VENOM HEMOLYSIS AFTER SPLENECTOMY, INCLUDING THE RESISTANCE OF THE ERYTHROCYTES OF NORMAL DOGS TO THE HEMOLYTIC ACTIVITY OF COBRA VENOM.

By JOHN A. KOLMER, M.D.

(*From the McManes Laboratory of Experimental Pathology of the University of Pennsylvania, Philadelphia.*)

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Several investigators have shown that the resistance of erythrocytes to various hemolytic agents is increased after splenectomy. The earlier investigations by Pugliese and Luzzatti (1), Vast (2), Joannovics (3), and Banti (4) were conducted by injecting such hemolytic agents as pyrodin and toluylendiamine into animals before and after splenectomy, and noting in a general manner the increased resistance to hemolysis *in vivo* following the removal of the spleen.

Later investigations by Chalier and Charlet (5), and more particularly by Pearce and his associates (6), have shown the increased resistance of the blood cells to hemolytic agents after splenectomy by experiments *in vivo* and *in vitro*. Chalier and Charlet (5) first drew attention to a slight increase in the resistance of dog erythrocytes to hypotonic salt solutions 10 to 12 days after splenectomy. In a series of researches by Pearce and his associates, Karsner, Austin, Peet, Krumbhaar, and Musser (6, 7, 8, 9), it was shown that the erythrocytes of dogs after splenectomy acquire an increased resistance to hypotonic salt solutions as determined by experiments *in vitro*, and to the influence of specific anti-dog hemolytic serum as shown by experiments *in vivo* and *in vitro*. These investigations have also shown that the increased resistance to hemolysis is due to changes in the erythrocytes themselves.

The relation of splenectomy to the resistance of erythrocytes to the hemolytic activity of cobra venom has not heretofore been investigated. Since the phenomenon of venom hemolysis appears to be intimately associated with the lecithin or ether-soluble activators present in erythrocytes, particularly fatty acids and their soluble soaps, and since the removal of the spleen has been shown to alter the content of fatty substances in the blood, it would appear that an investigation concerning the resistance of erythrocytes to venom before and after splenectomy may yield additional information in this problem.

While the exact nature of venom hemolysis is as yet unknown, the researches in this field indicate that the substance or substances within the erythrocytes acting with the hemolysin in the venom is of a lipoidal nature. Flexner and Noguchi (10), who originally showed that cobra venom is the most active hemolytically of a number of venoms studied, and that the erythrocytes of the dog are susceptible to its influence, believed that the venom hemolysin was activated by serum complement. Later researches by Kyes (11, 12) showed that venom causes the hemolysis of erythrocytes in the absence of serum, and Kyes and Sachs (13) believed that the activating principle was a lecithin contained in the erythrocytes, for which they proposed the name "endocomplement." Noguchi (14), on the other hand, claims that the fatty acids, neutral fats, and soluble soaps in the serum and erythrocytes are the active agents; while other investigators have attempted to correlate these opposing views on the basis that the fatty acids and soaps act as indirect activating agents in venom hemolysis, in that they possess the power of modifying the cell and rendering the intracellular lecithin available for the activation of the venom hemolysin.

The investigations of King (15) indicate that the spleen is concerned in regulating the quantity of unsaturated fatty acids, which are active hemolytic agents, in the blood, and that the benefits derived by splenectomy in pernicious anemia and other diseases, as described by Eppinger (16) and others, is to be ascribed to a reduction of the quantity of these agents in the blood. King has found in experiments conducted with dogs, that the removal of the spleen is followed by a reduction of the unsaturated fatty acids in the serum with an increase of total fats and cholesterol, and that the presence of both the latter and especially of cholesterol, which is well known to possess antihemolytic properties, may account for the increased resistance of the blood to hemolytic agents after splenectomy. However, as shown by Pearce and his associates, the resistance of the blood to hemolytic agents after splenectomy is due to changes in the erythrocytes themselves, which is not explainable on the basis of King's results, especially since King found that the quantity of antihemolytic cholesterol in the corpuscles was decreased after splenectomy, which, it would appear, should render the cells more susceptible to hemolysis, instead of the reverse. Recently Dubin and Pearce (17) reported that analysis, before and after splenectomy, of the blood of dogs shows practically no change in the amount of total fats and unsaturated fatty acids, as expressed by the iodine value.

