THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

XIV. CHANGES IN THE BLOOD FOLLOWING DIVERSION OF THE SPLENIC BLOOD FROM THE LIVER. A CONTROL STUDY OF THE EFFECTS OF SPLENECTOMY.

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In the earlier publications from this laboratory (1) have been described the various phenomena which follow splenectomy in the dog. In the discussion of the most striking of these,—anemia, increased resistance of the red cells, decreased tendency to jaundice, and prolongation of the recovery from anemia—it was assumed that, inasmuch as the spleen was absent, these changes were probably due to the loss of some function peculiar to this organ. As to the exact nature of the function lost, no definite opinion has thus far been given. Three possibilities, however, readily suggest themselves: (1) the loss of some function of blood destruction or regeneration resident in the spleen itself; (2) the loss of an internal secretion acting on the distant hemopoietic tissues, such as the bone marrow; and (3) the alteration of the venous drainage of the spleen, which, in that it is an important source of the portal blood, may have an essential relation to some peculiar function of the liver.

It is obvious that the value of some of these hypotheses might be tested without removal of the spleen, by diverting its venous outflow to the liver. We have, therefore, repeated many of our experiments, but instead of removing the spleen, the splenic vein has either been ligated or transplanted into the vena cava, or an Eck fistula has been established. As far as we know, exactly similar experiments

have not previously been attempted, though two observations have a bearing on the problem. Contrary to our results, Nassau (2) found that the number, color, and resistance of the red cells remained unchanged after simple Eck fistula; but as he gives only one count before and one after the operation, his observations are not conclusive. The other report which concerns us is Pribram's (3) artificial constriction of the splenic vein with resultant passive congestion of the spleen. The slight anemia that this caused is analogous to some of the results we report below.

If the anemia following splenectomy is caused by the removal with the spleen of some necessary factor in blood formation or of a hormone essential to the hemopoietic tissues, this factor should not operate in the vein transplantation experiments and Eck fistula, unless it is also essential that such a substance have direct approach to the liver for its proper functioning or activation. If, on the other hand, the anemia is largely due to the interference with the supply of splenic blood to the liver, it should occur in all animals tested. These problems, and also that concerning the influence of the experimental procedures on increased resistance of the red cells, will be considered in this paper. Other problems such as the decreased tendency to jaundice and the prolonged repair of anemia in the splenectomized animal when hemolytic agents are given, will be presented in a later communication.

Methods.

In the ligation experiments it was found necessary, on account of the numerous branches and the anastomoses of the splenic vein with veins from the stomach, to tie all branches of the splenic vein shortly after they left the spleen. In the earlier operations one of the subdivisions of the artery was also ligated, to lessen the supposed danger of rupture. This, later, was abandoned when it was found to be unnecessary, as well as undesirable, on account of infarct production. Postmortem examination of animals subjected to this operation showed in each case that all branches had been tied, that the vessel beyond the ligation was much diminished in size or completely obliterated, and that there was usually little attempt at compensatory development of new veins from the adherent omentum. When such

new-formed veins were present it was found that they seldom emptied into the portal system, and therefore, as a rule, did not complicate the experiment. Eck fistula was performed in the usual manner, the proximal end of the portal vein being tied off above its new anastomosis with the vena cava just before its entrance into the liver. Transplantation of the splenic vein into the inferior vena cava presented considerable difficulty on account of the smallness of the vein and the necessity of stretching it a little to make it reach the vena cava. In only one experiment, however, was it found that the transplanted vein had been occluded by thrombus. The success of the operation was always determined by examination of the vessels at autopsy, and in some instances injection specimens were prepared and dissected to make doubly sure that no new anastomosis had formed.

The particular phenomena studied were: (1) the quantitative changes in the red blood cells, leukocytes, and hemoglobin; (2) the resistance of the red cells to hypotonic salt solution; (3) the general condition as indicated by the weight of the animals and condition of the urine; and (4) the gross and microscopical appearance of the spleen and other viscera at autopsy.

