

THE EFFECT OF DENERVATION OF THE SPLEEN ON THE SERUM VITAMIN B₁₂ LEVEL IN DOGS

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The influence of the nervous system of the circulation of the blood has been widely studied in recent years by Soviet researchers [7]. Numerous investigations [3-7] have shown that after denervation of certain internal organs, changes develop in the peripheral blood picture and in the bone marrow.

A prominent place among these investigations is occupied by studies of the effect of denervation of the spleen in the activity of the blood system. It has been shown [1,3-7] that, after denervation of the spleen, anemia develops in every case. The times of development and the character of the anemias observed by different authors, however, have not shown any uniformity.

I. S. Rozanova and E. A. Zhukova [6], for instance, observed a decrease in the hemoglobin concentration and the red cell count only during the first two weeks after denervation of the spleen. These indices subsequently rapidly regained their original level, whereas the changes in the bone marrow continued to progress.

E. L. Kan [5] observed a hypochromic anemia in cats after denervation of the spleen, with the maximum fall in the hemoglobin concentration and red cell count on the 8th-14th day after operation, and lasting for several months.

R. A. Durinyan [3] showed that in cats this operation leads to the development of a hypochromic microcytic anemia of regenerative type. This author also pointed out the existence of a wide amplitude of variations in the red cell count and the hemoglobin level throughout the duration of the anemia.

According to N. N. Beller's findings [1], after denervation of the spleen in the cats the anemia follows a characteristically undulating course, and develops as a hyperchromic type.

Finally, M. A. Kalnyn' [4] observed in dogs an anemia with a maximum fall in the hemoglobin level and red cell count on the 9th-14th day after denervation of the spleen, and lasting for 2-3 months.

Denervation of the spleen thus leads to the development of anemia. In some cases the fall in the hemoglobin

level and the red cell count is slight and the anemia does not last longer than 2-3 weeks, but in others, on the contrary, a considerable and prolonged fall is observed in these indices; some authors have observed anemia of a hypochromic character, others hyperchromic.

In the present investigation we studied the influence of denervation of the spleen on the vitamin B₁₂ concentration in the serum of dogs, i.e., the changes in the blood concentration of a powerful antianemia factor such as vitamin B₁₂ during the development of experimental anemia.

METHOD

In chronic experiments on dogs (males), we studied the hemoglobin concentration (Sahli), the red and white cell counts, and the vitamin B₁₂ concentration before and after operation. Blood was taken daily, from the veins of the hind limb, with the animal in a fasting condition.

When we calculated the color index for each animal we used the mean values of the hemoglobin concentration and the red cell count obtained from examination of the blood before operation.

The determination of vitamin B₁₂ was based on a microbiological method [2] in which a strain of *Escherichia coli*, obtained from Prof. V. I. Bukin's laboratory, was utilized as test organism. After seeding on a liquid medium containing vitamin B₁₂, the strain of *E. coli* gave increased turbidity, which was proportional to the concentration of vitamin B₁₂ in the medium. Estimation of the turbidity was carried out by means of a photoelectric colorimeter, using a light blue filter.

After the normal fluctuations in the above indices had been studied for 4 to 6 weeks, in 5 dogs the spleen was denervated. The operation was performed under general ether anesthesia. All the nerve branches in the main vascular bundle of the spleen were divided. The vessels were exposed, the adventitia was carefully removed and their walls were then painted with 10% phenol solution.

As a control operation, laparotomy was performed, followed by dissection of the corresponding nerves and

Table 1. Changes in the Blood Composition and the vitamin B₁₂ Concentration in a Control Dog, not Undergoing Operation

Serial no.	Date of observation	Hemoglobin concentration (Sahli) in %	Red cell count (in thousands)	Color index	White cell count	Vitamin B ₁₂ concentration, $\mu\text{g}/\text{ml}$
	1956					
1	7/XII	78	6 220	1.04	9 000	0.400
2	11/XII	78	6 780	0.93	9 500	0.432
3	18/XII	76	6 055	1.046	10 000	0.372
4	25/XII	82	6 900	0.99	8 700	0.334
	Mean	78	6 490	—	9 300	0.384
	1957					
6	7/I	80	6 900	0.96	8 000	0.390
7	15/I	80	6 870	0.96	9 450	0.468
8	24/I	81	7 000	0.96	8 700	0.456
9	5/II	82	7 200	0.94	7 900	0.372
10	9/II	80	7 000	0.95	9 600	0.252
11	20/II	82	7 125	0.95	1 000	0.314
12	25/II	83	7 200	0.96	9 500	0.334
13	5/III	83	6 850	1.00	9 000	0.390
14	13/III	82	6 900	0.99	8 250	0.372
15	20/III	84	7 000	1.00	9 300	0.390

vessels of the spleen, which were not, however, divided.

