VITAMIN B_{12} CONTENT OF THE BLOOD OF DOGS DEVELOPING ANEMIAS AFTER THE FORMATION OF SMALL STOMACH POUCHES USING THE METHOD OF KLEMENTSIEVICH-HEIDENHAIN

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Many investigators have shown that denervation of some internal organs in animals will produce various types of anemia [2, 3, 4, 7, 8, 9, 11, 12].

This phenomenon has been viewed as a consequence of the disappearance of afferent and efferent impulses.

R. A. Durinian [2], O. M. Preger [7 and 8], S. I. Iakovlev [12] and others have shown that partial gastric denervation produced by the formation of a small stomach pouch employing the method of Klementsievich-Heidenhain will cause in animals the development of a macrocytic hyperchromic anemia of the hyporegenerative type.

The studies of N. A. Fedorov and his co-workers [10], S. I. Iakovlev [12] have also demonstrated that the gastric juice obtained from these Klementsievich-Heidenhain pouches in dogs in the period of hyperchromic anemia had very little of the anti-anemic factor, it being at times completely absent.

It is universally agreed that vitamin B_{12} is a very potent "external" anti-anemic factor. The utilization of vitamin B_{12} by the organism is closely associated with normal secretory activities of the mucous membranes of the entire gastrointestinal tract and to a large degree of the stomach itself.

We sought an answer to the question of whether the anemia so produced was caused by a disturbed absorption into the blood stream of the vitamin B_{12}; we therefore conducted experiments during the course of which we determined the level in the blood serum of the vitamin in dogs which had partially denervated stomachs.

EXPERIMENTAL METHODS

Under the conditions of a chronic experiment the blood picture was studied in male dogs, before and after the operation. As indicators of the conditions of the blood in the animals, counts were made of hemoglobin and vitamin B_{12} and the numbers of erythrocytes and leucocytes.

The blood was drawn from the fasting animal's femoral vein into a sterile centrifuge tube for vitamin B_{12} determinations and into ordinary test tubes for later use in Sahli hemoglobinometer and w.b.c. and r.b.c. determinations.

When calculating the color index for each animal we used the averages obtained for the hemoglobin and erythrocytes before the operation. This was done in an attempt to duplicate the methods and conditions of the work of R. A. Durinian [2]; using his findings as a guide, we found it necessary to perform completely only the biochemical studies.

The blood drawn from the vein under sterile conditions was centrifuged; to the serum was added an equal volume of acetate buffer, pH 4.6, and 4 volumes of distilled water [6]. After boiling for 1/2 hour on a water bath,

the protein precipitates were separated by centrifuging and the vitamin B_{12} content of the clear supernatant liquid was determined. The microbiological method of determining vitamin B_{12} was employed [1]. As the test microbe we used the intestinal bacillus whose original strain we obtained from the laboratory of Prof. V. I. Bukin. This strain of the intestinal bacillus, when seeded on a liquid medium containing vitamin B_{12}, will grow and cloud the solution in direct proportion to the amount of contained vitamin B_{12}. The degree of turbidity was determined in a photoelectrometer FEK-M, using a blue light filter.

Three dogs were studied for four weeks in order to observe the normal range of variations, after which they were operated and Klementsievich-Heidenhain gastric pouches were made. One dog served as a control and was not operated. It should be stated that the pouches were made much larger than usual so that almost the entire body and all of the bottom of the stomach was included. The postoperative studies were conducted for 5 months. The blood studies were begun 40 days after the operation by which time the postoperative anemia had disappeared and the specific hyperchromic macrocytic anemia had begun to develop.

EXPERIMENTAL RESULTS

Our studies demonstrated that 40-60 days after the formation of the Klementsievich-Heidenhain pouches the blood of the dogs underwent observable changes while in the control animal the variations stayed within normal limits, i.e., 2-4% Hb and up to 1/2 million erythrocytes.

In the three operated dogs the amount of hemoglobin dropped an average of 11.8%, the numer of erythrocytes diminished by an average 1,180,766, while the number of leucocytes increased by 2968.

It could be supposed that along with the lowering of the hemoglobin and decrease in erythrocytes the blood of the dogs would show also a decrease in the amount of vitamin B_{12}. However, in two of the operated dogs, Bars and Dobry, the amount of B_{12} did not diminish, but remained on the preoperative level. As an example, the results with dog Bars as presented in Table 2 may be studied. Table 2 demonstrates that the developing anemia is not paralleled by a decrease of the blood level of B_{12} so that, apparently, it does not depend on any disturbance in the absorption of vitamin B_{12} from the outside.

