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ON THE SOCALLED "NEW ELEMENT" OF THE BLOOD AND ITS RELATION TO COAGULATION.

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Whenever a figure appears in heavy type, the number refers to the corresponding number in the Bibliography under the name of the author.

In 1878 Hayem called attention to an element of the blood, whose existence and significance seem to have been almost wholly unrecognized. Hayem called these elements *hæmatoblasts*, and endeavored to show that they were early stages in the development of the red corpuscles. He also pointed out that they probably are connected in some way with the coagulation of blood; but as the theory of their hæmatoblastic function did not meet with general acceptance, the whole work seems to have been passed over with less attention than it merited.

Much greater prominence was given to the subject, when, in 1881, Bizzozero claimed independent discovery of the same elements, and emphasized particularly their connection with coagulation. He attacked the theory then in vogue as to the relation of the leucocytes to coagulation, and called forth many severe criticisms, which in turn were answered; thus the question was brought prominently to the attention of histologists. The result was several important investigations; but the irreconcilable results obtained by different observers, rendered it desirable that the whole matter should be made the object of further research. I was therefore led to take up the study of this question, and the results of my work thus far, form the material for this paper.

Before going into a discussion of the subject, which will involve the results of other observers, I will give a brief description of the "new element" as observed by myself, in order that what is to follow may be better understood.

In describing it I shall adopt the French name *plaque*, as it is short, more or less appropriate, and not liable to be confused

with any other name which may be used in connection with this subject.

If a drop of osmic acid be placed on the finger, and the finger pricked with a needle through the drop, the elements of the blood will all be hardened and preserved in their natural appearance immediately upon leaving the vessel.

If now a thin film of the blood mixed with osmic acid be examined under the microscope with a *good lens* magnifying about 600 to 800 diameters, the plaques may be seen floating in the plasma among the red corpuscles and leucocytes.

They are very pale and homogeneous structures, varying greatly in size, but mostly about one-third or one-fourth the diameter of the red corpuscles. When seen in surface view, they may appear either circular or elliptical, and seem at first sight to be flat, but a more careful observation with a fine objective, will reveal the fact that they are biconcave, although not as much so as the red corpuscles. (See Plate, Fig. 1, and photograph.) This is more plainly marked when we examine them seen on edge, in which case they show the characteristic dumb-bell shape presented by biconcave bodies when viewed in this position.

The form of the plaques, when hardened by the above method, never undergoes change. This is not the case, however, in blood drawn and allowed to clot. To study the plaques under these circumstances, the following method may be adopted: The finger is pricked and a good-sized drop of blood squeezed out. This is taken immediately upon a cover-slip, and then as quickly as possible most of it is washed off by a jet of .75 per cent. NaCl solution from a wash-bottle. The cover-slip is now placed on a slide, and transferred to the microscope stage with as little loss of time as possible. The plaques have the property of sticking to the slip, while the other elements are easily washed away by the jet, so that upon examination the whole field will be seen to be filled with plaques, some of them isolated, but most of them grouped in masses consisting of from two or three to a dozen or more. (Plate, Figs. 2 and 3, and photograph.)

They are now not pale and homogeneous, with a symmetrical outline, but appear glistening and granular, and their contour, instead of being regularly oval or circular, has become jagged. These changes are the more marked the longer the time which has

elapsed before the preparation is observed; and they may be seen to take place step by step while a preparation is being watched. The form of the plaques continues to undergo change, until finally, where they are grouped together, only a granular mass is found, in which the individual plaques can no longer be clearly distinguished. (Plate, Figs. 4 and 5.) *Pari passu* with these changes, processes are seen which run out from the granular masses; and when coagulation sets in, these processes are nearly always found to be continuous with threads of fibrin. (Plate, Figs. 5 and 6.)

The threads of fibrin are sometimes deposited as long needle-shaped crystalloids which are often seen lying free in the field, and not in connection with the granular masses; but the greater number are formed most thickly around these masses, from which they often radiate as centres. (Plate, Figs. 5, 6 and 7.)

If too much of the blood has been washed away by the jet of salt solution, no formation of fibrin will take place; if there is any part of the field where the blood still remains thick, the best observations can be made on the edges of this area.

The preceding description, I think, is given in sufficient detail to indicate what I mean by the term *plaques*.

§ 1.

Historical.