In the investigation by Kolmer and Pearce (18), on the influence of splenectomy upon the phenomenon of non-specific complement fixation sometimes shown by normal rabbit and dog serum, which has been found by Kolmer (19) to be due largely to lipoid substances in the serum, the removal of the spleen did not materially alter the non-specific complement-binding power of the serum, whereas lipoid-solving anesthetics, such as chloroform and ether, temporarily removed this property of dog and rabbit sera.

The results of my studies herein reported show that the erythrocytes acquire a temporary increased resistance to venom hemolysis after splenectomy; whether or not this increased resistance is due to a decrease of the lipoidal activator within the cells consequent to the removal of the spleen has not been investigated, but the investigations mentioned show that the phenomenon of venom hemolysis is intimately associated with lipoids and that the spleen may exert an influence upon the lipoidal substances in both corpuscles and serum. Further researches along these lines may explain in definite terms the nature of the increased resistance of erythrocytes after splenectomy, which, at present, is not understood.

Method of Study.

Two separate series of dogs were used in the present investigation; in Series A four animals were splenectomized and two used as controls; in Series B three were splenectomized and two used as controls.

Two or three preliminary tests of the resistance of the washed erythrocytes of each dog to cobra venom were made before the splenectomies were performed, in order to obtain a venom index for each dog. In addition, a large number of normal dogs were examined in order to gain more definite information regarding the normal resistance of the erythrocytes of the dogs with the technique employed.

All the animals were kept on a mixed diet of table scraps, consisting of meat, bread, cereals, and vegetables. As shown by Pearce, Austin, and Pepper (20), the anemia following splenectomy (21) is most marked on this cooked diet as contrasted with a diet of raw meat.

Venom Tests.—One lot of cobra venom was used throughout the study.[1] The dried venom was kept tightly stoppered at room temperature and a stock 1:1,000 solution in normal salt solution (0.85 per cent) was freshly prepared each time the venom tests were conducted. From this stock dilution further dilutions with normal salt solution ranging from 1:5,000 to 1:70,000 were prepared.

Twelve dilutions of venom in amounts of 1 cc. each were used

[1] The venom was kindly furnished by Dr. Hideyo Noguchi.

routinely with each blood. To each tube was added 1 cc. of a 4 per cent suspension of washed cells; this doubled the dilutions so that they now ranged from 1:10,000 to 1:140,000. After gentle mixing, the test-tubes were incubated at 37°C. for 2 hours and placed in a refrigerator over night when the readings were made.

In each experiment a hemolytic scale was prepared by dissolving 1 cc. of the mixed cells of four to six dogs in 49 cc. of distilled water. From this 100 per cent solution of 2 per cent of cells further dilutions of hemoglobin were prepared ranging from 5 to 100 per cent. With these the degree of hemolysis in the venom tests were read off and recorded according to the amount of hemoglobin in the supernatant fluid.

Each dog was bled from an external jugular vein through a dry needle in the proportion of 4 cc. of blood in 16 cc. of a 2 per cent solution of sodium citrate in normal salt solution. These suspensions of cells were set aside in the refrigerator over night and washed three times with normal salt solution by low speed centrifugalization. After the last washing a 4 per cent suspension of each lot of cells was prepared in normal salt solution.

Every effort was made to insure uniformity in preparing the dilutions of venom and the suspensions of cells. As venom in dilution tends to deteriorate, it was considered better to prepare a fresh stock dilution for each experiment.

In Series B parallel tests with hypotonic salt solutions were conducted after the method used by Karsner and Pearce (7), except that I used 0.1 cc. of the sediment of washed cells to 3 cc. of each hypotonic salt solution, whereas they used 0.1 cc. of the corpuscular mass secured by centrifuging the blood after defibrination by gentle whipping.

RESULTS.

Venom Hemolysis of the Erythrocytes of Normal Dogs.—The results of venom tests with the blood of normal dogs and the degree of maximal and minimal resistance are shown in Table I.

As shown in Table I, the resistance of dog erythrocytes to venom hemolysin varies, and the blood of the same dog may vary to a slight extent at different periods. Apparently there is a relation

TABLE I.

Venom Hemolysis of the Washed Erythrocytes of Normal Dogs (1 Cc. of a Four Per Cent Suspension of Cells + 1 Cc. of Venom Solution).

