THE RATE OF REGENERATION OF BLOOD PLATELETS.*

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The experiments to be reported in this paper were performed to determine the rapidity with which blood platelets reappear in the circulation of healthy animals after they have been more or less completely removed by repeatedly withdrawing, defibrinating, and reinjecting blood. Since defibrinated blood is almost free from platelets, the number in the circulation can be reduced to an extremely low level by repeating such a process a number of times at short intervals.

METHODS.

For the purpose of removal of platelets, dogs were anesthetized and, under aseptic technique, were bled from the carotid artery in amounts of 200 to 300 cubic centimeters. This blood was defibrinated by shaking for five minutes with glass beads, strained through gauze, and reinjected slowly into the jugular vein. In experiments I and II, this process was repeated ten and six times respectively, so that at the end of the operation blood obtained by cutting an ear vein showed only a very small clot on standing. In experiments III, IV, and V, the defibrination was less complete.

The animals showed few ill effects following this procedure and except for slight anemia and occasional wound infection, their condition remained good throughout the experiments. During the first few hours after the operation, animals I and II showed a marked tendency to hemorrhage. Bleeding from pricks in the ear veins was so profuse after the obtaining of blood for the platelet counts as to necessitate clamping. There was little bleeding, however, from the

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operation wounds. The latter observation can be accounted for by the fact that the wounds were made before the blood was de-

EXPERIMENT I.

Fox terrier; weight 17 pounds. Ten defibrinations. 200 c.c. of blood withdrawn at each defibrination. Duration of operation 4 hours.

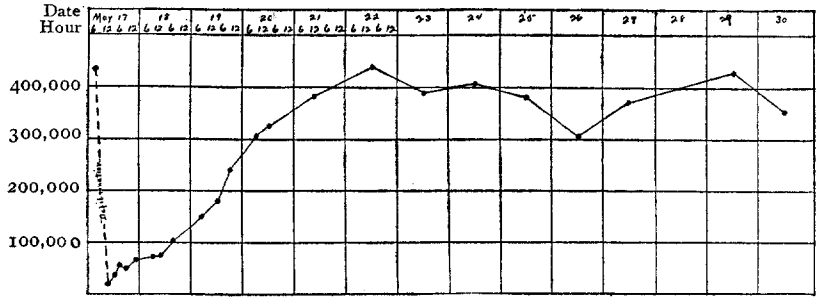

CHART I.

Date.	Platelet count.	Clot.	White count.	Remarks.
May 17				
8.30A.M.	436,000	Firm.	20,000	Hemoglobin 110 per cent. Sahli.
		Blood defibrinated.		
12.15P.M.	21,300	Very slight.	36,000	Bleeds excessively from small cuts in ear.
3 P.M.	38,000	Slight.	38,000	Bleeds excessively from small cuts in ear.
5 P.M.	51,000	Slight.		Bleeds excessively from small cuts in ear.
8 P.M.	43,000	Slight.		
May 18				
1 A.M.	69,000	Not firm.	30,000	Bleeding not markedly prolonged.
10 A.M.	72,000	Firm.	24,700	Bleeding not prolonged.
12 M.	75,000	Firm.	40,000	
6 P.M.	103,000		29,000	
May 19				
8 A.M.	150,000		26,000	Condition of animal good.
3 P.M.	179,000		31,000	
9 P.M.	240,000		40,000	
May 20				
10 A.M.	310,000		26,000	Wound breaking down.
3 P.M.	321,000		21,000	
May 21	383,000		33,000	Wound open.
May 22	444,000		22,400	
May 23	395,000		24,500	Hemoglobin 70 per cent.
May 24	404,000		20,000	Hemoglobin 80 per cent.
May 25	380,000		12,200	Hemoglobin 80 per cent.
May 26	306,000			
May 27	374,000			
May 29	434,000			
May 30	358,000			
May 31	464,000			

fibrinated. The severed vessels therefore became thrombosed while the blood was normal. Animals III, IV, and V, in which the defibrination was not so complete, showed no marked tendency to bleed.

EXPERIMENT II.

Young fox terrier; weight 19.5 pounds. Six defibrinations. 300 c.c. of blood withdrawn at each defibrination. Duration of operation 4 hours.