The dogs have been kept on the usual mixed diet of table scraps. This has been shown to maintain properly normal dogs as well as those convalescent from operations other than splenectomy, without the development of anemia (4).

RESULTS.

Our studies have been made on 15 dogs, divided as follows: ligation of splenic vein, 4; transplantation of splenic vein, 4; Eck fistula, 3; and, as controls, splenectomy, 3; transplantation of the inferior mesenteric vein, 1. In the case of the last dog, it was intended to transplant the splenic vein, and it was not until autopsy that we found that the inferior mesenteric vein had been used by mistake. As some of these dogs were used also for the study of the effect of hemolytic agents, all are not presented in this paper.

Changes in the Spleen.

In those dogs in which all branches of the splenic vein had been ligated and in which adequate new venous channels have failed to develop, the spleen shows considerable change. Before the operation is completed the spleen increases to almost double its size and takes on a dark purple color. If the animal is allowed to survive two or more months, the spleen at autopsy is found to be considerably smaller and much firmer than normal. The capsule is slightly thickened and puckered and the organ has a pale bluish color. On section the tissue cuts with increased resistance and a few small shrunken infarcts are occasionally found. The cut surface is less bloody than usual and shows an increase of fibrous tissue, with indistinct Malpighian corpuscles. Histologically, the tissue appears to be condensed with collapsed sinuses rather than to exhibit any marked fibrous tissue hyperplasia. Some hemosiderin pigment is present, the Malpighian corpuscles are small, and here and there are several areas of hyaline degeneration, which do not respond to the test for amyloid. Thromboses are not found, except in connection with the old post-operative infarcts. In the earlier animals, in which a branch of the artery was also ligated, a bulging hemorrhagic infarct appeared in the corresponding area. The abdominal lymph nodes and the liver are apparently unchanged. Speculation as to the adjustment of the splenic circulation in these cases must remain unsatisfied. The most probable explanation seems to be that the elastic spleen is able not only to accommodate the increasing pressure without rupture, but, with the aid of the minute new-formed capillaries in the adherent omentum, to maintain sufficient degree of nutrition to prevent necrosis. We have seen, however, that the exchange is not sufficient to prevent atrophy.

At the time of the operations for Eck fistula and the splenic vein-vena cava anastomosis, there is necessarily a temporary occlusion of the large veins. The spleen and intestine become very dark and turgid, but when the clamps are removed, these organs quickly return to normal color. At autopsy on animals so treated, except for numerous adhesions and frequently perisplenitis, no noteworthy changes are found.

Changes in the Blood.

An example of the changes in the blood which occur after ligation of the splenic vein is given in Table I.

It may be seen that this animal developed a moderate degree of anemia lasting several weeks and similar to but less severe than that following splenectomy. The anemia is accompanied by a temporary slight increase in resistance of the red cells to hypotonic salt solution.

TABLE I.
Blood Changes Following Ligation of Splenic Veins.

Dog 47.	Hemoglobin. <i>per cent</i>	Red blood cells per cmm.	Hemolysis.		Leukocytes per cmm.	Polymorpho- nuclears.	Small lympho- cytes.	Large transi- tionals.	Eosinophils.	Weight. <i>kilos</i>
			Begins.	Com- plete.						
Before	102	6,275,000	0.475	0.35	9,800	7,200	700	1,800	100	6.0
1 day after	98	6,110,000			19,000	15,200	950	2,850	0	5.7
4 days "	92	5,350,000	0.475	0.275	16,500					5.6
1 wk. "	90	5,520,000	0.425	0.275	16,800	13,600	1,300	1,600	300	5.4
2 " "	72	4,900,000	0.425	0.3	18,800	15,400	1,900	1,500	0	
3 " "	70	4,600,000	0.45	0.3	21,000	16,800	3,100	300	300	
4 " "	78	5,000,000			18,600					6.4
5 " "	74	4,380,000			15,000					
7 " "	80	5,040,000	0.475	0.275	12,800	10,000	1,700	1,100	0	7.3
10 " "	82	5,050,000	0.45	0.3	12,400					
12 " "	95	5,830,000	0.475	0.325	14,800	9,500	3,400	1,500	400	7.5
13 " "	92	6,410,000	0.475	0.35	14,900	9,200	3,600	1,200	900	
14 " "	95	6,150,000			15,600	12,200	2,500	600	100	7.4