In one dog, in addition, without any operative procedure of any kind, the blood indices were systematically studied in order to show any possible changes in the blood picture associated with the conditions under which the animals were kept, or any seasonal variations.

The investigations of the experimental and control animals lasted 5-6 months.

RESULTS

Variations in the blood indices of the dogs before operation were slight. The maximum amplitude of the variation in the red cell count, hemoglobin level, white cell count and vitamin B₁₂ concentration was 800,000, 2-5%, 1000 and 0.1-0.2 $\mu\text{g}/\text{ml}$ respectively.

No essential changes were observed in the blood picture of the control animal, on which no operation was performed, throughout the period of investigation (Table 1). The control operation led to a brief (7-8 days) and slight fall in the red cell count and hemoglobin level, but had no effect on the serum vitamin B₁₂ concentration (Table 2).

The red cell count thus fell by 1,200,000, the hemoglobin concentration by 12%, and the white cell count on the average was doubled.

Denervation of the spleen caused a more prolonged and pronounced change in the blood picture. In all the experimental dogs, during the first two weeks after the operation, a fall in the red cell count by on the average

1,575,000, and in the hemoglobin concentration by 16% took place; the white cell count was increased two- or threefold. On the 15th-20th day all these indices returned to their initial values or even slightly exceeded them, and remained at a higher level than before the operation throughout the period of investigation.

On careful examination of the results obtained (Table 3), it will be seen that the most characteristic change in the blood picture of the animals undergoing denervation of the spleen was the presence of considerable fluctuations in the values of the indices investigated from one experiment to another. It must be mentioned that the amplitude of the variations in the red cell count was greater than the amplitude of variation of the hemoglobin concentration, which resulted in periodic changes in the color index. As shown by the figures in Table 3, the variation in the vitamin B₁₂ concentration in the serum before operation could be measured in tenth parts of a $\mu\text{g}/\text{ml}$, whereas after operation the vitamin B₁₂ concentration changed considerably (three- or fourfold) from one determination to another.

We were unable to show any parallel trend in the increases in the red cell count and vitamin B₁₂ concentration, and in many determinations, when an increase or decrease was observed in the vitamin B₁₂ concentration, the red cell count was decreased or increased respectively.

It is apparent from the figures given that after denervation of the spleen in dogs the development of a true anemia is not in fact observed, but there is merely a

TABLE 2. Changes in the Composition of the Blood and the Vitamin B₁₂ Concentration in a Control Dog Undergoing Laparotomy

Serial no.	Date of observation	Hemoglobin concentration (Sahli) in %	Red cell count (in thousands)	Color index	White cell count	Vitamin B ₁₂ concentration $\mu\text{g/ml}$
1	1956 18/XII	75	6 640	0.99	8 250	0.504
2	20/XII	80	6 600	1.07	9 300	0.480
3	25/XII	76	6 800	0.98	8 000	0.390
4	1957 2/I	76	7 000	0.958	9 000	0.468
	Mean	76.9	6 760		8 638	0.460
	1957		Laparotomy performed			
6	3/I					
7	7/I	65	5 300	1.08	14 000	0.444
8	15/I	70	6 500	0.95	9 000	0.456
9	24/I	76	6 900	0.96	7 800	0.390
10	29/I	76	6 500	1.03	8 500	0.366
11	3/II	80	7 000	1.00	9 300	0.468
12	9/II	75	6 600	0.99	9 600	0.366
13	20/II	76	6 800	0.48	8 750	0.408
14	28/II	76	7 000	0.95	8 900	0.390
15	5/III	78	6 400	1.07	9 300	0.375
16	13/III	80	6 800	1.00	10 000	0.314
17	20/III	79	6 600	1.05	9 700	0.390
18	27/III	76	7 000	0.95	9 300	0.400
19	2/IV	78	6 500	1.06	8 500	0.390
20	9/IV	75	6 600	0.99	9 500	0.420
21	24/IV	77	6 900	0.98	9 250	0.318
22	7/V	76	6 950	0.97	8 750	0.368

slight and brief fall in the hemoglobin level and the red cell count.