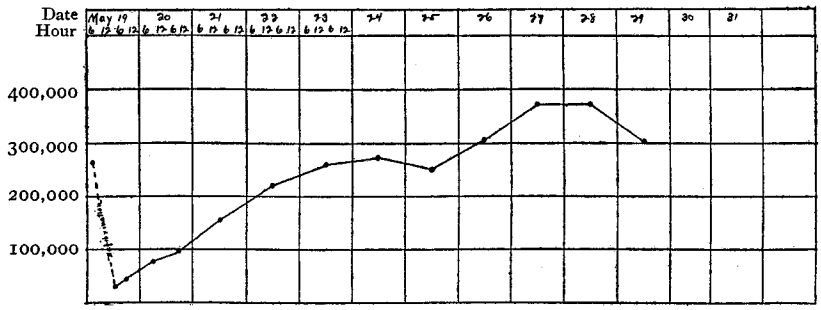

CHART 2.

Date.	Platelet count.	Clot.	White count.	Remarks.
May 19				
9 A.M.	256,000	Firm.	9,900	Hemoglobin 105 per cent. Sahli.
		Blood defibrinated.		
2 P.M.	33,000	Very slight.	21,000	Bleeds excessively from small cuts in ear.
9 P.M.	48,600	None.	26,500	Bleeds excessively from small cuts in ear.
May 20				
10 A.M.	80,000	Soft.	21,000	Hemoglobin 95 per cent.
3 P.M.	97,000	Firm.	31,000	Bleeding from cuts not excessive.
May 21	186,000		9,500	Wound healing. No infection.
May 22	223,000		13,500	
May 23	268,000		9,200	Hemoglobin 75 per cent.
May 24	272,000		8,200	
May 25	258,000			Hemoglobin 70 per cent.
May 26	310,000		20,700	
May 27	375,000		23,000	
May 28	378,000		26,000	
May 29	300,000		23,000	

The platelet counts were made by Wright and Kinnicutt's method.[1] In this method the blood is diluted in a blood pipette (1 to 100 dilution) with a mixture of two parts of a solution of brilliant cresyl blue (aqueous solution 1 to 300) and three parts of a solution of potas-

[1] Wright and Kinnicutt, *Jour. Am. Med. Assn.*, 1911, lvi, 1457.

sium cyanid (aqueous solution 1 to 1,400). This mixture must be filtered and used immediately. The exact proportion of stain and cyanid used in the mixture is of the greatest importance, and in studying blood of different species of animals the proportion must be varied somewhat. If the amount of cyanid is excessive, both the

EXPERIMENT III.

Fox terrier; weight 21 pounds. Three defibrinations. 250 c.c. of blood removed at each defibrination. Duration of operation 1 hour.

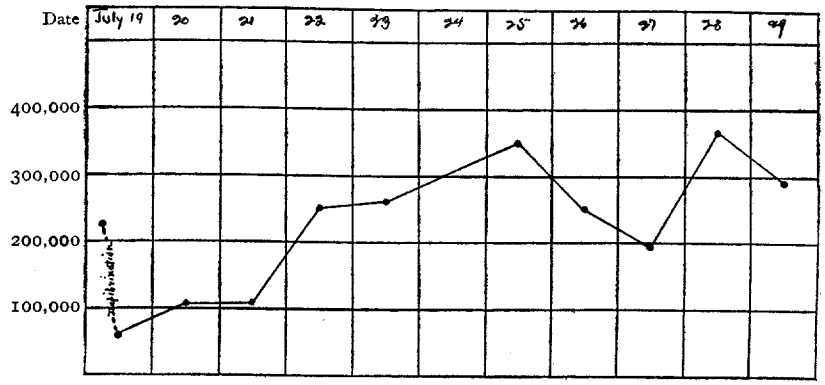

CHART 3.

Date.	Platelet count.	Clot.	Remarks.
July 19	225,000	Firm.	Blood defibrinated.
July 19	84,000	Soft.	Bleeding from small cuts in ear slightly prolonged.
July 20	108,000	Firm.	Bleeding not excessive.
July 21	110,000		Wound breaking down.
July 22	254,000		
July 23	264,000		
July 25	352,000		
July 26	249,000		
July 27	192,000		
July 28	370,000		
July 29	290,000		

platelets and leucocytes disintegrate, while if the amount is deficient the red cells are not completely laked and the platelets clump. Requisites for a good mixture are that the red cells must be laked, the protoplasm of the leucocytes well stained, and the platelets evenly distributed and present a blue or violet color. For dog

blood, a mixture of two parts of stain to five parts of cyanid solution gives the best results. The platelets are counted in a .100

EXPERIMENT IV.

Fox terrier; weight 12 pounds. One defibrination. 200 c.c. of blood removed.

