

THE INFLUENCE OF VITAMINE B<sub>12</sub> AND CHOLINE UPON THE CONTENT  
OF PHOSPHOLIPIDS IN THE BILE AND UPON ITS SECRETION IN HEALTHY  
DOGS AND IN DOGS WITH EXPERIMENTAL LIVER DEGENERATION

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In view of reports published by Soviet and foreign authors concerning the lipotropic properties of vitamine B<sub>12</sub> and choline [1, 3-7], we used the above preparations therapeutically on 120 patients suffering from various forms of liver cirrhosis. Clinical investigations showed that in the majority of cases, simultaneously with the improvement in the patients' general condition, the blood phospholipid level decreased [2].

It could be assumed that one of the possible factors which caused a decrease in the lipid infiltration of the liver, following treatment with vitamine B<sub>12</sub> and choline, consisted in a more intensive bile secretion by the liver including a more intensive secretion of phospholipids with the bile. To check this assumption we carried out the present experimental investigation on dogs, in an endeavour to establish the influence of vitamine B<sub>12</sub> and choline upon the secretion of bile, and upon the phospholipid level in the bile under normal conditions as well as in pathological states of the liver.

TABLE 1. Average Phospholipid Levels and Bile Secretion per hour in the Control Group and after the Administration of the Preparations

Name of dog	Preparation	Quantity of bile (in ml)		Phospholipid level (in mg/ml)		Total phospholipid secretion (in mg)	
		control	after the adminis- tration of the prepa- rations	control	after the adminis- tration of the prepa- rations	control	after the adminis- tration of the prepa- rations
Ryzhik .....	B <sub>12</sub>	4.2	7.8	0.44	1.06	1.6	7.78
Jim .....	B <sub>12</sub>	4.1	6.0	0.66	1.35	2.67	7.4
Shustryi .....		3.6	4.6	0.62	1.52	2.3	7.01
Prima .....		3.7	4.4	0.52	1.3	1.9	6.03

#### METHODS

The experiments were carried out on 4 dogs with a gall bladder fistule in which the common bile duct had been ligated. The animals were kept on the usual diet. The external opening of the fistule was kept permanently open to secure the free outflow of the bile and to enable the animals to lick it up. No acholic stools were observed. During the experiments the dogs were kept in a stand. The secretion of bile was measured over a period of 8 hours

and the phospholipid content of the bile was estimated in hourly collected portions of bile. The phospholipid level was estimated by the method of King and Wootton. This method is based on the fact that, when proteins are precipitated with trichloroacetic acid, the phospholipids are precipitated with the proteins and their quantity can be estimated on the basis of the phosphorus content in the precipitate. The phosphorus content was estimated in a photoelectric colorimeter (FEK-1). The preparations were administered at the beginning of the experiment: vitamin B<sub>12</sub> was given by intramuscular injection in a dose of 10 mg/kg (dogs "Jim" and "Ryzhik"), choline was given perorally through a gastric tube in the shape of a 10% solution in a dose of 0.3 g/kg (dogs "Shustriy" and "Prima"). 132 experiments were carried out on healthy dogs, including 71 control experiments, 39 experiments with vitamin B<sub>12</sub> and 22 experiments with choline. 56 experiments were carried out on sick dogs.

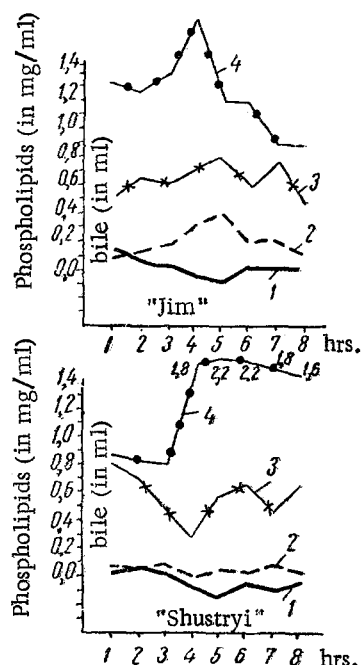


Fig. 1. The influence of vitamin B<sub>12</sub> and choline upon the bile secretion and the phospholipid content of the bile in healthy dogs (average data of 12 experiments on the dog "Jim", treated with vitamin B<sub>12</sub> and on the dog "Shustriy", treated with choline). 1) Normal rate of bile secretion; 2) bile secretion after administration of the substance; 3) normal phospholipid content of the bile; 4) phospholipid level of the bile after administration of the substance.