It must be mentioned that our findings differ considerably from those in the literature [1,3-5]. This discrepancy may probably be accounted for by the fact that our investigations were carried out on dogs, in contrast to those of the previous authors, who performed their experiments on rabbits and cats. It is also known that the results obtained in experiments on the same species of animal [1,3,4,6,] differ considerably from each other. For example, the degree, character, duration, and course of the anemias observed by different authors as a result of denervation of the spleen in cats are not the same. It may be suggested that denervation of the spleen in dogs does not lead to such profound changes in hemopoiesis as are observed in cats.

It is difficult for us to give a convincing explanation of the discrepancy between our own results and those of M. A. Kalnyn' [4], who, like us, carried out experiments on dogs and observed the development of anemia in these animals after denervation of the spleen.

On the question of the interpretation of experimental anemias, N. N. Beller [1] points out that the term "anemia"

applies also to observations in which the red cell count and the hemoglobin level fell relatively to their initial values, but in absolute values these indices were maintained only a little below their mean values. If this definition is used as a starting point, in our own cases we may speak of the appearance of a brief anemia which, in the 3rd week, gave way to a slight increase in the level of these indices by comparison with the original values.

In our opinion the most characteristic feature of the blood picture in dogs after denervation of the spleen is the presence of fluctuations in the hemoglobin concentration and, in particular, of the red cell count and the vitamin B₁₂ concentration from one determination to another. This feature suggests that denervation of the spleen leads to a disturbance of the mechanism maintaining these indices in normal conditions at a more or less stable level.

SUMMARY

An insignificant anemia of short duration develops following splenic denervation in dogs. The most characteristic feature of the blood picture in this condition is

TABLE 3. Changes in the Composition of the Blood and the Vitamin B₁₂ Concentration after Denervation of the Spleen in the Dog

Serial no.	Data of observation	Hemoglobin concentration (Sahli) in %	Red cell count (in thousands)	Color index	White cell count	Vitamin B ₁₂ concentration mμg/ml
1	1956 18/XII	78	7 100	0.94	8 500	0.318
2	20/XII	78	6 640	1.01	9 000	0.420
3	25/XII	79	6 900	0.98	9 700	0.368
4	1957 2/I	80	6 600	1.04	9 500	0.385
	Mean	78.75	6 810		9 175	0.373
6	1957 3/I	Operation of denervation of the spleen				
7	7/I	60	5 050	1.03	17 600	1.4
8	15/I	65	6 000	0.92	12 500	0.264
9	24/I	68	6 900	0.84	13 600	0.111
10	29/I	76	7 100	0.92	10 500	0.318
11	5/II	80	6 000	1.14	9 200	0.129
12	9/II	80	6 800	1.01	10 300	0.202
13	20/II	86	7 400	1.00	9 000	0.420
14	28/II	83	6 400	1.01	9 625	0.108
15	5/III	86	7 000	1.05	8 700	0.420
16	13/III	80	6 200	1.10	7 000	0.111
17	20/III	80	7 000	0.9	7 000	0.129
18	27/III	79	6 200	1.00	9 000	0.420
19	2/IV	77	7 100	0.90	9 500	0.385
20	9/IV	73	6 200	1.00	10 000	0.204
21	17/IV	83	7 400	0.9	9 000	0.385
22	24/IV	85	7 500	0.9	10 500	0.186
23	7/V	85	7 500	0.9	8 250	0.132
24	14/V	73	6 250	1.00	11 000	0.480

the presence of fluctuations in the hemoglobin level and especially in that of the red cell count and vitamin B₁₂ concentration, varying from one test to another. It is suggested that splenic denervation tends to disturb the mechanism maintaining a more or less stable level of the mentioned indices within the normal limits.

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