HEMATOPOIESIS AND VITAMIN B_{12} CONTENT IN DOGS AFTER GASTRECTOMY AND IN EXPERIMENTAL HEPATITIS

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Since the role played by disturbance of the formation of Castle's intrinsic factor in the pathogenesis of B_{12}-deficiency anemias has been explained [16, 18, 19, et al.), there has been greater interest in the experimental and clinical study of remote consequences of extensive resections of the stomach. Many descriptions of so-called agastric pernicious anemia in people who have undergone a total gastrectomy operation have appeared in recent years [2-5, 7, 8, 9, 23, 24, et al.]. Aside from Bence's attempts [12] to induce megaloblastic anemia in pigs after resection of the stomach, analogous experiments performed on other animals (dogs, monkeys) have shown the impossibility of developing a similar type of anemia experimentally [1, 6, 13, 14, 17, 20, et al.].

It was interesting in this connection to study the changes which occurred in hematopoiesis in an experiment on animals with a resected stomach and in which the vitamin B_{12}-depositing function of the liver was disturbed. Our experimental observations were performed on gastrectomized dogs in which we subsequently induced the development of toxic hepatitis.

EXPERIMENTAL METHODS AND RESULTS

Eleven of the twenty dogs used for the experiment survived. Six of the 11 surviving dogs had undergone total resection of the stomach; in the other five, the resection of the stomach was subtotal.

After the operation, we observed the development of a moderate hypochromic, normocytic anemia, which lasted 20-70 days and then usually subsided spontaneously. The hemoglobin decreased 10-23% – an average of 17.33%, – and the number of erythrocytes decreased 700,000-2,000,000, or an average of 1,350,000; no changes were observed in the number of reticulocytes and leukocytes. Only one dog (Bobka) died five months after total resection of the stomach, showing symptoms of pronounced hypochromic anemia (hemoglobin–32%, erythrocytes–4,060,000; see table). Examination of peripheral blood smears showed hypochromia and anisocytosis.

Examination of hematopoiesis in the bone marrow of the gastrectomized dogs disclosed the development in the animals of a hypogenerative reaction characterized by a decrease in the total number of erythroblasts and, to some extent, by disturbance of their maturation (relative increase of basophilic normoblasts and macroblasts and, sometimes, of proerythroblasts). The average erythrocyte diameter remained normal throughout the observation period in spite of a shift to the right (7.24-7.69 μ as against the initial 6.71-7.36 μ). According to the data of our department, the normal fluctuations for the average erythrocyte diameter in dogs are 6.2-7.7 ($M \pm 3\sigma$): $M = 6.95\ \mu$. We also observed this in our study of the vitamin B_{12} content in the blood serum and liver of the gastrectomized dogs (see table). The vitamin content was determined by V. N. Bukin's biological method. The content in the blood serum ranged between 252-381 $\mu\gamma$/ml (with background indices ranging from 319 to 532 $\mu\gamma$/ml). We determined the vitamin B_{12} content in the liver of the dogs at different times, depending on when the animals died (on the 74th day, after eight months and after one year), and established the following values: 251.26 mγ/g, 287.08 mγ/g and 259.06 mγ/g (the norm being 200-300 mγ/g). Using the dogs of this same group, we also studied certain of the liver functions. We found moderate disturbances of the functions studied – the proteinogenic function in particular. For example, the amount of total protein in the blood serum decreased 1-2% (from 7.2-6.89 to 5.9-5.25%); the mercuric chloride test decreased to 1.02-1.16 (the norm being 1.7-2), and the prothrombin content fluctuated between 83 and 89%. In a majority of analyses, Himans van den Bergh's test was negative, but the bilirubin content increased in a few cases to 1.8-2 mg%.

We induced the development of acute toxic hepatitis in some experiments in order to intensify the hematopoietic disturbances in the gastrectomized dogs. In this way, we proposed to effect disturbances of the vitamin B_{12} storage function of the liver and, on a background of gastrectomy, to be able to observe a megaloblastic shift of hematopoiesis.