the bile phospholipid level could be observed; the increase became manifest in the 2nd and 3rd hour and persisted throughout the experiment.

Fig. 1 shows that both vitamin B<sub>12</sub> and choline cause a marked increase in the bile secretion. Simultaneously the phospholipid content of the bile increased. In consequence the total phospholipid secretion increased to an even higher degree.

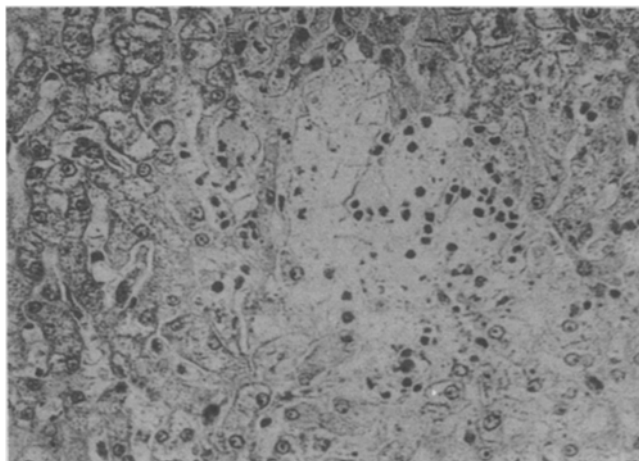


Fig. 2. Focal liver cell necrosis. The control dog "Prima", killed 2 months after the beginning of treatment with carbon-tetrachloride. (Microphotograph, magnification x 270).

## RESULTS

Experiments on healthy dogs: In 15-20 experiments the average quantity of bile secreted in 1 hour and its phospholipid content were established. Normally the bile secretion of the dogs varied between 4-9 ml per hour. During the experiments (3-8 hours) the variations in the quantity of bile secretion were insignificant. Towards the end of the 8 hours of the experiment the bile secretion usually decreased. The findings obtained in the control experiments showed that the phospholipid content in the bile was at the normal level, established for hepatic bile: 0.05% (0.5 mg/ml, or 50 mg%). In the course of the experiment only small variations in both directions within a range of 0.03-0.08% could be observed. The total quantity of phospholipids secreted per hour constituted, on the average, 2.67 mg.

After the injection of vitamin B<sub>12</sub> and choline (the preparations were administered at the beginning of the experiments as described above) an increase in the bile secretion and particularly in

TABLE 2. Changes in the Bile Secretion and the Bile Phospholipid Level under the Influence of Vitamine B<sub>12</sub> and Choline in Dogs with Toxic Liver Lesions

Character of experiment	No. of experiment															
	1		2		3		4		5		6		7		8	
	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P
Control group.....	5.3	0.5	4.5	0.64	4.2	0.6	3.5	0.7	2.9	1.1	4.2	0.6	4.2	0.8	4.5	0.5
Treatment with vitamine B <sub>12</sub> .....	4.4	1.52	4.65	1.88	4.95	1.92	5.05	1.82	4.69	2.0	4.2	2.16	4.2	2.2	4.4	2.16
Control group.....	4.5	0.82	4.7	0.74	4.5	0.52	3.5	0.3	3.1	0.6	3.7	0.68	3.4	0.5	3.5	0.7
Treatment with choline .....	3.5	1.83	3.9	1.56	4.1	1.72	4.9	1.18	4.2	1.92	4.0	2.0	4.5	1.6	4.0	1.92

Remark: B) quantity of bile (in ml) secreted per hour; P) phospholipid content (in mg/ml) of portions collected every hour.