In the third dog, Ryzhik, Table 3 shows a 3.2-fold decrease in the blood level of vitamin B_{12} at a time when the number of erythrocytes and amount of hemoglobin diminished within approximately the same limits as shown for the first two dogs. It should be noted that the dog Ryzhik had a severe irritation of the skin of the abdomen associated with rather luxuriant granulation tissues all due to the constant copious flow of gastric juice from the gastric pouch. For this reason we could not assume that the above described phenomena were due solely to the presence of a gastric pouch. Still, we considered that the anemia and decrease of vitamin B_{12} in Ryzhik was produced by disturbance in B_{12} absorption. In that case the blood picture could be altered by intramuscular injections of vitamin B_{12}.

Vitamin B_{12} in amount of 15 γ was given intramuscularly to all the dogs every third day for 4 weeks.

The results of these experiments showed that in all the dogs except Ryzhik, injection of vitamin B_{12} after the first or second injection increased the amount of hemoglobin and the blood erythrocyte count which then stabilized at the higher levels.

However, in the operated dogs Bars and Dobry the amount of hemoglobin and the number of erythrocytes did not reach preoperative levels. In the control dog the injection of vitamin B_{12} caused a rise in the figures for hemoglobin and erythrocytes which lasted 4-6 days after which the indicators returned to the base levels existing before the introduction of vitamin B_{12}.

Along with the rise in the amount of hemoglobin and number of erythrocytes, the number of leucocytes diminished after the introduction of the vitamin B_{12}, falling even below the preoperative level.

The operated animals failed to show a rise in the blood level of the vitamin B_{12} after the first two injections while the later injections gave a noticeable increase. When the tables are examined carefully, it can be observed that the vitamin B_{12} level in the blood of the control animal (Table 1) differed rather markedly from the corresponding figures for the operated animal (Table 2) throughout the period of injecting the vitamin. In the control dog, Sever (Table 1) during this period, the average for the serum content of B_{12} did not differ from the figures before the treatment (0.359-0.329 m μg), while at the same time in the dogs having the gastric pouch a marked

TABLE 1

Alterations in the Composition of the Blood and the Vitamin B_{12} Content in the Control Dog Sever

Date of study	hemo-globin	erythro-cytes	color index	leuco-cytes	Vitamin B_{12} content in m μg/cc
19/VI 1956	78%	6 500 000	1.0	10 000	0,356
2/VI 1956	76%	6 800 000	0.96	9 000	0,402
22/VI 1956	77%	6 400 000	0.98	9 500	0,379
3/VII 1956	76%	—	—	8 750	0,400
Average	76.7	6 570 000		9 312	0.384
29/VIII 1956	80%	6 900 000	0.98	8 750	0,374
5/IX 1956	80%	6 800 000	0.99	9 500	0,374
13/IX 1956	80%	6 800 000	0.99	10 000	0,396
17/IX 1956	82%	6 900 000	0.99	—	0,396
27/IX 1956	78%	6 700 000	0.98	9 625	0,339
2/X 1956	78%	6 700 000	0,98	—	0.300
10/X 1956	72%	6 400 000	0.94	10 000	0,149
17/X 1956	80%	6 820 000	0.97	9 750	0,305
Average	78%	6 752 000	—	9 004	0.329
23/X 1956					[1]
26/X 1956	85%	7 540 000	0.96	8 100	0,249[1]
30/X 1956	85%	7 500 000	0.96		—[1]
2/XI 1956					[1]
5/XI 1956	80%	—	—	—	0.375[1]
9/XI 1956	80%	6 900 000	0.98	10 000	0.373[1]
12/XI 1956					[1]
13/XI 1956	80%	6 800 000	0.99	8 170	0,438[1]
16/XI 1956					[1]
19/XI 1956	80%	6 840 000	0.99		0,413[1]
22/XI 1956	80%	7 040 000	0.96	8 750	0.309
Average	81%	7 103 000		8 750	0.359
27/XI 1956	78%	6 800 000	0.98		0.309

[1] Intramuscular injection of the 15 γ of vitamin B_{12}.

difference was seen. Thus, for example, in dog Dobry the difference was +0.122 m μg while in Bars it was +0.173 m μg.

