EFFECT OF PROLONGED ABSENCE OF BILE FROM THE INTESTINE ON VITAMIN B_{12} METABOLISM AND THE STATE OF THE BLOOD SYSTEM

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In dogs with an internal biliary fistula and in rats with a ligated common bile duct no regular decrease was found in the absorption of physiological doses of vitamin B_{12}, labeled with Co^{60}, in the intestine. In dogs with an internal biliary fistula the elimination of parenterally administered radioactive vitamin B_{12} with the urine was increased, but its elimination with the feces was lowered, although the total excretion was increased, presumably on account of disturbance of the enterohepatic circulation. The vitamin B_{12} concentration in the blood serum was reduced during the first and second months after formation of an internal biliary fistula; subsequently the level of this vitamin was unstable, but its mean value was much below the initial level. At late stages after this operation the vitamin B_{12} concentration in the liver tissue was reduced in most animals, and in some cases a macrocytic anemia developed.

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Excretion of vitamin B_{12} with the bile into the intestine has frequently been observed, much of it then being reabsorbed into the blood stream and entering the liver [13, 18]. The effect of bile on the intestinal absorption of vitamin B_{12} has been investigated in man and the rat, but the number of observations is small; bile has been found to have no significant effect on this process [15, 17].

The object of the present investigation was to test experimentally the possibility that hypovitaminosis B_{12} may arise following prolonged disturbance of bile excretion. They were carried out because of reports of the development of hyperchromic macrocytic anemia, which is often the result of vitamin B_{12} deficiency, under these conditions [2, 9, 11, 19]. The results have already been mentioned in a number of short communications [4-7], but they are now presented together.

EXPERIMENTAL METHOD

Observations were made on 24 dogs with an internal biliary fistula (anastomosis between the gall bladder and the right renal pelvis combined with ligation of the common bile duct) and on 10 rats with ligation of the common bile duct. The dogs' diet before and after the operation consisted of bread, oatmeal porridge, boiled potato, fat-free meat, and milk was given infrequently. The rats received milk and white bread. The intestinal absorption of vitamin B_{12} labeled with Co^{60} was investigated in the rats. In the dogs the intensity of absorption of vitamin B_{12} labeled with Co^{60} or Co^{58} in the intestine, the excretion of parenterally administered vitamin B_{12} labeled with Co^{60} in the urine and feces, the concentration of ordinary vitamin B_{12} in the blood serum and liver tissue, the content of gastromucoprotein in the gastric juice, the concentration of labile iron in the blood serum, the morphological composition of the blood and of the bone marrow obtained by puncture (myelogram), and the histomorphology of various organs were determined. The intestinal absorption of vitamin B_{12} was investigated by a modified method of Heinle and co-workers [13]. Radioactive vitamin B_{12} was given to the dogs in a dose of 0.3-1 μg (specific activity about 0.1 or 0.5 μCi/μg) with a piece of cooked meat, and to the rats through a gastric tube in a dose of 25 mμg. During the next 5 days all the feces were collected and incinerated at 500-600°. The radioactivity of the ash thus obtained was measured with a scintillation counter (NaI crystal) and expressed as a percentage of the activity of the administered dose. The percentage excretion thus found was used as an index of the intensity of ab-

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sorption of the vitamin. The content of gastromucoprotein was determined by the method of Glass and Boyd [11]; gastric juice was collected through a fistula from the whole organ after intravenous injection of 14-20 units of insulin. The vitamin B_{12} concentration in the blood serum and liver tissue was determined from the intensity of growth of Escherichia coli 113-3 [3]. The concentration of labile iron in the blood serum was determined by Barkan's method [10].

EXPERIMENTAL RESULTS AND DISCUSSION

Intestinal absorption of radioactive vitamin B_{12} was studied in 6 dogs with an internal biliary fistula (17 observations), and similar investivations were carried out on 12 healthy dogs. Between 1.5 and 8 months after the operation the intensity of absorption in 4 animals was close to normal, as shown by the percentage excretion of radioactive vitamin with the feces: 22-62%, with a mean value of $41.4 \pm 7.2\%$ (23.7-45.9% for healthy dogs, mean value $36.5 \pm 2.3\%$), while radioactivity of the feces of the other dogs with an internal biliary fistula after a test dose of vitamin B_{12} labeled with Co^{60} was sharply increased. In the later stages after the operation the excretion of radioactive vitamin with the feces increased to 63.8-87.8% of the administered dose, indicating lowering of the intensity of its absorption.

Before the operation on the rats, 22.6-71.9% (mean 42.3%) of the internally administered radioactive vitamin B_{12} was excreted with the feces, and two weeks after ligation of the common bile duct the figure was 22.8-57.6% (mean 35.2%).

The concentration of gastromucoprotein in the gastric juice of the healthy dogs varied from 46.6-112.2 mg%. In two dogs examined 6 and 11 months after the formation of a biliary fistula, its concentration remained within normal limits; in a third animal gastromucoprotein could no longer be found 14 months after the operation. These observations are in agreement with the findings of Ordina [8] and El'kina [1], who discovered atrophic changes in the gastric mucosa of some dogs which had lost bile for a long time through an external or internal biliary fistula.