The effect of vitamin  $B_{12}$  became manifest somewhat earlier than the effect of choline. This can be explained by the different rate of resorption due to the different route of administration. Statistical evaluation of the findings showed that the results were significant. For comparison Table 1 shows data concerning the phospholipid level and the bile secretion in the control animals, and after administration of the substances.

Experiments on healthy dogs thus showed that vitamin  $B_{12}$  and choline increase the bile secretion and cause an even more marked increase in the phospholipid level of the bile. The total quantity of phospholipids secreted with the bile in the course of 1 hour was on the average 2-3 times higher than the normal quantity.

Studies on dogs with experimental liver degeneration. Being now in the possession of reliable evidence for the considerable increase in the bile phospholipid level, which takes place under the influence of vitamin  $B_{12}$  and choline in healthy dogs, we found it necessary to investigate the effect of these substances in case of pathological conditions of the liver. The experiments were carried out on the same dogs, after they had been given carbontetrachloride for a prolonged period. All animals were given the latter substance by subcutaneous injections in doses of 0.05 mg/kg for one month and in doses of 0.1 mg/kg for the second month, every second day. During this period the dogs were kept on a choline-free diet.

2 months after the beginning of the treatment with carbontetrachloride one dog (Prima) was killed for the morbid anatomical investigation of the liver. It appeared (Fig. 2) that the bile ducts and the hepatic blood vessels were surrounded by great quantities of fibrous tissue. In some places proliferation of the small bile ducts could be observed. The protoplasm of the liver cells was of foamy structure; the cells were frequently dissociated from each other and had lost their cord arrangement. Frequently small foci of liver necrosis could be seen. In these areas fragments or shadows of liver cells were visible. In some necrotic foci polyblasts, histiocytes and lymphocytes could be found in addition to leucocytes; in this case the development of delicate collagenous fibers could frequently be observed. Fat stain revealed the presence of fine fat droplets in the protoplasm of numerous liver cells.

It could thus be established that, under the experimental conditions, mentioned above, cirrhotic liver lesions developed in the dogs. This fact enable us to test vitamin  $B_{12}$  and choline on two of the remaining three dogs:

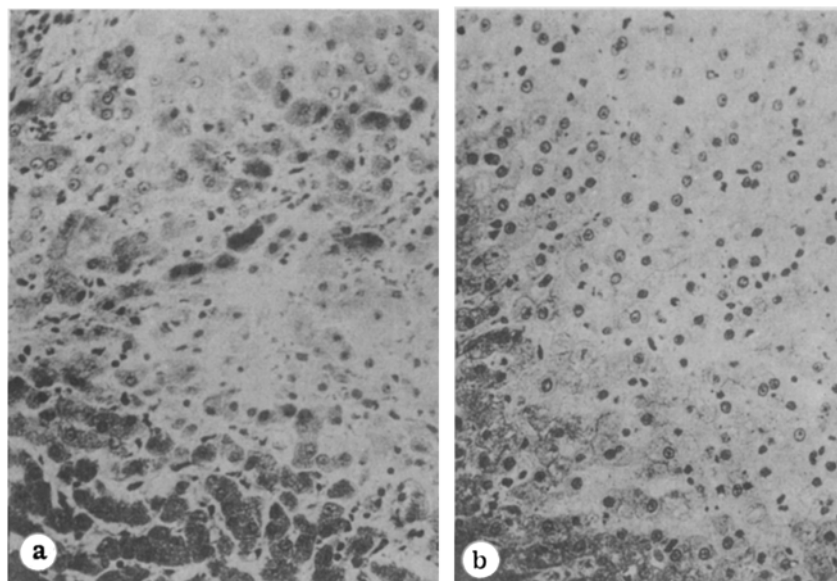


Fig. 3. Degenerative changes in the liver of the dogs "Shustriy" (a) and "Jim" (b); (microphotograph, magnification  $\times 270$ ). a) Focal necrosis of the liver cells; b) focal degeneration of the liver cells.