As in splenectomy, there is an immediate leukocytosis, due to polymorphonuclear and transitional forms, but prolonged by a more persistent lymphocytosis and eosinophilia. A very slight drop in weight occurs after operation, but later there is a distinct increase above the original weight. It has been shown elsewhere (4) by the study of control operations such as nephrectomy, that the changes here described are not merely postoperative. Other dogs with ligated veins gave similar results; such as Dog 51, with a maximum drop of 14 per cent

of hemoglobin and 1,200,000 red cells per cmm.; Dog 74, hemoglobin loss 24 per cent, red blood cell loss 1,800,000 per cmm.

The effect of diverting the splenic venous blood from the liver by transplanting the splenic vein into the vena cava is shown in Table II.

Much the same changes as after ligation are found here. In another dog (No. 71) similarly treated, the increased resistance was much more marked: before operation, hemolysis began at 0.50 and was complete at 0.35; soon after operation, it began at 0.425 and was complete at 0.25. Anemia was also present (a drop of 20 per cent in hemoglobin and of 1,800,000 red blood cells). A third animal (No. 16) lost 35 per cent hemoglobin and over 2,000,000 erythrocytes, and a fourth (No. 24) 22 per cent hemoglobin and over 1,000,000 erythrocytes.

The changes in an Eck fistula experiment are seen in Table III.

In another Eck fistula dog (No. 68), the increased resistance of the red cells was more marked (before operation, hemolysis began at 0.45 and was complete at 0.3; soon after operation it began at 0.425 and was complete at 0.225) and the animal lost 33 per cent hemoglobin and 2,000,000 red blood cells, in four weeks' time after operation. In a third (No. 5) with an even greater anemia, the blood picture was complicated by the occurrence of infection, so the figures are not given.

That interference with inflow to the portal circulation, from organs other than the spleen is responsible for changes in the blood is shown in another experiment (Table IV) in which by accident an anastomosis of the inferior mesenteric vein was done.

This dog developed an anemia of mild grade and recovered from it sooner than did the other dogs of this series. Although little weight can be placed on a single experiment such as this, the observation tends to support the theory that the supply of portal blood to the liver is an important factor in our problem.

The urine remained without bile in all the animals used.

There was an occasional postoperative loss of weight (especially in some of the Eck fistula dogs), but this was by no means constant, and bore no apparent relation to the anemia. This was frequently followed by an increase to more than the original weight, just as is frequently found after splenectomy.

TABLE II.

Blood Changes Following Transplantation of the Splenic Vein.

Dog 4.	Hemoglobin.	Red blood cells per cmm.	Hemolysis.		Leukocytes per cmm.	Polymorpho- nuclears.	Small lympho- cytes.	Large transi- tionals.	Eosinophils.	Weight.
			Begins.	Com- plete.						
	<i>per cent</i>									<i>kilos</i>
Before	97	6,960,000	0.45	0.35	16,100	8,400	4,700	1,600	1,400	9.4
1 day after	104	7,040,000	0.425	0.3	34,000	29,600	3,100	1,300	0	
1 wk. "	75	5,550,000	0.45	0.3	19,300	12,400	3,700	1,900	1,300	9.7
2 " "	68	5,020,000	0.475	0.275	16,900	11,800	2,700	2,200	200	9.4
3 " "	70	4,530,000								9.6
4 " "	80	4,510,000	0.475	0.3	14,700	9,600	2,800	1,500	800	10.4
5 " "	74	5,440,000	0.475	0.325	14,000	9,800	2,800	420	1,080	10.5
6 " "	78	5,630,000	0.45	0.3	11,200	6,300	4,000	560	360	
7 " "	82	5,770,000	0.45	0.325	11,100	7,400	2,900	200	600	12.9
8 " "	85	6,060,000	0.45	0.325						
9 " "	96	6,500,000								13.2

TABLE III.