Among the earlier observers, Müller, Mandl, Henle, Wharton Jones and others, have described various colorless elements existing in the blood; and it is not unlikely that they saw the plaques, more or less modified; but their descriptions are not sufficiently minute to enable us to make a positive decision.

Gerber[1] has described in the blood of mammals, bodies which he calls free nuclei, having a diameter of $\frac{1}{500}$ line ($= 4.1\ \mu$). Arnold[2] and Andral have also been recognized as possible discoverers of the plaques.

[1] Gerber. Allgemeine Anatomie. Quoted by Zimmerman, 2, 226.

[2] Arnold (Anatomie, Band I, p. 181) has been referred to by Zimmerman (2, 226) and Hayem (8, 581) as having probably seen the plaques. Hayem gives Arnold's measurement as $\frac{1}{1500}$ foot (which I take to be a misprint) and Zimmerman gives it as $\frac{1}{1500}$ zoll ($=$ about $16.7\ \mu$), which is entirely too large for a plaque. Zimmerman thinks that the structures described by Andral (Essai

Simon,[1] in studying blood drawn into a solution of potassium ferrocyanide, found small bodies which he took to be molecules of fibrin, but which were probably plaques, and other elements deformed by the reagents that he used.

Donné[2] (1842) described and figured the plaques so as to leave no doubt as to their identity. He calls them *globulins*, and says they belong properly to the chyle, from which they are supplied to the blood. He describes them as small white particles, or little rounded grains, isolated or irregularly agglomerated, with a diameter of not more than $\frac{1}{300}$ millimeter ($= 3.3\ \mu$), and says they are important as being the "premiers éléments des globules sanguins." He also noticed the tendency of the plaques to adhere to each other.

The *globulins* of Donné are not the same as the globulins of Milne-Edwards, nor of Robin; the former using the word to denote small, fatty particles found in the blood especially after a meal, and the latter using the word with reference to the smallest variety of leucocytes.[3]

To Zimmerman (1846) belongs the credit of first having studied the plaques with the aid of micro-chemical reagents. He (**2**, 227) repeated the experiments of Simon by drawing blood into a solution of potassium ferrocyanide, which prevents clotting, and then examining with the microscope. He found small bodies, occurring "by the billion," which he describes as quite colorless and more or less strongly refractive; they have not a well-defined contour, and when out of focus appear as dark points. They vary in size from $\frac{1}{1000}$ to $\frac{1}{400}$ of a line ($=$ about $2\ \mu$ to $5.2\ \mu$). He does not agree with Simon that they are molecules of fibrin, and calls them "Elementarkörperchen," because they cannot be

d'hématologie pathologique, p. 32), as molecules of fibrin are the same as those mentioned by Arnold, while Hayem (**8**, 582) thinks that Andral's molecules of fibrin are likely plaques, because of their small size. I was not able to refer to the papers of Arnold and Andral, so that I cannot reconcile the statements of Zimmerman and Hayem. Possibly the measurements referred to granular masses, which Arnold may have explained, without the explanation being quoted by Zimmerman or Hayem.

[1] Simon. Anthropochemie.

[2] Donné. Comptes Rendus de l'Académie des Sciences, 1842 (Vol. 14, pp. 366–368), and Cours de Microscopie, etc., 1844, p. 85; and Atlas, Plate 6, Fig. 21.

[3] See Hayem, **8**, 583.

broken down into any other smaller-formed element, and because, as he claims, they have power to grow by intussusception and form new cells. As to their origin, he supposes that they, like the white corpuscles, belong properly to the lymph, from which they are supplied to the blood.

It is more than probable that under the name of elementary corpuscles, Zimmerman has included not only the plaques, but also other bodies, especially certain granules often found in the blood, which are partly normal and partly the result of some methods of preparation. He speaks of elementary corpuscles as occurring in the blood of a frog; and describes in addition the nucleated plaque (found by later observers in the blood of all ovipara) as an intermediate stage in the formation of red corpuscles from the elementary corpuscles. He also claims to have seen the elementary corpuscles in defibrinated blood.

These inaccuracies have led some histologists to question whether Zimmerman really found anything but detritus, resulting from his methods of preparation; but his description of the behavior of the elementary corpuscles with certain reagents—*e. g.*, water, caustic potash, salt solutions, etc.—corresponds so nearly with the reactions of the plaques under similar conditions, that we can hardly doubt that he had plaques under observation.