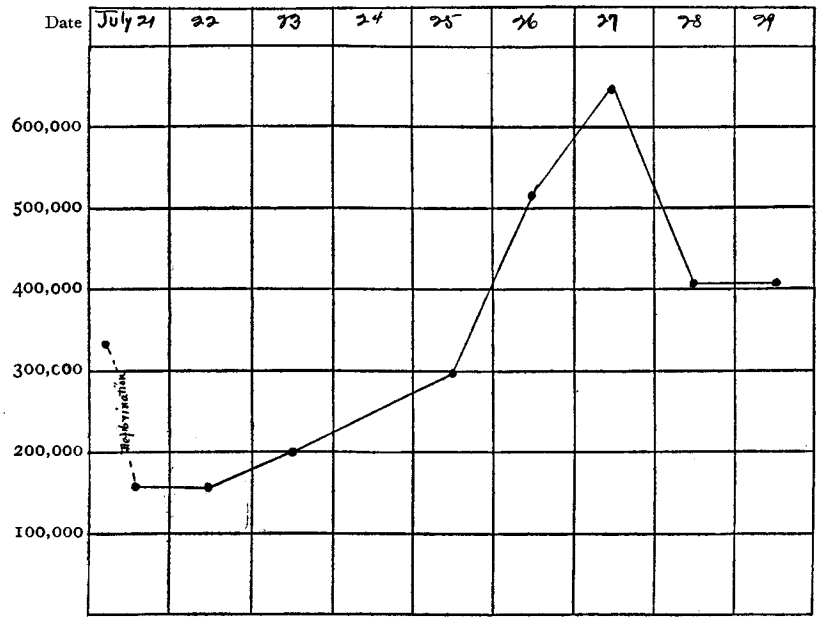

CHART 4.

Date.	Platelet count.	Remarks.
July 21	328,000	Hemoglobin 105 per cent. Sahli.
		Blood defibrinated.
July 21	161,000	
July 22	158,000	Wound open.
July 23	200,000	
July 25	300,000	Animal not lively. Losing weight.
July 26	520,000	Slight infection of ear where pricked.
July 27	644,000	Animal seems sick.
July 28	412,000	Hemoglobin 65 per cent.
July 29	404,000	Hemoglobin 70 per cent.

millimeter Zeiss chamber with a high dry lens. It is hardly necessary in this paper to discuss the relative merits of the various methods for counting platelets. It may be said simply that if the

technique is carried out quickly and if the blood used for the determination is flowing freely, constant results can be obtained by this method.

The platelet count in different dogs varies considerably. In carrying out experiments in another connection, we have noted

EXPERIMENT V.

Fox terrier; weight 12 pounds. Two defibrinations. 300 c.c. of blood removed at each defibrination.

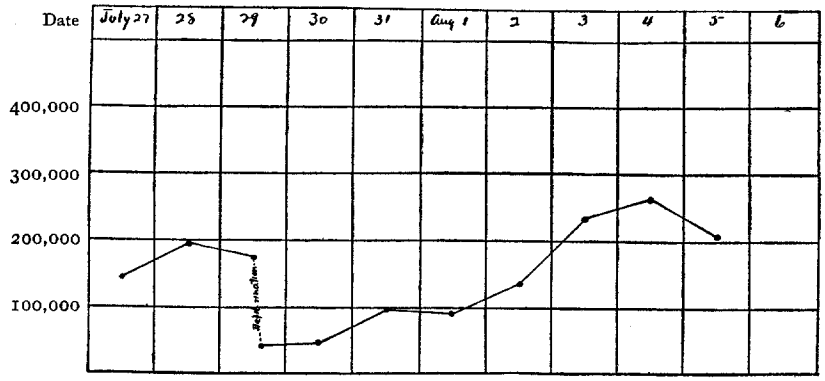

CHART 5.

Date.	Platelet count.	Remarks.
July 27	140,000	
July 28	194,000	
July 29	177,000	Hemoglobin 100 per cent. Sahli.
		Blood defibrinated.
July 29	39,200	Hemoglobin 95 per cent.
July 30	48,300	Hemoglobin 85 per cent.
July 31	92,000	Hemoglobin 80 per cent.
Aug. 1	88,000	Wound breaking down. Animal looks rather quiet and sick.
Aug. 2	135,000	
Aug. 3	236,000	
Aug. 5	263,000	
Aug. 8	204,000	

counts as high as 800,000 and as low as 180,000. In an individual dog, however, under normal conditions the count taken from day to day rarely varies as much as 100,000.

In the first two experiments, in order to detect slight changes, four counts were made at each determination; in the last three experiments, two. The average results are recorded in the charts.

DISCUSSION.