Table 1 shows that in the control animal the vitamin B_{12} level approached base level 2-3 days after the injection while in the operated animals 4-5 days elapsed after the injection before normal levels were reached. This lag is due, apparently, to a disturbance in the process of depot storage and utilization of vitamin B_{12} in the operated animals.

This idea has been expressed previously by I. B. Likhtsier and his co-workers [5] who investigated the amount of vitamin B_{12} present in the blood and urine of patients having cirrhosis of the liver and chronic enterocolitis with complicating anemia. Within an hour after intramuscular introduction of vitamin B_{12} these

TABLE 2

Alterations in the Composition of the Blood and the Vitamin B_{12} Content in Dog Bars with a Partially Denervated Stomach

Date of study	hemo-globin	erythro-cytes	color index	leuco-cytes	Vitamin B_{12} content in m μg/cc
12/VI 1956	93%	7 500 000	0.95	9 000	0.400
19/VI 1956	89%	7 000 000	1.0	10 000	0.310
26/VI 1956	89%	7 080 000	0.99	8 750	0.381
3/VII 1956	93%	7 220 000	1.02	9 000	0.400
Average	91%	7 200 000		9 187	0,372
10/VII 1956 Date of operation when the Klementsievich – Heidenhain pouch was made					
22/VIII 1956	69%	5 160 000	1.07	17 500	—
29/VIII 1956	73%	5 640 000	1.03	15 000	0.320
5/IX 1956	76%	6 000 000	1.00	15 125	0.320
13/IX 1956	78%	5 900 000	1.04	9 000	0.418
17/IX 1956	80%	6 300 000	1.00		0.534
27/IX 1956	73%	5 600 000	1.03	10 500	0.378
2/X 1956	74%	5 800 000	1.00	9 000	0.377
10/X 1956	75%	5 620 000	1.05	—	0.387
17/X 1956	73%	5 656 000	1.03	10 500	0.301
Average . .	74.5%	5 741 700		12 364	0,379
23/X 1956					[1]
26/X 1956	85%	6 500 000	1.03	—	0.356[1]
30/X 1956	85%	6 600 000	1.03	7 000	0.350[1]
2/XI 1956					[1]
5/XI 1956	82%	6 340 000	1.03	6 800	0.520[1]
9/XI 1956	82%	6 340 000	1.03	6 700	0.513[1]
12/XI 1956					[1]
13/XI 1956	80%	6 040 000	1.05	6 900	0.600[1]
16/XI 1956					[1]
19/XI 1956	80%	6 000 000	1.05		0.520[1]
22/XI 1956	80%	6 000 000	1.05	9 750	0.505[1]
Average . .	82%	6 260 000		7 430	0.552
27/XI 1956	80%	6 000 000	1.05		0.480
7/XII 1956	80%	6 000 000	1.05		0.390

[1] Intramuscular injection of 15 γ of vitamin B_{12}.

authors observed in these patients a sharp rise in the content of the vitamin in the blood. The B_{12} level in the serum of these patients did not return to base level even after 3 days.

In our experiments with dog Ryzhik, in spite of the introduction of vitamin B_{12} and the rise of its blood level, the r.b.c. count and the amount of hemoglobin continued to fall. In this specific instance it might be thought that the secondary manifestations such as the large inflammatory surface of the skin of the animal had more influence on the hemogram in general and the vitamin B_{12} level in particular than the presence of a gastric pouch.

TABLE 3

Alterations in the Composition of the Blood and the Vitamin B_{12} Content in Dog Ryzhik with a Partially Denervated Stomach