Beale (1864), (**1**, 42) describes as existing in the blood, "numerous corpuscles differing very much from the ordinary red corpuscles in size, color, and refractive power. They are much smaller than the latter; they exhibit a granular appearance and are colorless. They might be described as small white corpuscles, but many are much smoother than the colorless corpuscles. It is not easy to see these corpuscles unless the blood is examined by powers magnifying upwards of 1000 diameters. Such corpuscles are exceedingly faint, and can only be distinguished if great care be employed. . . . . *The small, faintly granular corpuscles are colored by carmine*,[1] while the ordinary red corpuscles . . . . are not."

At first, from the above description, it seems as if Beale had reference to the plaques; but the fact that the structures he describes stain with carmine excludes this supposition.

Beale (**2**, 48) also describes other structures which occur "*only*

[1] The italics are mine.

in the blood of man and the higher animals," and which are of the same refractive power as the white corpuscles. These particles he thinks may develop into white or red corpuscles. He further mentions (**2**, 60) small bodies, which he believes to be a kind of white corpuscle, which stick to the slide, while the red corpuscles are drawn hither and thither by the current. These descriptions may well apply to the plaques.

The "granular masses" described by Max Schultze (1865), which have been more or less well known to histologists ever since, are undoubtedly plaques, as may be seen from his description. He says (**1**, 36 and 37), "I find more or less plentiful in my blood and the blood of many others, . . . clumps of colorless balls varying in size accordingly as they are made up of few or many individuals. The latter measure .001—.002 mm., and occur also singly in the blood, but more frequently they are found sticking together to form an irregular, finely granular mass. The balls are quite colorless, homogeneous or finely granular and pale, ... and hence, as also on account of their small size, they can only be made out with a good lens.

"They are, however, not always regularly ball-shaped: they are often angular and drawn out, and then they present a sharper contour and a clearly granular appearance. . . . . It looks as though they may have come from the leucocytes, but of this we must remain uncertain as long as we are in the dark as to the fate of the latter."

He also obtained the characteristic reactions of plaques with water, acetic acid and other reagents.

Riess (1872), (**1**, 240) examined blood drawn by venesection, with as little loss of time as possible, and found "Stäbchen und helle Kügelchen von ähnlichem Glanze mit den farblosen Körperchen."

The "Kügelchen" are mostly round, but sometimes angular. They are usually about $\frac{1}{10}$ the diameter of red corpuscles (.7–1.5 μ), but they vary from very minute bodies to half the size of the red corpuscle. This last statement makes it somewhat doubtful whether Riess has made the necessary distinction between the plaques and the smaller varieties of granules found in the blood. He also claims to have found all stages between leucocytes and granular masses of plaques.

In 1873 Ranvier laid a communication (**2** and **31**) before the Société de Biologie, on the "Formation of Fibrin in Blood Removed from the Vessels." He describes the threads of fibrin as radiating from granules, or groups of granules, which he thinks are chemically identical with fibrin. From references made to these granules in later publications we know that he had reference to the plaques, which he has also seen free (**1**, 215).

In connection with Ranvier's communication, Vulpian (**1**, 94) gave the results of some observations which he made on blood. He examined the blood of many persons, and found that there were always present small corpuscles, either singly or in groups, which quickly adhered to the slide, and were not carried about by currents, like the other elements. When coagulation takes place, the threads of fibrin are often seen to radiate from prolongation of these corpuscles, or from the edge of groups into which they have become collected. He identifies these corpuscles with those described by Riess, and takes occasion to combat the statement of Riess that they are the result of pathological conditions, maintaining that he has found them in the blood of healthy persons as well as in the blood of the sick.

Bearing also on the relation of the plaques to the fibrinous network are the observations of Nedsvetzski (**1**, 147–150), made independently of the work of Ranvier and Vulpian, and published about the same time. He describes small, homogeneous bodies about the size of the granules of the leucocytes, which he regards as normal constituents of the blood, and gives them the name of "Blutkörnchen" or "Hæmococci."

After fibrin has been formed, these small bodies are found like knots in the meshes of the network. He also says that these bodies possess the power of movement.

The Proceedings of the Royal Society of London for 1874 contain "An Account of Certain Organisms Occurring in the Liquor Sanguinis," by Osler, in which he describes certain interesting changes which take place in the granular masses when heated in serum to 37°C. In the same communication Osler secures for himself the credit of having first discovered the plaques in the bloodvessels. His observations were made on connective tissue from the back of young rats, in the vessels of which the plaques were seen scattered freely in the blood among

its other elements. The figures which accompany Osler's article are very good.