The vitamin B_{12} concentration in the liver tissue of 12 dogs before the formation of the internal biliary fistula was 127-328 mμg/g fresh tissue (mean 230 ± 25 mμg/g), falling to 57-201 mμg/g, mean 129 ± 13 mμg/g ($P < 0.01$) 6-22 months after the operation, and in 9 animals the vitamin concentration at these times was much lower than initially.

The concentration of vitamin B_{12} in the serum of most dogs fell during the first months after formation of the biliary fistula: before the operation this index was 402 ± 23 μμg/ml, falling to 294 ± 32 μμg/ml 1-2 months after the operation and to 260 ± 20 μμg/ml 3-4 months after the operation. Subsequently the vitamin level in the serum was unstable, returning at times to its initial state, although the mean concentration was below normal.

Our observations show that the decrease in vitamin B_{12} concentration in the liver tissue and blood serum of dogs with an internal biliary fistula is due not only to inadequate absorption of the vitamin in the intestine, but also to an increase in its excretion. After intravenous injection of vitamin B_{12} labeled with Co^{60} in a dose of 5 μg (one week previously 3 injections of the ordinary vitamin, in doses of 200-300 μg, had been given in order to saturate the tissue depots), its excretion in the urine and its total excretion from these animals were much higher than from healthy animals (21.6-37.2, mean 28.4%, and 13.8-19.6, mean 17.6%, respectively). The excretion of radioactive material with the feces was reduced but did not cease altogether. This shows that vitamin B_{12} is excreted into the lumen of the bowel not only with the bile, but also with other digestive secretions. Its increased elimination with the urine in dogs with an internal biliary fistula is explained by interruption of the enterohepatic circulation: in these animals all the vitamin present in the bile passed through the anastomosis into the urinary tract and was discharged outside, whereas in healthy dogs it was eliminated into the intestine from which it was reabsorbed into the blood.

All the dogs with an internal biliary fistula developed anemia 1-14 months after the operation. However, we were unable to confirm the observations of Crandall and co-workers [11] who found that such animals regularly develop a hyperchromic macrocytic type of anemia. Of the 24 dogs which we had under observation for 5-23 months, only 2 developed this type of anemia. In the other cases the anemia was macrocytic or normocytic hypochromic or normochromic. The bone marrow of some dogs showed a decrease, and of others an increase in the number of erythroblasts, but the index of their maturation was lowered. In the late stages after the operation the number of reticulum cells was increased, in some cases to 19-32% of the total number of myelokaryocytes. These changes in the composition of the myelogram, together with the

absence of reticulocytosis in the circulating blood, indicate that the anemia is hypoplastic in character. The state of anemia was not permanent, for spontaneous remissions and relapses were observed.

These observations show that bile does not participate in the assimilation of vitamin B_{12} from the food, but its prolonged absence in the intestine leads to a decrease in the absorption of this substance on account of changes of a secondary character. These include structural changes in the intestinal wall demonstrated in our dogs by Ordina [8] and El'kina (unpublished data) in the late stages after the operation, such as marked coarsening of the stroma of the villi, thickening and homogenization of the blood vessel walls, an increase in the number of goblet cells, and degenerative changes in the nervous system. Assimilation of the vitamin may be prevented by the presence of an unusual microflora in the intestinal contents, evidence of the development of which is given by the frequent cases of hemorrhagic enteritis in dogs with a biliary fistula. In some animals in the late stages after the operation the secretion of gastromucoprotein was sharply reduced, and this could interfere with the passage of vitamin B_{12} through the intestinal mucosa.

The decrease in concentration of vitamin B_{12} in the liver tissue and blood serum of dogs losing bile for long periods through a fistula indicates that hypovitaminosis B_{12} can develop in association with chronic disturbances of bile secretion as a result of impairment of absorption and cessation of the enterohepatic circulation: the latter is evidently an important factor in the safeguarding of the vitamin B_{12} reserves in the body and in maintenance of its normal concentration in the serum.

As well as the vitamin B_{12} deficiency, iron deficiency probably plays a role in the mechanism of development of the anemia arising after the prolonged absence of bile from the intestine. The concentration of labile iron in the blood serum of our dogs with an internal biliary fistula was 73 ± 7.6 μg% before the operation, 66 ± 8.4 μg% 7 months after the operation, and 34 ± 6.8 μg% at a later period ($P < 0.01$). The reason for this decrease could be inadequate absorption of exogenous iron [20] and cessation of the enterohepatic circulation of endogenous iron excreted with the bile. The development of anemia in dogs with an internal biliary fistula surviving for about a year or longer after the operation was probably facilitated by general disturbances of metabolism due to the lowered assimilation of several other food substances (lipids, fat-soluble vitamins, proteins, etc.). The weight of these animals fell by 30-40% of its initial level, and at autopsy atrophic changes were found in various organs, indicating depression of synthetic processes in the tissues. In the early stages after the operation, no rigid correlation could be observed between the body weight and the state of the blood system. In some cases anemia developed in well nourished animals or animals with only slight emaciation, while other animals had no anemia despite a fall of 20-25% in their body weight.

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