No.	Dilution of venom and degree of hemolysis.											
	1:10,000	1:16,000	1:20,000	1:32,000	1:40,000	1:50,000	1:60,000	1:70,000	1:80,000	1:100,000	1:120,000	1:140,000
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	100	100	100	55	70	50	5	—	—	—	—	—
2	100	100	100	30	60	45	10	8	8	—	—	—
3	100	100	100	60	50	40	18	20	20	15	—	—
4	100	100	100	60	50	45	10	5	5	—	—	—
5	100	100	100	95	90	80	20	10	10	5	—	—
6	100	100	100	80	25	10	5	5	—	—	—	—
7	100	100	100	90	15	10	—	—	—	—	—	—
8	100	100	100	100	100	90	10	10	10	—	—	—
9	100	100	50	5	—	—	—	—	—	—	—	—
10	100	100	100	100	90	60	25	25	25	8	—	—
11	100	100	100	100	100	100	40	40	40	5	—	—
12	100	100	100	90	15	10	—	—	—	—	—	—
13	100	100	100	100	100	100	80	80	20	10	—	—
14	100	100	100	90	20	15	—	—	—	—	—	—
15	100	100	100	90	90	20	—	—	—	—	—	—
16	100	100	100	60	60	60	30	30	—	—	—	—
17	100	100	100	20	—	—	—	—	—	—	—	—
18	100	100	100	20	10	5	—	—	—	—	—	—
19	100	100	100	90	20	15	—	—	—	—	—	—
20	100	100	100	100	90	40	—	—	—	—	—	—
21	100	100	100	80	30	—	—	—	—	—	—	—
22	100	100	100	100	50	5	—	—	—	—	—	—
23	100	100	100	100	30	5	—	—	—	—	—	—
24	100	100	100	100	90	40	—	—	—	—	—	—
25	100	100	100	100	80	60	30	20	—	—	—	—
26	100	100	100	100	100	100	80	60	50	10	—	—
27	100	100	100	90	70	30	10	5	—	—	—	—
28	100	100	100	100	90	60	30	10	—	—	—	—
29	100	100	100	100	100	90	80	40	10	—	—	—

between the degree of anemia which an animal may have due to intestinal parasitism, distemper, or other causes, and the resistance to venom, as the cells become more susceptible to venom in the presence of an anemia. This is important in interpreting the results

of resistance tests after splenectomy on account of the temporary anemia following the removal of the spleen.

On the basis of the examination of the dogs listed in Table I, the maximal resistance of washed dog erythrocytes to cobra venom in our tests may be stated as follows:

Dilution of venom 1: 10,000 gave complete hemolysis in 100 per cent.
" " " 1: 16,000 " " " " 100 " "
" " " 1: 20,000 " " " " 97 " "
" " " 1: 32,000 " " " " 41 " "
" " " 1: 40,000 " " " " 17 " "
" " " 1: 50,000 " " " " 10 " "
" " " 1: 60,000 does not produce complete hemolysis.

In general terms, the erythrocytes of the majority of the dogs were not able to withstand an exposure to venom lower than 1: 20,000 before complete hemolysis resulted; hemolysis may begin in dilutions as high as 1: 100,000 of venom, and the point of minimal resistance is more variable, or at least more difficult to read than the point of maximal resistance.

Venom Hemolysis of the Erythrocytes of Splenectomized Dogs.— In Tables II and III are shown the results of venom tests with the blood of two of the six dogs belonging to Series A; Text-figs. 1 to 6 were plotted according to the point of maximal resistance, or the

TABLE II.

Venom Hemolysis of the Erythrocytes of Dog 4 before and after Splenectomy.

Date.	History.	Dilution of venom and degree of hemolysis.										
		1: 10,000	1: 16,000	1: 20,000	1: 32,000	1: 40,000	1: 50,000	1: 60,000	1: 70,000	1: 80,000	1: 100,000	1: 120,000	1: 140,000
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Oct. 19	1st preliminary.....	100	100	100	90	90	40	5	—	—	—	—	—
" 29	2nd "	100	100	100	60	50	45	10	5	5	—	—	—
Nov. 4	3rd "	100	100	100	50	40	20	10	—	—	—	—	—
" 10	5 days after splenectomy...	100	90	25	—	—	—	—	—	—	—	—	—
" 17	12 " " "	100	50	30	5	—	—	—	—	—	—	—	—
Dec. 7	32 " " "	100	100	100	100	90	20	10	5	—	—	—	—
" 15	40 " " "	100	100	100	100	90	80	80	60	—	—	—	—

TABLE III.