Date of study	hemo-globin	erythro-cytes	color index	leuco-cytes	Vitamin B_{12} content in m μg/cc
12/VI 1956	74%	6 000 000	0.97	11 750	0.244
19/VI 1956	76%	6 200 000	0.98	11 900	0.360
26/VI 1956	75%	6 000 000	1.0	11 250	0.342
3/VII 1956	75%	6 000 000	1.0	12 400	0.204
Average	75%	6 050 000	—	11 825	0.287
10/VII Date of operation when the Klementsievich – Heidenhain pouch was made					
21/VIII 1956	69%	5 160 000	1.08	17 000	0.128
29/VIII 1956	72%	5 900 000	0.97	14 625	0.144
5/IX 1956	72%	5 600 000	1.03	14 125	0.144
13/IX 1956	65%	5 000 000	1.04	14 250	0.07
17/IX 1956	58%	5 000 000	0.90	18 250	0.128
27/IX 1956	60%	4 060 000	1.20	18 750	0.022
2/X 1956	63%	4 680 000	1.09	12 500	0.022
10/X 1956	58%	5 360 000	0.87	14 350	0.072
17/X 1956	56%	4 760 000	0.95	17 000	0.072
Average	63.7%	5 057 000		15 650	0.089
23/X 1956					[1]
26/X 1956	60%	4 480 000	1.06		0.033[1]
30 X 1956	60%	4 600 000	1.04	16 250	0.072[1]
2/XI 1956					[1]
5/XI 1956	61%	5 300 000	0.90		0.264[1]
9/XI 1956	58%	5 080 000	0.90	18 750	0.233[1]
12/XI 1956					[1]
13/XI 1956	60%	4 400 000	1.09	18 750	0.233[1]
16/XI 1956					[1]
19/XI 1956	60%	5 000 000	0.96	17 300	0.072[1]
22/XI 1956	58%	4 400 000	1.05		0.072[1]
Average	59.5%	4 750 000		17 636	0.139
27/XI 1956	57%	4 400 000	1.03		
7/XII 1956	50%	4 000 000	1.0		

[1] Intramuscular injection of 15 γ of vitamin B_{12}.

The decrease in the vitamin B_{12} level in this animal is the result of disease which produces alterations in metabolism, these processes interfering with storage and utilization of the vitamin B_{12}.

Summarizing, our experiments show that partial gastric denervation in dogs leads after 40-60 days to a slight hyperchromic anemia not accompanied by a drop of the blood level of the vitamin B_{12}, this latter phenomenon being seen in two experimental dogs. This proves that the anemia in these cases is not due to disturbances in the absorption of the extrinsic factor.

This is corroborated also by the fact that intramuscular injection into the animals of vitamin B_{12} does not restore the hemogram completely.

SUMMARY

Anemia of the hyperchromic type appears in dogs 40-60 days after partial denervation of the stomach (formation of the small stomach pouch by Klementsievich-Heidenhain's method). It is not connected with decrease of vitamin B_{12} in the blood. Intramuscular introduction of vitamin B_{12} does not normalize the blood completely. These data permit the assumption that in this case development of anemia does not depend on disturbance of absorption of vitamin B_{12} introduced externally.

LITERATURE CITED

[1] V. N. Bukin, L. Ia. Aneshkina and L. S. Kutseva, Biokhimiia, XIX, 6, 713-720 (1954).

[2] R. A. Durinian, Experimental Analysis of Anemias Developing After Denervation of Some Internal Organs,* Dissertation, Moscow, 1957.

[3] E. L. Kan, Materials for Studying the Influence of the Nervous System Upon the Composition of the Blood,* Dissertation, Leningrad, 1953.

[4] M. A. Kalnyn', The Effect of Spleen Denervation Upon Hemopoiesis* Candidate's Dissertation, Riga, 1955.

[5] I. P. Kikhtsier, B. M. Braginskii and V. N. Gershanovich, in: Vitamin B_{12} and its Clinical Applications, Moscow, 1956, pp. 164-175.

[6] Iu. L. Milevskaia, ibid, pp. 146-152.

[7] O. M. Preger, Transactions Third Pavlovian Conference at Tomsk Medical Institute,* Tomsk, 1953, pp. 58-61.

[8] O. M. Preger, Transactions Fourth Pavlovian Conference at Tomsk Medical Institute,* 1954, 67-72.

[9] N. A. Fedorov, Contemporary Problems of Hematology and Blood Transfusions,* Moscow, 1955, section 31, pp. 75-77.

[10] N. A. Fedorov, A. M. Namiatysheva and A. M. Kakhetelidze, Problemy Hematol., I, 1, 10-16 (1956).

[11] V. N. Chernigovskii and A. Ia. Iaroshevskii, Problems of Nervous Regulation of the Blood System,* Moscow, 1953.

[12] S. I. Iakovlev, The Role and Significance of the Nervous System and B-Group Vitamins in Erythropoiesis* Dissertation, Leningrad, 1955.

* In Russian.