From this time until the publication of Hayem's work, the only literature bearing on the subject are the papers by Schmidt (**2** and **3**), Semner (**1**) and Boettscher (**1**). Schmidt and Semner examined "the granular masses of Max Schultze," and described them as being derived from a peculiar corpuscle not at all like the plaques.

Boettscher (**1**, 298 and plate), who believes the red corpuscles to be nucleated, has figured structures found by him among the red corpuscles, which he regards as nuclei set free from the latter; but which, judging from his figures, are probably plaques.

To show what a confusion of ideas prevailed in regard to this subject before Hayem's work came out, I cannot do better than follow Hayem in quoting from Robin (*Leçons sur les humeurs normals et pathologiques*, 1874). After reviewing the work of Riess and Vulpian, he says: "Il est de fait qu'avec un peu d'attention on trouve de ces globules sur presque tous les sujets, bien qu'en très petit nombre. On en voit même circuler de loin en loin entre les hématies et les leucocytes, sur les batraciens et les poissons vivants. Ces corpuscules se trouvent soit isolés, soit réunis en petits groupes. Ils sont larges de 0.002 à 0.006, c'est-à-dire que certains atteignent presque le volume des hématies et même des leucocytes. Ils sont incolorés, hyalins, homogènes ou à peine grenus. Parmi ceux-ci il en est qui se déforme lentement comme les leucocytes. Pour ces derniers l'action de l'eau et de l'acid acétique sous un fort grossissement montre aisément qu'il ne s'agit là que des leucocytes encore petits et en voie d'evolution, qu'il s'agit en d'autres termes de ceux des leucocytes que M. Donné (1844) a décrits sous le nom de *globulins*, larges de 0.003."

With Hayem's work (1878) begins a new era in the history of the plaques, for, by the careful and thorough investigation to which he subjected them, he was not only able to remove all doubts as to their existence and identity, but he also traced their close connection with the process of coagulation, and has advanced some very suggestive speculations regarding their affinities to the red corpuscles.

Bizzozero (1881 and 1882) so confirmed and extended the

observations of Hayem by new and ingenious methods, that all the work done later, has been carried on with a more definite understanding of the subject, and the results of the observations may be considered as confirming or conflicting with those of Hayem or Bizzozero.

Before concluding the historical chapter of this article, the claim of Norris to the discovery of the plaques should be considered.

Norris bases his claim on a communication read before the Birmingham Philosophical Society, in 1879, in which he says (**2**, 163): "There exist in the blood of mammalia . . . . colorless, transparent, biconcave discs *of the same size*[1] as the red ones. Between these two kinds of biconcave discs others are demonstrable having every intermediate grade of *color*.[1] . . . They often present themselves as small spheres, at other times as discs, and at others, liquid-like, they take the shape of the interstices in which they lie."

In a later article (**3**, 562), Norris speaks of Hayem's haematoblasts as fragments of one form of his corpuscles.

From a comparison of these descriptions with those given at the beginning of this paper, I think it will appear evident that what Norris saw was not the plaques, but red corpuscles which had lost their haemoglobin.

§ 2.

Histology of the Plaques in the Blood of Mammals, with Methods of Preparation.

The plaques have been found under different circumstances by so many observers (see historical section), that it is now needless to enter into a discussion as to their presence in the blood—at least after it has been removed from the bloodvessels.

The method of preparation may be varied to advantage according to the point which we wish especially to make out. The following may be recommended as giving very satisfactory results:—

A drop of the preservative fluid is placed on the finger, and the finger pricked through the drop, so that the blood may come

[1] The italics are mine.

in contact with the reagent immediately upon leaving the vessel. The drop of blood thus mixed with the reagent is next taken on a cover-slip, and the latter laid on a slide and examined. To prevent evaporation, melted paraffin may be painted around the edges of the slip.

The part of the finger from which the blood is to be drawn should be washed with water, alcohol and ether, in the order named.

Whenever it is necessary to run any fluid under the slip by suction with filter-paper, it can most easily be accomplished by putting the minutest part of a drop of very thick balsam on each of the four corners of the cover-slip. When laid on the slide the balsam should be on the under side of slip. By this arrangement the slip is held to the slide by the balsam, and will not be floated about by an excess of the fluid run under. It also prevents the slip from lying so close to the slide as to interfere with the filter-paper readily absorbing the fluid. Other observers have used paraffin, but I find thick balsam rather superior.