Venom Hemolysis of the Erythrocytes of Dog 2 Used as a Control.

Date.	History.	Dilution of venom and degree of hemolysis.											
		1:10,000	1:16,000	1:20,000	1:32,000	1:40,000	1:50,000	1:60,000	1:70,000	1:80,000	1:100,000	1:120,000	1:140,000
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Oct. 19	1st examination.	100	100	100	100	90	80	60	20	10	—	—	—
" 29	2nd "	100	100	100	30	60	45	10	8	8	—	—	—
Nov. 4	3rd "	100	100	100	90	90	80	60	30	20	10	—	—
" 10	4th "	100	100	100	80	70	60	30	20	20	—	—	—
" 17	5th "	100	100	100	60	40	35	10	—	—	—	—	—
Dec. 7	6th "	100	100	100	100	80	40	10	5	—	—	—	—
" 15	7th "	100	100	100	100	60	50	5	—	—	—	—	—

highest dilution of venom causing complete hemolysis in the various tests. In this series venom tests were conducted 5, 12, 32, and 40 days after splenectomy.

In Series B venom tests were conducted at intervals varying from 4 days to 4 months after splenectomy; in Table IV are shown

TABLE IV.

Venom Hemolysis of the Erythrocytes of Dog 25 before and after Splenectomy.

Date.	History.	Dilution of venom and degree of hemolysis.											
		1:10,000	1:16,000	1:20,000	1:32,000	1:40,000	1:50,000	1:60,000	1:70,000	1:80,000	1:100,000	1:120,000	1:140,000
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Feb. 1	1st preliminary.	100	100	100	100	80	60	30	10	—	—	—	—
" 8	2nd "	100	100	100	90	40	20	—	—	—	—	—	—
" 14	4 days after splenectomy.	100	90	30	10	—	—	—	—	—	—	—	—
" 23	13 " " "	100	95	80	60	30	10	—	—	—	—	—	—
" 29	19 " " "	100	100	90	80	40	5	—	—	—	—	—	—
Mar. 14	33 " " "	100	100	100	90	40	10	—	—	—	—	—	—
" 22	6 wks. " "	100	100	100	100	90	30	10	5	—	—	—	—
Apr. 20	10 " " "	100	100	100	100	100	80	40	10	—	—	—	—
May 25	4 mos. " "	100	100	100	100	100	80	60	10	—	—	—	—

the results observed with one dog of this series, and a summary of the results is shown in Text-figs. 7 to 11, according to the point of maximal resistance, or the highest dilution of venom in each test causing complete hemolysis of the cells.

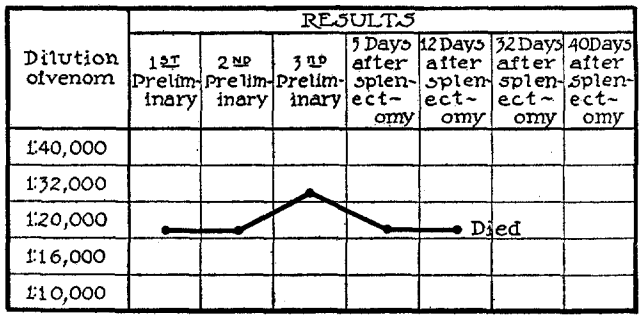
At the time of the venom tests parallel tests with hypotonic salt solutions were conducted for the purpose of a comparative study. Solutions of sodium chloride ranging from 0.3 to 0.575 per cent were used in each experiment; Table V shows the results observed with one dog of this series. In Text-figs. 7 to 11 I have included a summary of the experiments; the percentage of salt solution given being the point of maximal resistance, or the highest percentage of salt causing just complete hemolysis.

TABLE V.

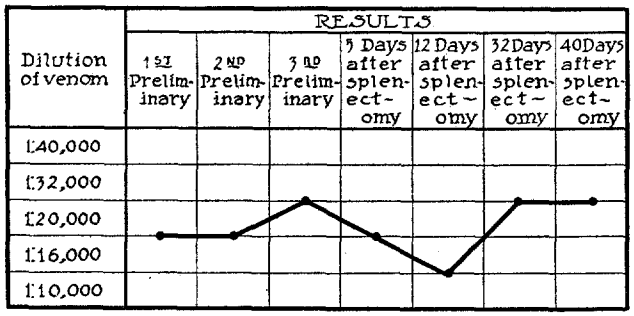
Resistance of the Erythrocytes of Dog 25 before and after Splenectomy, to Hypotonic Salt Solutions.