The experiments were performed on three dogs (two with total, one with subtotal resection of the stomach). Hepatitis was induced by the intramuscular administration of a 1% yellow phosphorus solution in apricot oil,

injected in a dose of 0.01 g/kg. The injections were given twice a week; the course consisted of 4-5 injections.

The development of acute injury of the liver tissue was verified by an increase in the bilirubin content to 10.5-16.5 mg%, a decrease in the prothrombin content to 62.5-55% and a decrease in the mercuric chloride test to 0.8-1. The total protein content decreased to 4.2%.

Examination of the peripheral blood showed the development in these animals of hyperchromic macrocytic anemia, lasting 65-133 days (Fig. 1 a and b). The hemoglobin content fell 36-46% of the original figures; the number of erythrocytes decreased 2,000,000-2,790,000. There was a considerable increase in number of reticulocytes: this index had increased from 3-10‰ (before administration of the phosphorus) to 57-145.5‰ by the 31st-44th day of the experiment. As before, no changes in the number of leukocytes were observed. By the 65th-103rd day of the experiment, the average erythrocyte diameter had increased 0.7-0.92 μ, reaching 7.92-8.12μ. On erythrocytometric curves, the minimal diameter was 5-6μ, the maximal, 10-10.5μ. A few erythrocytes were as large as 11.5-12.5μ.

On supravitally stained peripheral blood smears, we also established that formations like Heinz bodies began to appear in the erythrocytes after the administration of the yellow phosphorus solution to the animals (Fig. 2 and 3). They did not appear until the 15th day of the experiment; they were first observed as single, very tiny formations, which then increased in size and number. The maximum number of erythrocytes containing these bodies was observed on the 18th-20th day of the experiment (984-871‰), after which the number of such erythrocytes began to decrease until, on the 45th-78th day of the experiment, the bodies were no longer apparent.

Therefore, this hitherto unrecorded appearance of pathological structures in the erythrocytes indicates that phosphorus is a hemolytic as well as a hepatic poison.

Bone marrow punctates taken from the dogs Dzhonka and Al'fa after the administration of the phosphorus showed an increased number of erythroblasts of every type, the immature forms in particular (proerythroblasts increased from 1.4 to 2.6%, basophilic erythroblasts increased from 2.75-8.4 to 16-19% and macroblasts increased from 4.6 to 32.6%). The leukoblast-erythroblast ratio decreased from 1.95-2.5 to 0.46-0.66. Erythropoiesis was observed to be inhibited in the dog Ryzhii (erythroblast content decreased from 41.6 to 11.4%), but there was a sharp increase in the number of reticuloendothelial and plasma cells (the former increased from 0.5-1 to 22%, the latter, from 1.8 to 6.8%.

The changes in the vitamin B_{12} content of the blood serum were very definite in character. There was a marked increase in the vitamin B_{12} content on the 15th-16th day of the experiment. In the dog Ryzhii, for example, two weeks after the administration of the phosphorus, the

