THE BLOOD PLATELETS IN PNEUMOCOCCUS INFECTIONS.

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The subject of the variation in the number of blood platelets, or thrombocytes, in the blood during fever and various acute infectious diseases has been studied by numerous investigators. Many different conclusions have been drawn from the observations which have been made, owing probably to the fact that many different methods have been employed in making the counts. The great number of methods and modifications of methods found in the literature indicates a general distrust of the reliability of the methods heretofore employed. Although some of the procedures give results which seem consistent enough, discrepancies occur with such frequency, without accountable reason, that suspicion is often cast on any single count. The idea is prevalent that within physiological limits, the platelets vary more in number than do the other formed elements of the blood. For instance, the normal number of platelets per c.mm. as given by different investigators varies between 200,000 and 850,000. It is probable that this conception is the result of faulty methods. Indeed our studies with an improved method which is here reported indicate that the number of platelets in the circulating blood during health is fairly constant.

Owing to the absence of any consensus of opinion concerning the number of platelets which may be considered normal, the interpretation of variations in the counts during disease has been difficult. In general, however, it has been fairly well established that during the course of the acute infectious diseases the number of platelets in the blood is decreased, and that during convalescence the number becomes normal or even greater than normal. As regards the platelets in the blood during pneumonia, especially in lobar pneumonia associated with the pneumococcus, only a few studies have been made.

No investigators have reported the results of frequent observations in individual cases and, consequently, no information is at hand regarding the changes which occur during the various stages of the disease.

It, therefore, seemed of value to make a series of observations regarding the platelets in pneumonia, with a method giving consistent findings, and the present paper states the results of these observations.

Method.

In most of the older methods for counting platelets, the blood was obtained by pricking the finger, smears were made, the relative number of platelets and erythrocytes was determined and the absolute number of platelets was then calculated. The total number of red cells per c.mm. of blood was found by the usual method.

Possibly the greatest error in most of this previous work resulted from the faulty method of obtaining the blood. When blood is allowed to come into contact with tissue juices, clumping of the platelets to a greater or lesser degree almost always occurs. Therefore, if the specimen for examination is obtained by pricking the skin, under which condition contamination with tissue juices always occurs, one cannot expect to obtain correct or consistent counts. Moreover, when the attempt is made to estimate the relative number of platelets and red blood cells in the same microscopic preparation, frequently many platelets are covered by red cells and faulty estimations are obtained.

In 1919 Oluf Thomsen (1) devised a method in which the platelets are counted directly and this method we believe to be more reliable than those previously employed. He made use of a well known fact; namely, that if blood be mixed with a solution of sodium citrate to prevent clotting, and this mixture be allowed to stand until the red and white cells have settled to the bottom, the platelets remain for a long time uniformly suspended in the supernatant fluid. The platelets in this suspension may then be counted directly and, since the dilution employed is known, the number of platelets per c.mm. of undiluted blood may be readily estimated. This method has been employed by Gram (2) by Schenk and Spitz (3) and I (4) have also found it satisfactory in previous studies. Briefly the technique as described by Thomsen is as follows:

Blood is withdrawn from the vein and run into a graduated centrifuge tube containing 0.5 cc. of 10 per cent solution of sodium citrate until the 5 cc. mark is reached. The tube is inverted several times to facilitate thorough mixing. The tube is then allowed to stand until the red and white blood cells have settled to the bottom. The supernatant fluid containing the platelets in suspension is then diluted 1:20 in a leucocyte-counting pipette by means of a solution containing sodium chloride, formaldehyde, and brilliant cresyl blue, and the platelets are counted in a hemocytometer chamber. The number of platelets per c.mm. of

blood can then be readily calculated; the volume of the red cells is taken into account, being determined either directly or by the method of calculation from the hemoglobin content as employed by Gram (2).

Certain modifications in technique were made which render the method more simple without detracting from its accuracy. The following is a description of the technique I have employed.

