongly radioactive or inactive bile by mouth with milk, there is increase in bile formation by the liver and a rise (compared to the control) of the absolute phosphorus content of secreted bile, but this is not accompanied by increase or "dilution" of radioactivity: the specific activity of secreted bile remains unchanged. It follows from this that the phospholipids absorbed from the intestine from the bile introduced into it from a donor dog do not pass directly into the bile of the recipient dog [2, 4, 5].

Having established that bile phospholipids could be absorbed in the intestine without splitting, it was decided to check whether there was also absorption of phospholipids in the gall bladder where, as is known, considerable changes in the composition of the bile with respect to its individual components take place.

There are only scanty and conflicting references in the literature concerning the absorption through the gall bladder wall of various components of bile. It could be supposed that the isotope technique would furnish considerable help in the study of the absorptive capacity of the gall bladder wall [5].

### EXPERIMENTAL METHOD

Radioactive bile was collected from the donor dog and introduced in amounts of 5-10 ml into the gall-bladder of the recipient dog after preliminary ligation of the bile duct and aspiration of the bile from the

TABLE 1

Protocols of Experiments on Absorption of Labeled Bile Phospholipids from the Gall-Bladder in Dog (Short-term Experiments)

| Date of experiment | Dog's weight (in g) | Radioactive bile introduced into the gall bladder (in ml) | Total activity of introduced bile (in imp/min) | Residual activity in the gall bladder at end of experiment (in imp/min) | Remarks  |
|--------------------|---------------------|---|--|---|--|
| 15/I 1954          | 8 500               | 7,0   | $2,3 \cdot 10^6$                               | $2,3 \times 10^6$   | No radioactivity was detected in the liver and blood serum in these expts. |
| 1/IV 1954          | 11 000              | 7,5   | $6,86 \times 10^5$                             | $6,85 \times 10^5$  |  |
| 12/V 1954          | 6 000               | 5,0   | $7,2 \times 10^5$                              | $7,18 \times 10^5$  |  |

gall-bladder. In long-term experiments the bile was introduced into the gall bladder through a fistula, in amounts of 5-8 ml. In order to avoid increased pressure in the gall bladder the radioactive bile in the latter case was introduced in 3-4 divided doses with intervals of 60-90 minutes following preliminary evacuation of the gall bladder. Only recently operated dogs were taken for these experiments. As reported by us earlier [6] dogs with gall bladder fistulas often develop, in time, degeneration of the gall bladder mucosa and morphologic changes in the liver.

The total activity of the introduced bile was from 800,000 to 2,000,000 imp/minute. Blood was taken from the femoral vein, in long-term experiments from the saphenous vein, 5, 10, 15, 30, 60, 90, 120, 180, 240, 300 and 360 minutes after introduction of radioactive bile. Serum was examined for total, inorganic and lipid phosphorus and their radioactivity.

At the end of the experiment radioactivity of the liver was determined. Moreover the gall bladder was excized and placed into a crystallizer with water for 30-40 hours. During this period the water was changed 2-3 times. The summated radioactivity of the washings and the residual radioactivity in the gall bladder wall were determined. The percentage of  $P^{32}$  absorbed during the entire experiment was calculated from data on the amount of administered  $P^{32}$  and that remaining in the gall bladder at the end of the experiment.

This series included 4 short-term experiments and 4 long-term experiments, the latter on dogs with gall-bladder fistulas. The duration of each experiment was from 5 to 8 hours.

#### EXPERIMENTAL RESULTS

There was no passage into the blood of labeled bile phospholipids or inorganic radioactive phosphorus split off from them. Nor was any radioactivity detected in the liver (Table 1).

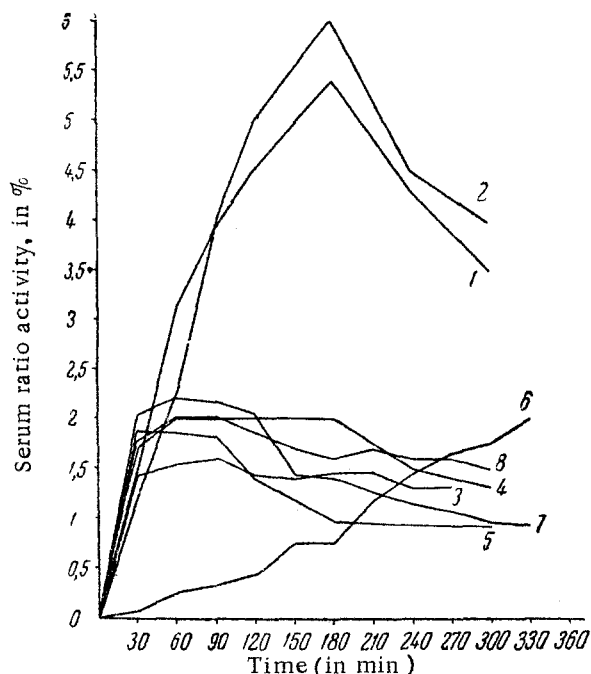
In the second series of experiments radioactive sodium phosphate ( $Na_2HP^{32}O_4$ ) was introduced into the gall bladder and the passage of radioactive phosphorus into the blood traced in time.

Since the content of total, inorganic and lipid phosphorus during the experiment usually showed only slight fluctuations, the calculation of blood serum radioactivity was carried out as follows: the number of impulses per minute of 1 ml serum was multiplied by one twentieth of the animal's weight in grams, taking this last value arbitrarily as the weight of the total amount of blood serum circulating in the given animal.