Date.	History.	Salt solution and degree of hemolysis.											
		0.3	0.325	0.35	0.375	0.4	0.425	0.45	0.475	0.5	0.525	0.55	0.575
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Feb. 1	1st preliminary.	100	100	100	100	90	80	40	30	10	5	—	—
" 8	2nd "	100	100	100	100	100	90	40	30	20	10	—	—
" 14	4 days after splenectomy.	100	100	100	90	90	80	50	40	10	5	—	—
" 23	13 " " "	100	100	100	90	60	50	30	5	—	—	—	—
" 29	19 " " "	100	100	80	30	10	10	5	5	—	—	—	—
Mar. 14	33 " " "	100	100	90	80	60	30	20	5	—	—	—	—
" 22	6 wks. " "	100	100	90	80	80	60	30	20	5	—	—	—
Apr. 20	10 " " "	100	100	100	90	80	60	60	20	10	5	—	—
May 25	4 mos. " "	100	100	100	90	90	80	40	20	10	—	—	—

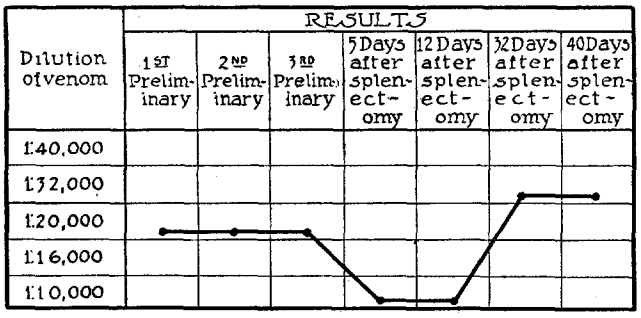
The results of my experiments with the two series of dogs may be summarized as follows: (1) The resistance of the erythrocytes of normal dogs to the hemolytic action of cobra venom is likely to vary to a slight degree in different tests, as shown in the preliminary tests with Dog 4 in Series A, and Dogs 25, 26, 28, and 29 in Series B. While every effort was made to insure a uniform technique in successive experiments, it is possible that these variations were due in part at least to variations in the strength of venom or injury to the



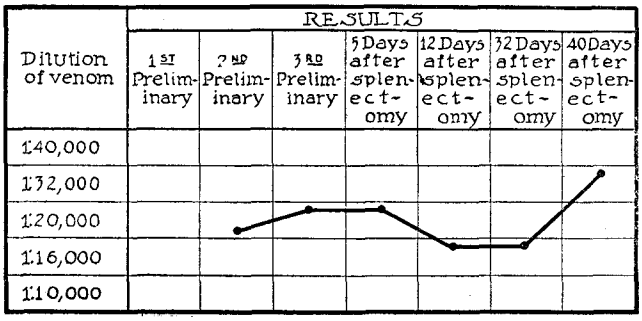
TEXT-FIG. 1. Maximal resistance of the erythrocytes of Dog 1 before and after splenectomy.



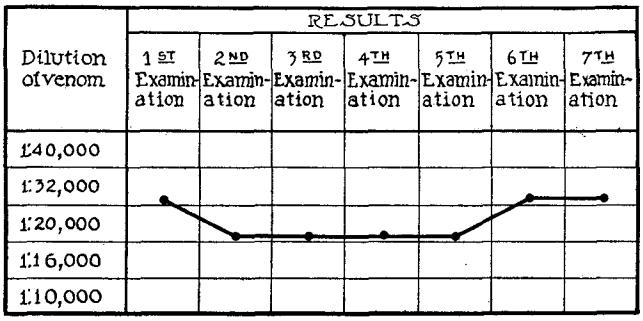
TEXT-FIG. 2. Maximal resistance of the erythrocytes of Dog 3 before and after splenectomy.



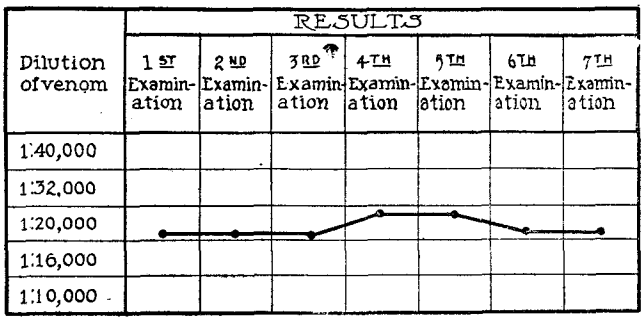
TEXT-FIG. 3. Maximal resistance of the erythrocytes of Dog 4 before and after splenectomy.