On collecting blood directly from a needle-prick into a suitable liquid, the plaques are preserved in their original form, but their relative number is small, and they do not readily attract attention lying among the more prominent corpuscles. Their presence in the blood may be demonstrated more strikingly by proceeding as follows: A large drop of blood is squeezed from the finger and taken directly on a cover-slip. The surface of the cover-slip to which the blood is adhering, should next be touched three or four times to the surface of some .75 per cent. salt solution in a watch-glass, or washed with a jet of the same solution from a wash-bottle, until nearly all of the blood is apparently washed off. This part of the proceeding should be finished as quickly as possible, and the cover-slip placed in a watch-glass of osmic acid for about twenty to thirty minutes or longer. It should then be washed by leaving it in a watch-glass of water for several minutes, after which it may be mounted in acetate of potash and examined.

The plaques are seen in great numbers all over the field. Sometimes they may be found singly, but more frequently they are seen in groups, the size of which depends upon the length of time between drawing the blood, and getting it into the osmic

acid. (See photograph.) If this be very short, the masses are small, and the individual plaques of which they are composed may be plainly distinguished; but the longer the time, the larger, as a rule, will be the masses, and the less distinctly can the individual plaques be seen.

For hardening and preserving the plaques, I think 1 per cent. osmic acid is the best reagent that can be employed. Instead of osmic acid we may use Hayem's solution, the formula for which is:—

Distilled water,	200
NaCl	1
Na_2SO_4	5
$HgCl_2$	0.5

I prefer, when using Hayem's solution, to dilute it with $\frac{1}{10}$ its volume of 75 per cent. NaCl solution. This avoids the disadvantage of a precipitate if the fluid should evaporate or become more concentrated, and preserves the plaques very well. The plaques show very plainly when examined in Hayem's solution, their appearance being more striking than when seen in osmic acid; but we are more apt to get very slight irregularities of outline, together with minute granules deposited on the face of the plaque, which are less frequently seen in osmic acid preparations. This is probably due to Hayem's fluid acting somewhat less quickly than osmic acid, thus allowing to appear the first changes which the plaques undergo in breaking down. I have also tried Bizzozero's fluid, and find it an admirable medium for studying the changes undergone by the plaques in breaking down, as the changes go on very slowly in this solution. Bizzozero's fluid is .75 per cent. NaCl solution, to which methyl violet is added. He recommends the ratio of methyl violet to salt solution 1 : 5000. I did not determine the ratio of the violet to the salt solution in the fluid which I found to give the best results, but I think it contained relatively more of the staining element than that recommended by Bizzozero.

I have also tried the method of preservation by drying. Preparations were dried both by spontaneous evaporation and carefully over an alcohol flame, but the results of this method were so far inferior to those obtained by hardening in osmic acid or Hayem's fluid, that I did not employ it to any great extent. If the blood is first hardened in osmic acid and then dried, the

results are much more satisfactory, although then the drying process is unnecessary.

As mounting media, I have tried the following:—Canada balsam, Dammar varnish, glycerine, glucose, acetate of potash (saturated solution) and Hayem's fluid.

Balsam and Dammar varnish were generally used with the dry specimens. They may either be mounted directly (if perfectly dry), or passed through turpentine or xylol. The latter method is rather preferable. Balsam and Dammar were also often used with specimens stained in an alcoholic fluid, but in general it is best to avoid the use of alcohol, as it appears to cause a slight shrinkage even after hardening with osmic acid.

Glycerine is not the best medium for mounting unstained plaques, especially when hardened in osmic acid, as they lose somewhat in clearness. For stained specimens it works very well.

Glucose makes a very satisfactory temporary mounting material. It is used in concentrated aqueous solution, and has the advantage of becoming perfectly hard and requiring no cement.[1]

Acetate of potash is probably the best mounting medium of all those employed. In it both plaques and fibrin threads stand out clearly and sharply defined.

In addition to these regular mounting media, the plaques may be preserved for a time in Hayem's fluid. This does very well for some weeks, but in specimens several months old there is often a coarsely granular precipitate, apparently from the plasma, and sometimes crystals are deposited.

The description which I have given of the plaques in the introductory pages of this article, agrees for the most part with the descriptions of previous observers, but there are some points of difference to which it is desirable to call attention.