Thus the amount of  $P^{32}$  present in the blood serum at the moment of examination could be determined as percentage of the total amount of  $P^{32}$  introduced into the gall bladder.

In order to find out the significance of the parasympathetic nervous system in the processes of absorption in the gall bladder, atropine in the dose of 0.05 mg per 1 kg body weight was given subcutaneously in some of the experiments; the injection was made 30 minutes prior to administration of  $P^{32}$ . In other experiments the gall-bladder receptors were excluded by irrigating the bladder wall, 30 minutes prior to administration of  $P^{32}$ , with 2% solution of dicaine (1-2 ml). The latter was aspirated from the gall bladder before the  $P^{32}$  was introduced into it.

There is a number of references to the action of dicaine on the peripheral and central parts of the nervous system [1, 3, 4, etc.]. The role of the nervous system in the processes of absorption in the digestive tract and in processes of permeability of glandular tissue has received much attention.



Blood serum radioactivity following administration of  $\text{Na}_2\text{HP}^{32}\text{O}_4$  into the gall bladder in dog (short-term experiments).

Curves 1 and 2 – control experiments; curves 3, 4, 5 – against the background of the action of preliminarily administered atropine; curves 6, 7, 8 – against the background of the action of preliminarily administered dicaine.

bladder during 5 hours. Inhibition of  $\text{P}^{32}$  absorption from the gall bladder was also observed after irrigation of its walls with dicaine (see Figure, Curves 6, 7, 8). In the experiment represented by curve 6 only part of the dicaine introduced into the gall bladder could be aspirated and in this case the inhibition of  $\text{P}^{32}$  absorption was the greatest.

The experiments carried out thus indicate that phospholipids are not absorbed in the gall bladder, whereas inorganic phosphate is absorbed rapidly. In the course of 5 hours up to 80% of phosphate introduced into the

M. Lindeman [6], working in I. T. Kurtzin's laboratory, investigated the effect of changes in the functional state of the cerebral cortex on absorption of water and sodium chloride from the gall bladder.

The results of our studies on the absorption of radioactive sodium phosphate in the gall bladder in control experiments and with administration of dicaine and atropine are presented in the figure.

In the control experiments slight blood serum radioactivity was observed 3 hours after the beginning of the experiment (curves 1, 2). All the activity was present in the inorganic phosphorus fraction of the blood serum. No activity was found in the lipid fraction of the serum.

In this way it was possible to trace, in relative units, the dynamics of absorption. In addition to this, the total amount of absorbed  $\text{P}^{32}$  was found from the difference between the introduced and residual  $\text{P}^{32}$  in the gall bladder at the end of the experiment using the method indicated above (Table 2). In these experiments about 80% of the introduced  $\text{P}^{32}$  was absorbed in 5 hours. Injection of atropine 30 minutes before the introduction of  $\text{P}^{32}$  caused sharp inhibition of  $\text{P}^{32}$  absorption from the gall bladder. In this case the curve for absorption of inorganic phosphorus differed markedly from the control (curves 3, 4, 5). Only 10-20% of the introduced  $\text{P}^{32}$  was absorbed from the gall-

TABLE 2

Protocols of Experiments on Radioactive Sodium Phosphate ( $\text{Na}_2\text{HP}^{32}\text{O}_4$ ) Absorption from the Gall Bladder in Dog

| Date  | Dog's weight (in g) | $\text{P}^{32}$ introduced (in imp/min) | Residual activity in the gall bladder at end of experiment (in imp/min) | Amount of $\text{P}^{32}$ absorbed from gall bladder as % of $\text{P}^{32}$ introduced |
|---|---------------------|---|---|---|
| Control experiments                         |                     |   |   |   |
| 2/III 1954                                  | 14 300              | $1.43 \times 10^6$                      | $2.86 \times 10^6$  | 80  |
| 16/III 1954                                 | 7 500               | $9.4 \times 10^6$                       | $2.02 \times 10^6$  | 73  |
| Experiments with administration of atropine |                     |   |   |   |
| 12/III 1954                                 | 13 350              | $1.35 \times 10^7$                      | $1.24 \times 10^7$  | 8   |
| 18/IV 1955                                  | 13 750              | $1.37 \times 10^7$                      | $1.21 \times 10^7$  | 12  |

gall bladder is absorbed. Injection of atropine or irrigation of the gall bladder walls with dicaine prior to introduction of phosphate into it causes sharp inhibition of absorption: in 5 hours only 10-20% of introduced phosphase is absorbed in these cases. This points to the importance of the parasympathetic nervous system and the receptors of the gall bladder mucosa in the process of absorption.

#### SUMMARY

These experiments were devoted to the study of the absorption of labeled  $P^{32}$  phospholipids and inorganic sodium phosphate from the bile duct of a dog. Labeled phospholipids were obtained by biosynthesis in the body of a donor dog. Phospholipids introduced into the recipient dog's gall bladder were present in the bile collected from the donor dog. The absorption from the bile duct was judged by the appearance of  $P^{32}$  in the blood serum and by the activity retained in the gall bladder. The effect of subcutaneous injection of atropine (in the dose of 0.05 mg per kilogram of body weight) and administration of 2% solution of dicaine into the gall bladder on the absorption of inorganic phosphate was also studied in short-term experiments.

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\* In Russian.