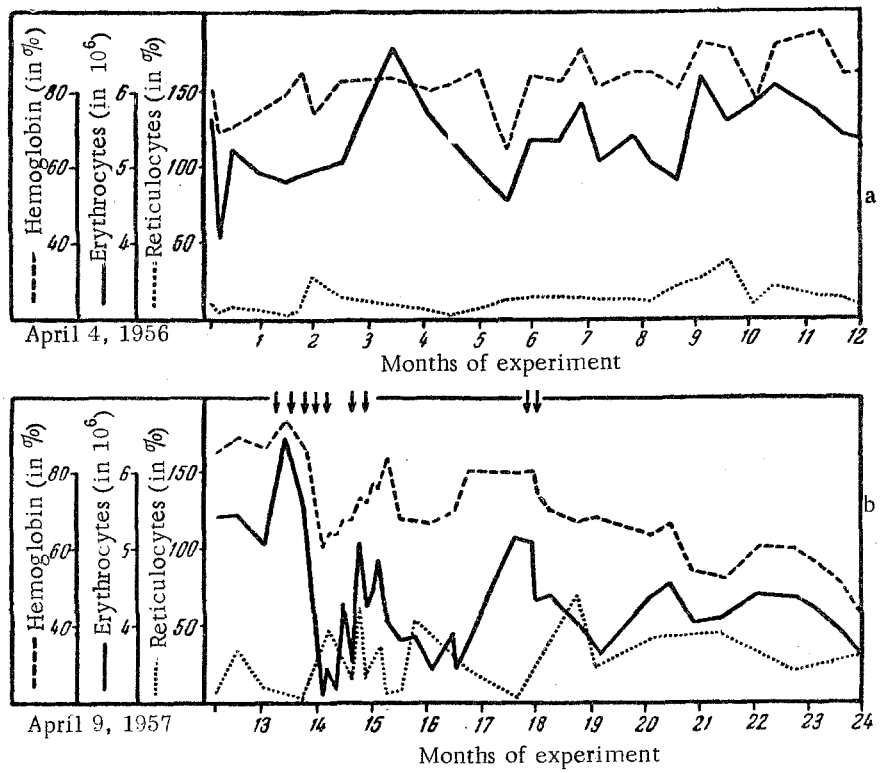

Fig. 1. Change in the blood indices of the dog Dzhonka (subtotal resection of the stomach and yellow phosphorus poisoning). a) During the 1st-12th months of the experiment; b) during the 13th-24th months of the experiment.

Maximal Changes in the Blood Indices and Vitamin B_{12} in Dogs Following Total or Subtotal Resection of the Stomach

Dog's name	Type of resection	Observation period	Hemoglobin content (in %)		Erythrocyte content		Average erythrocyte diameter (in μ)		Vitamin B_{12} content in blood serum	
			initial	minimum after resection	initial	minimum after resection	initial	minimum after resection	day of expt.	amount (in $\mu\gamma$/ml)
Dzhonka	Total	3 yr, 2 1/2 mos	81	68	5 600 000	4 120 000	7,09	7,33	396	305
Ryzhii	"	7 1/2 mos	106	75	6 000 000	3 820 000	7,36	7,69	90	243
Bobka	"	5 mos	80	32	6 220 000	4 060 000	7,26	7,57	—	—
Pudel'	"	50 days	97	80	5 890 000	4 210 000	—	—	67	293
Kukla	"	43 days	94	81	5 300 000	4 200 000	—	—	Bkgd.	387,5
Tarzan	"	28 days	88	72	5 750 000	5 380 000	—	—	—	—
Al'fa	Subtotal	1 yr, 4 1/2 mos (alive)	94	71	5 130 000	3 540 000	7,2	7,24	55	381
Pal'ma	"	1 yr	91	77	6 370 000	4 400 000	6,71	7,37	309	252
Pirat	"	7 1/2 mos	88	80	5 700 000	4 830 000	—	—	177	255
Naida	"	2 mos	90	79	5 180 000	4 380 000	—	—	—	—
Laima	"	34 days	10	90	5 810 000	5 050 000	—	—	Bkgd.	136

vitamin B_{12} content was twice the original (having increased from 243 to 580 $\mu\gamma$/ml) and by the 72nd day constituted 653.2 $\mu\gamma$/ml. In the dog Al'fa, the vitamin content had more than doubled by the 16th day, having increased from 381 to 776 $\mu\gamma$/ml, but the administration of phosphorus did not cause the same reaction in Dzhonka: in this dog, the vitamin B_{12} content did not increase until the 89th day of the experiment, and then only after an additional injection of the phosphorus was administered.

After involution of the symptoms of hepatitis and normalization of the liver functions, the vitamin B_{12} content in the blood of these dogs was back to normal. The normal hematologic indices and bone marrow picture were also restored. This restoration was not observed, however, in the dog Ryzhii, which died of poisoning on the 72nd day in a condition of serious hepatitis and anemia.