"Jim" and "Shustriy". The third dog ("Ryzhik") was left as control animal. 10 experiments lasting 8 hours and involving administration of the substances mentioned above were carried out on each of the dogs according to the method described above.

Previously 8 control experiments had been carried out to establish the rate of bile secretion and the phospholipid content in the bile without the administration of the lipotropic agents. The average results of these experiments are set forth in Table 2.

It appeared that both, vitamin  $B_{12}$  and choline caused an increase in the bile secretion in the dogs "Jim" and "Shustryi"; the phospholipid level in the bile showed a marked increase. The above experiments warrant the conclusion that vitamin  $B_{12}$  and choline cause a rise in the phospholipid level of the bile and increase the bile secretion in dogs with liver lesions to the same degree as in healthy dogs. The results of the control experiments showed that the indices characterizing the bile secretion and the bile phospholipid level in experimental liver degeneration were hardly different from the indices established on the same animals before the development of the liver lesions. The presence of liver lesions in the dogs in question was confirmed by the autopsy findings\*.

Histological investigation of the liver in the dog "Shustryi" (Fig. 3,a) revealed a marked proliferation of small bile ducts in the periportal layers. The large bile ducts and the blood vessels were surrounded by great masses of fibrous tissue. In some areas small groups of lymphoid cells, histiocytes and polyblasts could be seen. In some places, most frequently at the periphery of the hepatic lobules, the cord arrangement of the liver cells was disrupted; in these places the liver cells were of varying size, frequently much smaller than usual. In many cells the nucleus appeared to be smaller and darker, the protoplasm was of homogeneous appearance. Some cells had lost their nucleus but had preserved their outlines. Between cells of this type fragments of disintegrated necrotic cells could frequently be seen.

Fig. 3, b shows a microphotograph of the liver in the dog "Jim". This figure shows that the cords of liver cells seem to be separated from each other (edema). In the peripheral zone of the lobules the arrangement of the liver cells in cords seems to be disrupted in places. In these areas the liver cells are either swollen or, conversely, reduced in size. Some cells have lost their nucleus but have preserved their outlines. In some places the outlines of the cells have become indistinct and only fragments of disintegrated cells can be seen. In many cells the nuclei are smaller and hyperchromatic. Liver cells containing two nuclei can occasionally be seen. Almost everywhere, the protoplasm of the liver cells shows abundant fatty infiltration.

Histological investigations thus showed scattered foci of liver cell degeneration and necrosis, i.e. pathological lesions of cirrhotic character. Similar changes were found in the liver of the 4th dog ("Ryzhik") which had been left as control animal.

Our investigations showed that vitamin  $B_{12}$  and choline cause an increase in the bile secretion not only in normal livers (healthy dogs) but also in pathologically changed livers with artificially produced degeneration of cirrhotic character. At the same time the phospholipid content of the secreted bile was several times higher than normally. The more intensive bile secretion and the consequent greater secretion of phospholipids possibly represent one of the links of the complex mechanism responsible for the lipotropic effect of vitamin  $B_{12}$  and choline, two substances which have been employed, not without success, for the treatment of hepatic cirrhosis.

#### SUMMARY

The effect of vitamin  $B_{12}$  and choline on the phospholipid content in the bile and its secretion was studied on 4 dogs. As established, bile secretion increased and there is a rise of its phospholipid content under the effect of the aforementioned substances. Such effect is seen both in healthy dogs and in those with an experimentally induced affection of the liver. The latter was achieved by a two-month administration of carbon tetrachloride. Hepatic dystrophy was confirmed histologically. Possibly the mentioned effect of vitamin  $B_{12}$  and choline is one of the links in the lipotropic action of these preparations.

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