TEXT-FIG. 4. Maximal resistance of the erythrocytes of Dog 5 before and after splenectomy.



TEXT-FIG. 5. Maximal resistance of the erythrocytes of Dog 2 used as a control.



TEXT-FIG. 6. Maximal resistance of the erythrocytes of Dog 6 used as a control.

cells during the washing. At all events it would appear necessary to bear in mind these possible variations in interpreting the influence of the removal of the spleen upon the resistance of the erythrocytes.

Dilution of venom	RESULTS								
	1st Preliminary	2nd Preliminary	4 Days after splenectomy	13 Days after splenectomy	19 Days after splenectomy	33 Days after splenectomy	6 Weeks after splenectomy	10 Weeks after splenectomy	4 Mos. after splenectomy
1:50,000									
1:40,000									
1:32,000									
1:20,000									
1:16,000									
1:10,000									
Hypotonic salt solution	0.375	0.4	0.35	0.35	0.325	0.325	0.325	0.35	0.35

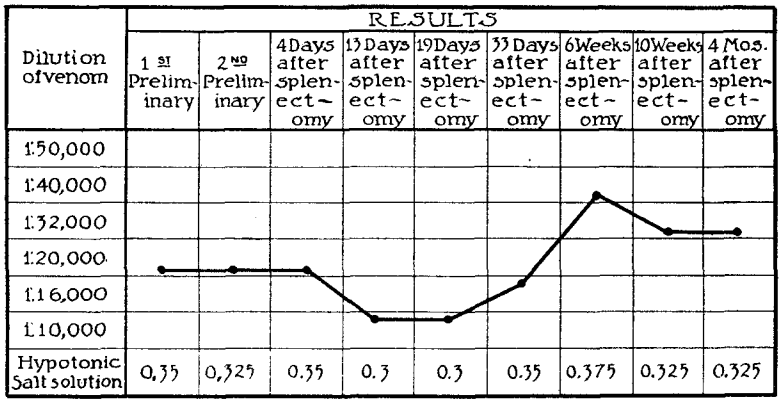
TEXT-FIG. 7. Maximal resistance of the erythrocytes of Dog 25 before and after splenectomy.

Dilution of venom	RESULTS								
	1st Preliminary	2nd Preliminary	4 Days after splenectomy	13 Days after splenectomy	19 Days after splenectomy	33 Days after splenectomy	6 Weeks after splenectomy	10 Weeks after splenectomy	4 Mos. after splenectomy
1:50,000									
1:40,000									
1:32,000									
1:20,000									
1:16,000									
1:10,000									
Hyptonic salt solution	0.35	0.35	0.35	0.3	0.3	0.35	0.35	0.35	0.35

TEXT-FIG. 8. Maximal resistance of the erythrocytes of Dog 26 before and after splenectomy.

(2) On the 4th and 5th days after splenectomy the resistance of the erythrocytes to cobra venom was increased to a well marked extent in the majority of the dogs. On the 12th and 13th days after splenectomy

this increased resistance was still well marked and persisted in most instances for a period of 19 days or 3 weeks, when the erythrocytes gradually became more and more vulnerable to the influence of venom. 5 to 6 weeks after splenectomy the erythrocytes of all the dogs were as susceptible to venom as before the operation and in some instances to a greater extent. (3) Apparently the decrease of resistance to venom noted in the majority of the dogs about 4 or 5 weeks after splenectomy was coincident with an anemia, as shown by the following results of blood examinations of dogs in both series.



TEXT-FIG. 9. Maximal resistance of the erythrocytes of Dog 27 before and after splenectomy.

Dog 3.—32nd day after splenectomy. Hemoglobin, 16 per cent; erythrocytes, 1,072,000. Maximal resistance to venom, 1:32,000 (a decrease of resistance). (Text-fig. 2.)

Dog 4.—32nd day after splenectomy. Hemoglobin, 51 per cent; erythrocytes, 3,952,000. Maximal resistance to venom, 1:32,000 (a decrease of resistance). (Text-fig. 3.)