An examination of the data shows that the reappearance of platelets in the circulation after their removal by repeated defibrination of blood is remarkably rapid. In each of the five experiments, the normal count was reached in from three to five days. This was true whether the removal of platelets had been almost complete (78 to 95 per cent.) or only partial (50 to 66 per cent.), whether the normal count for the animal was high (436,000) or low (180,000). The rate of reappearance was in some instances highly remarkable and amounted to as much as half the normal number of platelets in the blood in one day.

A rather striking difference can be seen between the first two and the last three experiments. In the first two, the rise in the platelet count began immediately after the operation and proceeded quite steadily to normal or a little above. In the last three, however, there was little change in the count until the second or third day, and then followed a rapid increase which in one case (experiment IV) rose high above the normal. It may be pointed out that in the experiments in which the rise appeared to be delayed, the platelets had been less completely removed from the circulation. Whether this delay was due to a less intense demand for platelet regeneration or to injury and premature disintegration of the platelets remaining in the circulation after the operation is a question which cannot be definitely decided, and it hardly warrants discussion.

The experiments were carried out mainly in the hope of throwing light on the rate of generation of platelets which obtains under physiologic conditions.

It is conceivable that after the removal of platelets from the blood by defibrination, the generation is continued at the usual rate and that the curves in experiments I and II (experiments in which the removal of platelets was almost complete) give an approximate picture of the normal rate of platelet formation in dogs. In view of our ignorance, however, as to whether or not a lack of platelets is a stimulus to platelet generation, such a conclusion is not justifiable. In fact, the existence of such a stimulus would seem most probable

and one would be almost safe in assuming that the rate of generation in the experimental condition was abnormally rapid.

The experiments show, however, that under conditions not markedly pathologic, the generation of platelets is extremely rapid and, in comparison with that of the red cells under a somewhat analogous state of stimulation (severe anemia[2]), is much more rapid. It may be added in this connection that fluctuations in the platelet count in human beings under both normal and pathologic conditions are greater and more rapid proportionally than those of the red count. In a case of tuberculosis,[3] for example, it was observed that just preceding an attack of purpura hemorrhagica, the platelet count fell from 210,000 to 4,000 within a period of three days. After remaining at this level for five days it suddenly began to rise at an average rate of 100,000 a day until a count of 720,000 was reached. The red count under no circumstances shows such marked and rapid changes. It has been noted also[4] that the platelets of normal individuals introduced into three patients having hemorrhagic disease of the type just mentioned disappeared within a period of about three days. The red cells introduced at the same time gave no evidence of rapid disintegration.

It is evident that under certain conditions the disappearance of platelets may be extremely rapid, and that under the influence of certain demands the rate of generation also may be extremely rapid. It is hard to believe that such rapid regeneration could take place if the demand for it was a rare one, and a view that rapid disintegration and replacement of platelets is a more or less physiological occurrence would seem to fit well with the data. It is possibly justifiable, therefore, to suggest that platelets under the usual conditions of life are rapidly disappearing either through utilization or disintegration and that the count is kept constant under a given set of conditions by as rapid a rate of formation. In comparison with the erythrocytes, both the rate of destruction and rate of genera-

[2] P. M. Dawson, Effects of Venous Haemorrhage and Intravenous Infusion in Dogs, *Am. Jour. Physiol.*, 1900, iv, 1.

[3] A complete report of this case is to appear in *Deutsch. Arch. f. klin. Med.*, 1911.

[4] W. W. Duke, The Relation of Blood Platelets to Hæmorrhagic Disease *Jour. Am. Med. Assn.*, 1910, lv, 1185.

tion would seem to be proportionately more rapid. If such is the case, the life cycle of the platelet must be considered extremely short and can possibly be reckoned in days.

CONCLUSIONS.

By repeatedly withdrawing, defibrinating, and reinjecting blood, the platelet count in dogs can be reduced to an extremely low level.

When the reduction in platelets and fibrinogen has been carried to an extreme degree the animals display a marked tendency to bleed.

The regeneration of platelets after their removal by defibrination is remarkably rapid and amounts, on an average, to about one fifth of the entire number in the blood per day.

From the experiments and from observations on the platelet count in human beings, it is believed that under the usual conditions of life platelets are utilized or destroyed and replaced in enormous numbers daily, and that the life cycle of the platelet is extremely short,—possibly a matter of a few days.

My sincere thanks are due to Dr. G. H. Whipple, of Johns Hopkins Medical School, and Professor M. A. Barber, of the University of Kansas Medical School, in whose laboratories the experiments were carried out, and also to Drs. J. H. Wright and Roger Kinnicutt for their excellent method of counting blood platelets.