0.9 cc. of blood is drawn from the vein into a tuberculin syringe containing 0.1 cc. of a 10 per cent solution of sodium citrate. It is important to employ a needle that is not too small; a No. 20 Luer needle is most satisfactory. After removing the needle the blood is drawn into the upper part of the barrel, the finger-tip is placed over the open end and the syringe is inverted several times to insure thorough mixing. Then an inch or more of rubber tubing is slipped over the open end, and the tubing bent over and laid back on the barrel of the syringe to which it is firmly attached by wrapping with a rubber band. The piston is then removed and the syringe placed in a vertical position to permit sedimentation. After this has occurred a dilution of 1:20 of the supernatant fluid is made in a blood-counting pipette with normal saline as a diluting fluid. A drop from the diluting pipette is placed on the hemocytometer slide and the platelets allowed to settle for about 15 minutes. The platelets may then be counted with as little difficulty as are erythrocytes, in the usual blood-counting technique. The platelets seen in 80 small squares are counted. Three ciphers are added to the number obtained and this gives the number of platelets per c.mm. of the citrated plasma. The syringe containing the citrated blood is then centrifuged for 20 minutes at 2,000 revolutions per minute. By means of the following proportion the number of platelets per c.mm. of citrated blood is calculated.

No. of platelets per c.mm. of citrated plasma	: x : :	Total amount of the mixture	:	Amount of citrated plasma

(x equals the number of platelets per c.mm. of citrated blood.) Then, if 10 per cent of this number is added on account of the original dilution with citrate, the result gives the approximate number of platelets per c.mm. of whole blood.

By the above method, the average number of platelets per c.mm. of normal human blood has been found to be 350,000, varying slightly from time to time in the same individual. In counting the blood platelets in rabbits it was found impossible to enter the ear vein with a needle which was large enough to obviate clumping of the platelets. Therefore the skin over the vein was smeared with vaseline, and the vein was cut across. As the blood flowed out rapidly, it was allowed to drop into the syringe, the mouth of which was also coated with vaseline. In spite of the precautions observed, clumping of the platelets was occasionally encountered, necessitating a repetition of the procedure. The number of platelets in normal rabbit blood was found to be considerably higher than that in normal human blood, from 500,000 to 600,000.

Platelets in Pneumococcus Infections.

There will be reported the fluctuations in the number of platelets in thirteen cases of lobar pneumonia, in three cases of bronchopneumonia, and in two rabbits in which pneumococcus septicemia was produced. All counts were made by Thomsen's method, with the modifications previously mentioned. As a rule, counts were made daily, or every other day during the 1st week; thereafter, counts were made at longer intervals. The charts are arranged so that the heavy line represents an arbitrary normal level of rectal temperature, normal number of leucocytes per c.mm., and normal number of platelets per c.mm. in the blood.

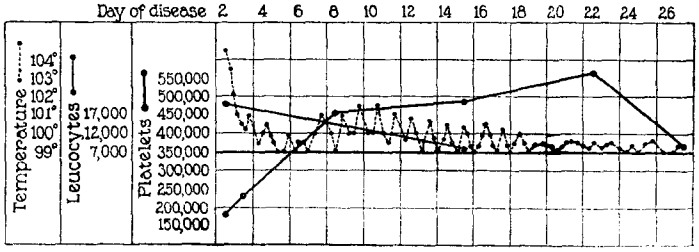
In all of the uncomplicated cases of lobar pneumonia with recovery, the curves made by charting the various counts are of similar contour. In each instance a thrombopenia of from 125,000 to 243,000 platelets per c.mm. occurred during the fever, and a subsequent thrombocytosis was observed after the temperature had reached normal. Usually the number did not begin to increase until several hours after the crisis had begun. Frequently, the increase commenced only after the temperature had been normal for several hours. In the subsequent 6 to 9 days, the number of platelets increased steadily until the normal limit was greatly exceeded. The greatest number observed was 748,000 per c.mm. After the highest point in the curve had been reached, the number again diminished until the normal level was regained in about the same number of days.