Dog 25.—10 weeks after splenectomy. Hemoglobin, 62 per cent; erythrocytes, 3,840,000. Maximal resistance to venom, 1:40,000 (a decrease of resistance). (Text-fig. 7.)

Dog 26.—10 weeks after splenectomy. Hemoglobin, 80 per cent; erythrocytes, 4,800,000. Maximal resistance to venom, 1:32,000 (normal resistance for this animal). (Text-fig. 8.)

Dog 27.—10 weeks after splenectomy. Hemoglobin, 24 per cent; erythrocytes, 1,800,000. Maximal resistance to venom, 1:32,000 (a decrease of resistance). (Text-fig. 9.)

On the other hand, the erythrocytes of the control or non-splenectomized animals did not show a decrease of resistance to venom according to the results of blood examinations.

Dog 6.—32nd day. Hemoglobin, 86 per cent; erythrocytes, 5,328,000. Maximal resistance to venom, 1:20,000 (normal for this animal). (Text-fig. 6.)

Dilution of venom	RESULTS								
	1ST Examination	2ND Examination	3RD Examination	4TH Examination	5TH Examination	6TH Examination	7TH Examination	8TH Examination	9TH Examination
1:50,000									
1:40,000									
1:32,000									
1:20,000									
1:16,000									
1:10,000									
Hypotonic salt solution	0.35	0.35	0.35	0.375	0.4	0.375	0.375	0.375	0.35

TEXT-FIG. 10. Maximal resistance of the erythrocytes of Dog 28 used as a control.

Dilution of venom	RESULTS								
	1ST Examination	2ND Examination	3RD Examination	4TH Examination	5TH Examination	6TH Examination	7TH Examination	8TH Examination	9TH Examination
1:50,000									
1:40,000									
1:32,000									
1:20,000									
1:16,000									
1:10,000									
Hypotonic salt solution	0.325	0.350	0.35	0.35	0.375	0.35	0.325	0.35	0.35

TEXT-FIG. 11. Maximal resistance of the erythrocytes of Dog 29 used as a control.

Dog 2.—32nd day. Hemoglobin, 125 per cent; erythrocytes, 5,600,000. Maximal resistance to venom, 1:32,000 (normal for this animal). (Text-fig. 5.)

Dog 28.—After 10 weeks. Hemoglobin, 82 per cent; erythrocytes, 4,200,000.

Maximal resistance to venom, 1:40,000 (a decrease for this animal, probably due to distemper from which the animal was suffering). (Text-fig. 10.)

Dog 29.—After 10 weeks. Hemoglobin, 87 per cent; erythrocytes, 5,100,000. Maximal resistance to venom, 1:32,000 (about normal for this animal). (Text-fig. 11.)

An increased resistance to hypotonic salt solutions was shown by the three splenectomized dogs belonging to Series B (Text-figs. 7, 8, and 9). This increase of resistance on the part of the erythrocytes to hypotonic salt solutions was apparent 13 days after the operation, and in two of the dogs (Nos. 25 and 27) persisted to a slight degree throughout the 4 months' period of observation. Dog 26 showed a slightly increased resistance on the 13th and 19th days after splenectomy, and after this time the resistance returned to the point found in the tests prior to splenectomy.

The increased resistance of the erythrocytes to venom and hypotonic salt solutions developed at or about the same intervals after splenectomy, but resistance to the latter appeared to persist for a longer time.

CONCLUSIONS.

1. The resistance of erythrocytes of dogs to the hemolytic activity of cobra venom is increased after splenectomy.

2. This increased resistance was observed as early as 4 days after splenectomy and usually persisted for a period of about 3 weeks, when the resistance gradually decreased to normal or slightly beyond.

3. The decrease of resistance to the hemolytic activity of venom for the erythrocytes of splenectomized dogs following the primary increase is apparently coincident with the anemia following splenectomy. An intercurrent infection, such as distemper, tends to reduce the resistance of erythrocytes to venom.

4. An increased resistance of the erythrocytes to hypotonic salt solutions was found with all the splenectomized dogs in which these tests were made. Increased resistance to hypotonic salt solutions apparently persists for a longer period than the increased resistance to cobra venom.

5. As the lysis of erythrocytes by venom is dependent upon the presence of certain lipoidal substances within the cells, and as the

spleen may exercise an influence over the lipoidal contents of corpuscles and serum, it is suggested that the increased resistance of erythrocytes to the hemolytic activity of venom after splenectomy is due to alterations in the lipoid content of the erythrocytes.

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