THE EFFECT OF SUBDIAPHRAGMATIC VAGOTOMY ON THE METABOLISM OF VITAMIN B_{12} AND THE STATE OF THE BLOOD SYSTEM IN DOGS

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The present investigation was undertaken on the basis of the previously established facts that: 1) transection of the vagus nerves below the diaphragm leads to the development of hyperchromic macrocytic anemia [3,4], which is a condition most frequently associated with vitamin B_{12} hypovitaminosis; 2) the vagus nerve stimulates the secretion of gastric mucoprotein [7-9,11], which is now identified with castle's intrinsic factor essential for intestinal absorption of vitamin B_{12}.

METHODS

The observations were made on 34 dogs. In 18 of these, the vagus nerves were completely severed below the diaphragm, in 2 this was done above the diaphragm at the level of the distal section of the esophagus, partial vagotomy was performed on 9 animals (retaining the branches of the vagus nerve posterior trunk below the diaphragm); 5 animals (control group) were subjected to laparotomy without section of the vagus nerves.

In 24 of the dogs with partial or total vagotomy and in the control dogs, during 1-2 months before operation and at various intervals after operation, we determined various hematological parameters: quantity of hemoglobin, erythrocytes, leucocytes, reticulocytes, color index, mean corpuscular diameter and mean volume of the erythrocytes (MCD and MCV), and the cellular make-up of aspirated bone marrow. In studying the metabolism of vitamin B_{12}, determinations were made of its concentration in blood and liver tissue, and also the intestinal absorption of vitamin $B_{12}Co^{60}$ was measured. The blood serum and liver tissue concentrations of vitamin B_{12} were determined by a microbiological method [1] based on growth activity of E. coli 113.

In determining the intestinal absorption of vitamin B_{12}, the animals were given a single oral 0.3 μg dose of vitamin $B_{12}Co^{60}$ (specific activity 0.09 $\mu C/\mu g$) after a 20-22 h fast. A piece of meat was used as a carrier to insure the swallowing of the radioactive vitamin solution. At 4-5 h after receiving the vitamin, the animals were given their usual allowance of food.

Evidence of vitamin $B_{12}Co^{60}$ absorption in the intestine was obtained by measuring the fecal radioactivity and the absorption was expressed as percent of the administered radioactivity. For the purpose of determining the excretion of the radioactive vitamin, the feces were accumulated during the 5 days after the test dose administration, these were ignited at 500° and the radioactivity of the resulting ash was measured with a scintillation counter (sodium iodide crystal). This was carried out before the operation and at various periods thereafter in 5 of the dogs with total subdiaphragmatic vagotomy. Another 5 animals were used for a single determination of intestinal absorption of the vitamin a long time after vagotomy (1-3 years).

A gastric fistula was prepared in 3 animals with complete subdiaphragmatic vagotomy and in 2 controls; this was done to obtain gastric juice for the determination of its mucoprotein content. The gastric juice was collected during fasting and 30-60 min after intravenous administration of insulin (12-16 units). The gastromucoprotein was determined by the Glass and Boyd method [10].

TABLE 1. Hematological Signs and Serum Vitamin B_{12} Concentrations in Dogs Before and After Total Severing of the Vagus Nerves Below the Diaphragm

Days following operation	Hemoglobin (%)	Erythrocytes (millions)	Color index	MCD (μ)	Reticulocytes (%)	Leucocytes 1000's	Vitamin B_{12} (μμg/ml)
	$M \pm m$	$M \pm m$	$M \pm m$	$M \pm m$		$M \pm m$	$M \pm m$
Preoperative	90,1±1,3	6,61±0,08	0,67±0,01	6,99±0,025	5,5	11,7±0,5	401±26
1 week	79,2±2,6	6,02±0,2	0,65±0,02	—	7,1	11,3±1,7	—
2 weeks	80,3±2,7	5,74±0,24	0,69±0,02	—	8,1	15,3±1,3	—
3-4 weeks	80,8±2,2	5,92±0,18	0,66±0,01	—	7,6	14,1±1,1	402±29
2 months	83,2±1,3	6,13±0,12	0,68±0,001	7,07±0,04	5,2	11,7±0,6	440±23
3 months	83,6±2,6	6,08±0,10	0,65±0,02	—	7,9	10,7±0,9	398±26
4 months	79,9±2,3	6,37±0,12	0,68±0,01	7,10±0,04	6,2	11,5±0,7	382±36
5-6 months	87,5±1,0	6,46±0,09	0,64±0,01	7,11±0,03	5,7	10,1±0,6	386±32
7-9 months	91,2±1,2	6,63±0,11	0,66±0,008	7,07±0,01	7,0	10,4±0,4	341±33
10-12 months	90,9±0,9	6,65±0,07	0,68±0,01	7,11±0,02	6,9	10,9±0,5	344±35
13-15 months	94,8±1,3	6,68±0,12	0,75±0,02	7,09±0,025	5,8	10,7±0,6	402±42
16-18 months	95,8±1,6	6,64±0,11	0,72±0,02	7,09±0,02	7,3	10,2±0,5	329±54
19-24 months	98,0±2,5	6,84±0,2	0,71±0,02	6,97±0,025	6,7	—	387±41

TABLE 2. Fecal Excretion of Vitamin $B_{12}Co^{60}$ in Dogs Before and After Sectioning the Vagus Nerves (% of administered dose)