It is of interest to note that in Case 1, in which fever persisted after the crisis, the rate of increase in the number of platelets was somewhat retarded, and that in Cases 1 and 2 the usual high number was not attained. Cases 2 and 3 are also of interest since a secondary rise in temperature, due to severe serum disease in Case 3 and to pleurisy in Case 2 was in each instance accompanied by a second diminution and subsequent increase in the number of platelets. Text-fig. 4 shows an example of the usual curve obtained.

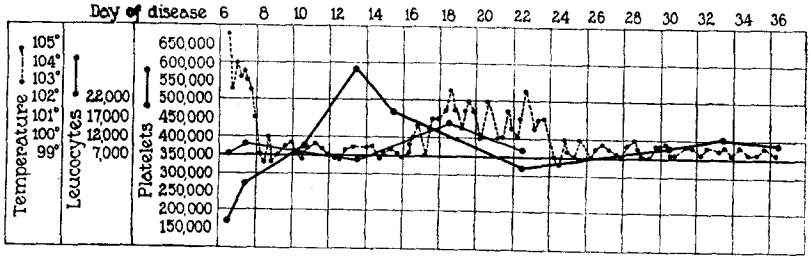
In one case the first count was made 22 hours after the initial chill and the number of platelets was then still within the normal limit. The diminution in the number of platelets appeared later. Judging from this and from observations on rabbits, it seems likely

that the diminution in the number of platelets occurs only after the infection is well established.

Two cases of lobar pneumonia which ended fatally were studied and in these no diminution in the number of platelets was observed. Both of these cases were admitted into the Hospital late in the disease and in both a pneumococcus septicemia was present. In one of these cases admitted on the 5th day of illness, the platelet count showed a tendency to increase, being 354,000, 386,000, and 437,000 on 3 successive days. Unfortunately we have no way of knowing if a thrombopenia preceded this rise or not.



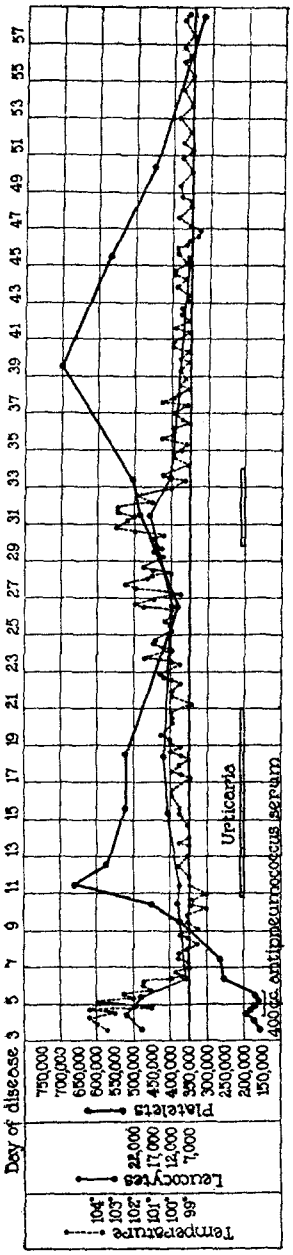
TEXT-FIG. 1. Case 1. Type IV lobar pneumonia.



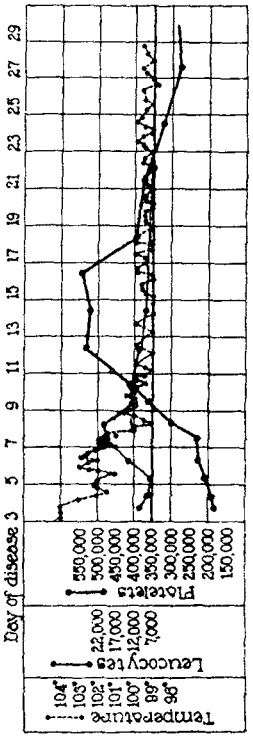
TEXT-FIG. 2. Case 2. Type IV lobar pneumonia; pleurisy.

In addition to the studies made on patients with lobar pneumonia, three patients with bronchopneumonia were studied, two mild cases and one ending in death. In one of the mild cases the number of platelets found was normal; in the other one a fluctuation in the number from 160,000 to 360,000 was observed. In the fatal case counts of 90,000 and 70,000 platelets per c.mm. were obtained.