Blood Changes Following Eck Fistula.

Dog 31.	Hemoglobin.	Red blood cells per cmm.	Hemolysis.		Leukocytes per cmm.	Polymorpho- nuclears.	Small lympho- cytes.	Large transi- tionals.	Eosinophils.	Weight.
			Begins.	Com- plete.						
	<i>per cent</i>									<i>kilos</i>
Before	99	6,500,000	0.45	0.325	13,200	9,800	2,700	500	200	16.1
1 day after	88	6,040,000	0.475	0.325	36,000	32,400	2,800	800	0	
3 days "	84	6,300,000	0.45	0.325	32,400	27,500	2,900	1,600	400	14.2
5 " "	72	5,400,000	0.425	0.3	24,000	17,300	3,800	2,400	500	14.2
1 wk. "	74	5,500,000	0.425	0.3	18,800	13,700	3,400	800	900	13.6
2 " "	68	5,040,000	0.425	0.275	19,600	15,500	3,900	600	600	13.5
3 " "	73	5,110,000	0.425	0.275						
4 " "	67	4,460,000	0.425	0.3	21,000	15,500	4,400	600	500	14.6
5 " "	72	4,880,000	0.475	0.325	18,400	12,900	3,700	600	1,200	14.2
6 " "	84	5,600,000	0.475	0.325	16,000	10,500	3,800	400	1,300	14.9
7 " "	90	6,200,000	0.45	0.3	15,200	10,300	2,800	600	1,500	15.3
8 " "	98	6,520,000	0.45	0.3	15,800	11,400	3,500	300	600	15.4

TABLE IV.

Blood Changes Following Transplantation of Inferior Mesenteric Vein.

Dog 55.	Hemo- globin.	Red blood cells per cmm.	Hemolysis.		Weight.
			Begins.	Complete.	
	<i>per cent</i>				<i>kilos</i>
Before	100	5,940,000	0.45	0.3	14.7
2 days after	76	5,240,000	0.4	0.25	
3 " "	75	5,100,000	0.4	0.25	13.9
10 " "	76	4,880,000	0.45	0.275	
2 wks. "	85	5,400,000	0.425	0.25	
3 " "	84	5,890,000	0.425	0.25	
4 " "	78	5,600,000			14.7
5 " "	88	5,720,000			
6 " "	92	5,800,000	0.425	0.275	15.3

SUMMARY.

1. In dogs whose splenic veins have been ligated or transplanted into the inferior vena cava, or in which an Eck fistula has been made, an anemia occurs which resembles that following splenectomy and shows the same general variation in degree and duration.

2. The resistance of the red cells to hypotonic salt solution is quickly increased, sometimes coincident with and sometimes preceding the anemia. As a rule, it gradually returns to normal in about the same length of time as it takes the anemia to disappear, but may remain increased for longer periods.

3. There is an initial leukocytosis, involving at first the polymorphonuclear leucocytes and transitional cells. As the total leukocytosis diminishes there is both a relative and actual increase of small lymphocytes and usually of eosinophils. This may either be temporary or last during the rest of the period of observation and differs from the ordinary postoperative leukocytosis.

4. Ligation of the splenic vein is followed by considerable atrophy of the spleen, but not by necrosis or thrombosis. There is rarely adequate new vein formation. The other operations cause little or no change in the spleen.

5. Whether the disturbances as described are due to the loss of a certain volume of blood to the liver, or, as has been previously

suggested, to the loss of a splenic hormone, it is impossible to say. If the former is true, the method of production of the anemia still remains unexplained. It is evident, furthermore, that the latter theory has also no value unless it is assumed that this hormone must be activated by passage through the liver.

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