Number of dog	Before operation	Period after Vagotomy (months)					
		1 – 2	3	4 – 5	6	7	12 or more
1	36,4	63,6	77,1	62,4	73,8	46,0	38,2
2	53,4[1]	68,8	—	65,7	—	51,1	53,2
3	34,9	43,3	—	65,5	—	56,0	57,1
4	42,5[1]	80,2	70,8	63,9	50,4	—	—
5	30,7	45	—	—	39,1	—	—
6	—	—	—	—	—	—	33,5
7	—	—	—	—	—	—	28,7
8	—	—	—	—	—	—	32,7
9	—	—	—	—	—	—	40,5
10	—	—	—	—	—	—	40,3

[1]Vitamin $B_{12}Co^{60}$ was given at a 0.6 μg level (in all other cases the dosage was 0.3 μg).

RESULTS

Examination of the vitamin B_{12} content of serum revealed that dogs with total subdiaphragmatic vagotomy had a tendency to decreased levels ($P > 0.05$) for 9-12 months after the operation (Table 1). In 7 of 14 animals, the serum vitamin B_{12} showed a brief temporary increase at 1-2 months after vagotomy, but the increase did not bring any levels above the upper limit of normal. Two animals, in which the vagus nerves had been cut above the diaphragm, showed variations in serum vitamin B_{12} concentration which did not vary from those observed in the preoperative period. Animals with partial vagotomy were tested for 5 postoperative months, and the serum vitamin B_{12} levels were found not to differ from the original. The vitamin B_{12} levels in liver tissue at 6 months, 1, and 2-3 years after subdiaphragmatic vagotomy do not differ substantially from normal.

Intestinal absorption of vitamin $B_{12}Co^{60}$ was found to be substantially decreased during the course of 5-6 months after subdiaphragmatic vagotomy, as revealed by increased fecal radioactivity during this period (Table 2). A year after the operation and at later times thereafter (2-3 years), the excretion of vitamin B_{12} following administration of a standard dose was practically the same as that observed in the normal dog (respectively 35.1 and 35.4%).

The study indicates a disturbance in intestinal absorption of vitamin B_{12} in dogs during the early months after total subdiaphragmatic vagotomy. The mechanism responsible for this disturbance was investigated by studies in two different phases. It was found that the oral administration of the vitamin $B_{12}Co^{60}$ together with 40-45 ml of fresh gastric juice, obtained from healthy dogs after intravenous insulin injection, significantly improves the intestinal vitamin $B_{12}Co^{60}$ absorption in animals that had been subjected to section of the vagus nerves. In this case, the fecal radioactivity fell to 40.1-53.4% of the administered dose, which is close to that observed in the normal dog. In this connection, it was established that there is a sharp fall in or complete absence of gastric mucoprotein secretion following vagotomy. In the course of 1-1½ years after operation, gastric mucoprotein was absent from gastric juice collected on fasting as well as from that obtained following insulin injection. The secretion of gastric juice in these animals in response to insulin was notably decreased. After 1-1½ years, the reaction of the gastric glands to insulin administration was restored in greater or less degree and gastric mucoprotein was found in the juice, though most often in very limited quantity. The research supports the view that decreased intestinal absorption of vitamin B_{12} in the dog during the early months after complete vagotomy is a consequence of the disruption of gastric mucoprotein secretion.

One of the earliest signs of B_{12} hypovitaminosis is macrocytosis [2,5,6]. In studies on peripheral blood of dogs with complete subdiaphragmatic vagotomy, there was found to be a small but statistically significant increase in MCD (see Table 1). In most of the animals (12 of 16), an anemia (slight to moderate in severity) developed in the month following operation, and during this time the color index remained substantially unchanged. In 4-5 months following the operation, the content of hemoglobin and red cells had risen to the original level (see Table 1). The reticulocyte count increased slightly, only during the first month, and subsequently it was normal. The aspiration material from the bone marrow, during the period of anemia development, was observed to increase in leucoerythroblastic ratio. The type of hematopoiesis became normoblastic. In dogs with partial vagotomy, the anemia was less definite and of short duration, the hemoglobin and erythrocyte levels increased to normal in 3 months after surgery. The increase in MCD and rise in color index in the animals with partial vagotomy were not statistically significant. The control dogs evidenced an insignificant fall in hemoglobin concentration and red cell count only in the first postoperative month (hemoglobin fell by 3 to 5%, erythrocyte count decreased by 200,000-500,000).

Our studies show that complete severing of the vagus nerves below the diaphragm leads to a decrease in intestinal absorption of vitamin B_{12} and a tendency to depressed levels of vitamin B_{12} in the serum during the first year after operation. In all probability, the basis for the disturbance in vitamin B_{12} absorption is the inhibition of secretion of the Castle intrinsic factor. In the later periods following vagotomy, this disturbance is not severe. The small but statistically significant increase in the MCD in dogs subjected to vagotomy is the sole evidence of hypovitaminosis B_{12}. Inasmuch as the restoration of normal hemoglobin concentration and erythrocyte count occur earlier than the return to normal of vitamin B_{12} absorption by the intestine or of the serum level of vitamin B_{12}, it may be postulated that the disturbance in vitamin B_{12} metabolism does not play a substantial role in the pathogenesis of the anemia.

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