One experimental rabbit received an overwhelming dose of pneumococci and the changes in the platelets were studied. 24 hours after

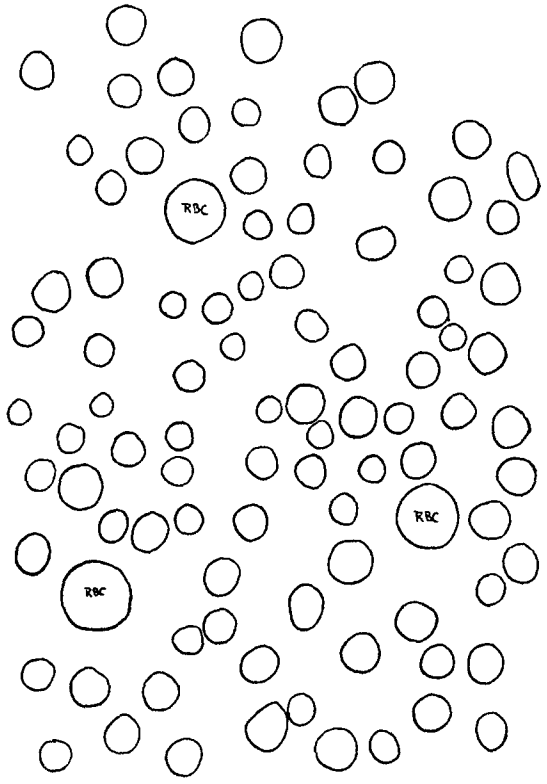


TEXT-FIG. 3. Case 3. Type I lobar pneumonia; serum sickness.



TEXT-FIG. 4. Type II lobar pneumonia.

infection there was found a diminution in the number of platelets. The number then further decreased rapidly until death occurred. In a second animal, which received a much smaller dose, only a very slight diminution in the number of platelets was observed, and a postfebrile rise occurred.



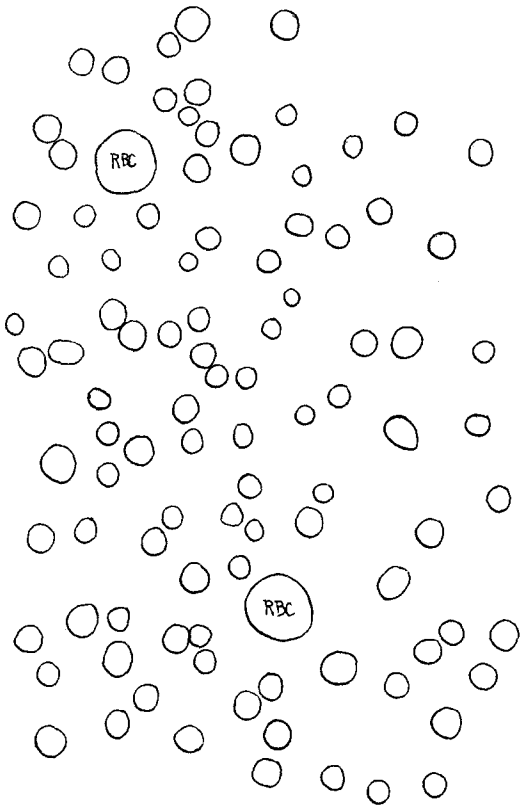
TEXT-FIG. 5. Normal blood platelets. 350,000 per c.mm.

Morphology of the Platelets.

A study was made of the morphology of the platelets at various times during the course of pneumonia and also during the postfebrile period. It is generally conceded that no nucleus is present in platelets and that normal intact platelets are round or oval discoid bodies consisting of a perfectly homogeneous substance, and that the granules

become visible in the platelets only after the death of the cell. According to various authorities normal blood platelets vary from 2 to 7 microns in diameter. Webster (5) states that the size varies inversely with the number.

I have made observations on the variations in size of platelets.



TEXT-FIG. 6. 2nd day of disease. Platelets 123,000 per c.mm.