The increase in the vitamin B_{12} content of the blood serum effected by phosphorus poisoning was due to the fact that the storage function of the liver was disturbed during hepatitis. This conforms with clinical observations recording weakening of the storage function in patients with cirrhosis of the liver and infectious hepatitis [10, 11, 21, 22]. If the functional ability of the liver is subsequently restored, the possibility of vitamin B_{12} storage in it is also restored (normalization of the vitamin B_{12} content of the blood serum).

We also examined three control (non-gastrectomized) animals which had received yellow phosphorus in the same dose as the gastrectomized dogs. Macrocytic, hyperchromic anemia similar to that observed in the first group of dogs developed in these animals. The average erythrocyte diameter increased to 7.89-8.12 μ. The base of the Price-Jones curve ranged between 5.5-6.5μ and 10-10.5μ. The bone marrow punctates showed some decrease in the leukoblast-erythroblast ratio (from 1.09 to 0.94) due to a slight increase in the total number of erythroblasts; the polychromatophilic normoblasts increased from 8.3 to 10.3%, and the proerythroblasts increased from 0.6 to 1.6%. By the 15th day of the experiment, the vitamin B_{12} con-

Fig. 2. Blood smear from the dog Dzhonka after yellow phosphorus poisoning. Degenerate hemoglobin structures (supravitally stained with Nile blue sulfate).

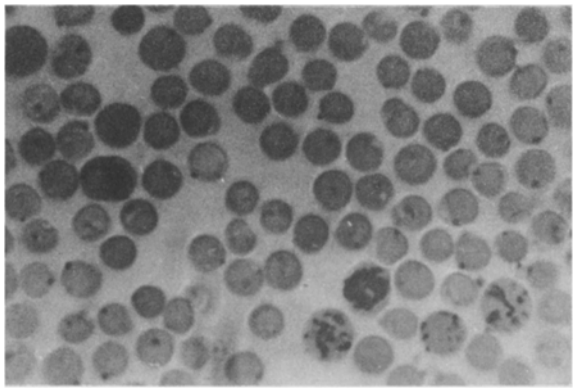

Fig. 3. Blood smear from the dog Al'fa after yellow phosphorus poisoning. Degenerate hemoglobin structures (supravitally stained with Nile blue sulfate).

tent in the blood serum of these dogs had increased from 532.2-516.5 to 1425-1212.5 $\mu\gamma$/ml, but was back to the original indices by the 51st day. In the liver of the dog Dina, which was killed on the 163rd day of the experiment, there was a decreased vitamin B_{12} content—94.83 mγ per 1 g fresh tissue.

From our observations, it is evident that phosphorus caused a more serious and protracted anemia in the animals which had undergone total gastrectomy. The administered dose of phosphorus proved lethal in the case of Ryzhii (total gastrectomy): the animal died, showing symptoms of acute macrocytic anemia, after receiving a dose easily tolerated by the non-gastrectomized dogs and the dog with subtotal gastric resection. Dzhonka (total gastrectomy) was killed by this same dose of the poison after its administration to her in the second experiment with phosphorus, performed almost a year after her first poisoning, with a picture of developed hepatitis and hyperchromic anemia.

Summing up the above, one can see that in spite of the absence of the stomach and the disturbance of the vitamin B_{12}-storing function of the liver, the macrocytic anemia and disturbance of the vitamin B_{12} content attending the experimental hepatitis were temporary changes, and no transition to the megaloblastic type of hematopoiesis was observed. This functional resistance of erythropoiesis is evidently due, to some extent, to the fact that in dogs Castle's intrinsic factor is formed in the small intestine as well as in the stomach.

SUMMARY

Experimental hepatitis was induced in gastrectomized dogs by the intramuscular injection of yellow phosphorus. Hepatitis development was associated with pronounced macrocytic anemia and disturbance of the B_{12} storing liver function. All these changes were only temporary. No transition to the megaloblastic type of hematopoiesis was observed. Analogous phenomena developed in dogs with experimental hepatitis and intact stomachs. Moreover, formations of the Heinz bodies type appeared in the erythrocytes as a result of the yellow phosphorus injection.

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