For this purpose I have taken tracings from stained smears by means of a camera lucida. Smears were made during the course of illness when the count was lowest, during the stage of so called regeneration, when the count was above normal, and again after the number had returned to normal. Tracings were made from each smear and compared. It is realized that with this method the platelets were observed when spread out on a slide; that is to say, not in their normal condition. However, no other satisfactory method is available.

The attempt was made to stain all films to approximately the same intensity by allowing 12 drops of Wright's stain to remain on the slide for 30 seconds, followed by 9 drops of distilled water, the mixture being allowed to stand for 2½ minutes and then washed off with distilled water. Even with this constant technique, considerable differences in intensity in depth of staining were encountered in different smears, probably because the films were of different thickness, or owing to the different physical characteristics of the films, the platelets, or their surrounding matrix.

TABLE I.

Differential Count of Platelets.

Case No.	Day of disease.	Large, many granules.	Small, many granules.	Large, few granules.	Small, few granules.	Large platelets.	Small platelets.	Platelets with many granules.	Platelets with few granules.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
2	6	18	58	9	15	27	73	76	24
	13	19	30	37	14	56	44	49	51
4	3	4	45	18	33	22	78	49	51
	9	14	31	26	29	40	60	45	55
7	3	4	51	25	20	29	71	55	45
	5	10	51	9	30	19	81	61	39
	14	2	10	8	80	10	90	12	88
	27	10	33	22	35	32	68	43	57
8	4	26	64	4	6	30	70	90	10
	9	12	28	31	29	43	57	40	60
9	2	8	69	7	16	15	85	77	23
	8	12	46	30	12	42	58	58	42
	27	15	41	18	26	33	67	56	44
Normal individual.		9	20	36	35	45	55	29	71
" "		21	25	34	20	55	45	46	54

The platelets were studied in this way in ten cases and in all cases it was found that the average size of the platelets was distinctly smaller during the thrombopenic period than when the count was increased or normal, as shown by the accompanying figures. In two of the cases the preponderance of the smaller size platelets persisted during the stage of thrombocytosis. In the other cases, during the period

of thrombocytosis, the average size of the platelets was that seen in normal individuals. In no instance, either during the stage of thrombocytosis or during that of thrombopenia, were the platelets very large. The largest one found on measuring 2,500 had a diameter of 6 microns. The studies of normal blood showed that the platelets vary in size between 2 and 5 microns.

An attempt was made to classify the platelets by a differential count in a number of instances. A very arbitrary classification was devised; namely, large with many granules, large with few granules, small with many granules, small with few granules. Roughly, the platelets over 3 microns in diameter were considered "large," and those under 3 microns were considered "small." Obviously, differences in intensity of staining affected the classification considerably. However, even with this crude method it is evident from Table I that a distinct preponderance of the so called well filled forms, or the forms containing many granules, occurred during the febrile period of pneumonia. This observation is in agreement with that of Zeller (6) who found platelets to be fewer and more granular during acute diseases.

DISCUSSION.

Relation of Platelets to Immunity.

It has been suggested upon numerous occasions that the blood platelets play a part in the defense of the body against bacterial invasion.

Govaerts (7) states that the platelets attach themselves to foreign bodies, such as bacteria, in the blood stream, and influence their destruction. Delrez and Govaerts (8) injected staphylococci and paratyphoid bacilli intravenously into rabbits and observed that the platelets soon attach themselves in large numbers to the bacteria and that the bacteria then disappear from view. But if instead of staphylococci and paratyphoid bacilli, pneumococci were injected into rabbits no agglutination with the platelets occurred, the number of colonies in the blood culture was not diminished, and the bacteria did not disappear from the blood. If the rabbit was then injected with antipneumococcus serum, clumping of the pneumococci occurred without association of the platelets and the bacteria disappeared from the blood.

Later, Bull and McKee (9) demonstrated that pneumococci circulating in an actively immunized rabbit, whose platelets have been removed from the circula-

tion by treatment with antiplatelet serum, agglutinate just as quickly and completely as they do in a rabbit which has the normal number of platelets. They conclude that the association of platelets with bacterial clumps is merely incidental.

Cramer and Drew (10) observed that when animals are raised in a dark room a thrombopenia occurs and that such animals are very liable to infections. After exposure to a mercury vapor lamp, there is an increase in the number of platelets and a decrease in the liability to infection. Similarly, Cramer, Drew, and Mottram (11) found that a withdrawal of soluble vitamine A from the diet of a rat also leads to a thrombopenia and a diminished resistance to infection, and that in animals treated in this way infections often develop spontaneously. When soluble vitamine A is again added to the diet, the platelets increase in number and the animals recover from the infections. They conclude from these experiments that the blood platelets represent a defensive mechanism against bacterial invasion. Bedson and Zilva (12) have been unable to confirm these observations. It would seem that the mere absence of light and withdrawal of an important factor of the diet may alone be sufficient to render the animals more liable to infection irrespective of platelet changes, and that this tendency may disappear upon reestablishment of normal conditions.

The observations on patients with pneumonia suggest that the diminution in the number of platelets is a result of the infection or the attendant fever rather than that thrombopenia is a factor in the occurrence of infection. These observations, moreover, do not indicate that the platelets play an important part in recovery from infection.

Relation of Platelets to the Coagulation Time of the Blood during Pneumonia.

In connection with the observations on the behavior of the platelet count, it is interesting to correlate them with the work of Dochez (13) and Minot and Lee (14), who showed that the coagulation time in pneumonia is lengthened, but tends to become shorter about the time of crisis. The blood used in their determinations was obtained by venepuncture. Anders and Meeker (15) state that the coagulation time is somewhat shortened during pneumonia. The contradictory findings are probably explained by the fact that these authors obtained the blood for their observations by pricking the skin. The admixture of "tissue juices" containing cytozyme or thrombogen which is rendered inevitable by this procedure vitiates the accuracy of the determination of the coagulation time.

It is highly probable that the diminution in the number of platelets during pneumonia plays an important rôle in the delayed coagulation time. Bordet (16) and others have shown that the platelets participate actively in the production of the coagulating principle. Minot and Lee (14), Duke (17), Lesourd and Pagniez (18) state that the clot does not retract, or retracts poorly, when the platelets are diminished in number. This phenomenon was observed in the blood during the thrombopenic stage in the cases of pneumonia reported in this investigation.

Behavior of the Platelets after Injection of Foreign Substances.

Numerous observers report that the parenteral injection of foreign substances is followed by a diminution in the number of platelets in the blood. For instance, Achard and Aynaud observed a diminution in number following the injection of albumin, Bedson one after agar serum, Zeller and Schiff and Matyas one after serum and milk, and Pickering and Hewitt a like phenomenon after the injection of nucleic acid. However, in the five cases observed in this study, which received repeated injections of 100 cc. doses of antipneumococcus (horse) serum, no immediate effect on the number of platelets was observed. In several instances counts were made immediately before, immediately after, and again 6 hours after the injection of the serum. Practically no changes in the platelet counts which could be attributed to the injection of the serum occurred. It is possible that the injection of foreign substances into normal individuals produces a fever with the usual concomitant reduction of the number of platelets.

SUMMARY.

1. A study was made of the behavior of the blood platelets during the course of pneumonia in man, as also in several rabbits experimentally infected with pneumococci.

2. The number of platelets begins to diminish after the infection has become established. A thrombopenia occurs during the febrile period. Immediately following the onset of the crisis in lobar pneumonia or after the temperature begins to fall by lysis, the platelets begin gradually to increase in number. In the postfebrile period, the

platelet count increases until the normal number is greatly exceeded, and there is a return to the normal number only after about 2 weeks.

3. Sequelæ, characterized by fever, following pneumonia are accompanied by a renewed diminution in the number of platelets. After subsidence of the temperature their number usually increases until the normal is again exceeded.

4. The platelets in general are smaller and contain more granules during the thrombopenic stage of pneumonia.

5. Intravenous injection of antipneumococcus serum during pneumonia ordinarily produces no variation in the number of platelets.

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