In the first place, their biconcavity is still a subject of dispute. Bizzozero (**7**, 18 and **3**, 357) asserts most positively that they are flat, but says they may become biconcave when treated with strong salt solution; while Löwit (**3**, 108) says that they are

[1] This was found to hold good for several months, but in most of the specimens examined after the lapse of a very hot summer, the glucose was found to have crystallized out, and the specimen consequently was ruined. This method cannot, therefore, be recommended for permanent preparations.

never biconcave in blood drawn into salt solutions, but that under certain conditions a biconcavity may be seen in them when examined in peptone blood. Hayem (**5**, 695 and 697) and Laker (**1**, 177), on the other hand, uphold the view that they are normally biconcave, while Schimmelbusch (**2**, 217) says they are not biconcave when examined circulating in the capillaries of the mesentery, but become so when the blood is drawn into Hayem's fluid. As I have never examined the circulation in the mesentery, I am not prepared to endorse or dispute this statement; but I can say that in addition to seeing them on edge and making out their characteristic dumb-bell shape, I have succeeded several times in observing them rolling over and over in a very slow current, so that at least in osmic acid and Hayem's solution I was able to convince myself beyond all question of their biconcavity. The biconcavity also appears in the photograph, which was made from a specimen stained with Bismarck brown.

Bizzozero also describes the plaques as granular, and not homogeneous. This is due, I think, to the method which he employed. If he had drawn the blood directly into osmic acid, he would have found the plaques homogeneous.

The sizes, as given by different observers, vary within wide limits, but the mean of each of them generally corresponds pretty well with the mean of the others.

Osler gives their measurement as one-eighth to one-half of that of the red corpuscles, which I think expresses with sufficient accuracy their extreme variations in size. Hayem (**5**, 703) at first describes the plaques as homogeneous and non-nucleated, but later (**17**, 480 and 481) he changes his opinion and states that they contain a nucleus, which may be brought into view by staining with haematoxylin. He gets his best results from drying a film of blood on the slip—a method which I have never considered as free from objection, especially when heat is applied to effect the desiccation.

In a later article (**18**, 372) he again reiterates his statement in regard to the nucleus of the plaques, and this time with more confidence than before. At the same time, he expresses himself in favor of the view that red corpuscles are also nucleated.

Afanasef investigated this question after Hayem, and comes

to the conclusion that what Hayem regards as a nucleus is really only a precipitation of granules in the centre of the plaque. Schimmelbusch (**2**, 225) takes a view very similar to that of Afanasef. I have some very satisfactory specimens of plaques stained with haematoxylin; some are mounted in acetate of potash, others in balsam, but none were preserved by the drying method. A thorough examination of these preparations has failed to reveal a nucleus, but the characteristic appearance of a concave body when seen full on the surface was presented. This was rendered more prominent than in the unstained plaque, and in so small a structure, it is possible that an appearance of this sort may have been taken for a nucleus. Preparations stained with Bismarck brown and magenta show the same thing.

The same question as to a cell-membrane may be raised with regard to the plaques, as in the case of the red corpuscles.

The plaques, under certain circumstances, will shrivel and become crenate, just as the red corpuscles; and it is quite a usual thing to see both plaques and red corpuscles caught at one end and drawn out by a current so as to present a long, pear-shaped or even threadlike appearance. (Plate XIX, Fig. 1, *d*.)

Hayem (**5**, 696 and 717) described the plaques as containing haemoglobin. In this he was supported by Mayet (**1**, 243 and 244), and by the work of Laptschinsky (**1**, 658), who describes corpuscles about one-third the size of the red corpuscles, which are sometimes more strongly colored than usual, and sometimes colorless.

Hlava also (**1**, 403) has seen circulating in the mesentery of rabbits, small, mostly round elements colored by haemoglobin, which are either young red corpuscles (which he doubts) or fragments of old ones. These are not the true plaques, for he has described the latter (**1**, 404), and says they are colorless.

Riess (**1**, 224), Bizzozero (**3**, 352), Laker (**1**, 200), Hlava (**1**, 403), Halla (**1**, 220), and Schimmelbusch (**2**, 217), all take the view that the plaques are without color.

Laker has noticed the "teinte verdâtre" of Hayem, but thinks it only a shimmer from the surface. He has seen the same thing in the leucocytes. Another proof that the color is not proper to the plaques, is the fact that when seen in groups, one lying over the other, the color is no more intense than when

seen singly or isolated. This has also been noticed by Laker (**1**, 179), and by Halla (**1**, 220), as well as by myself.

Another interesting relation between the plaques and red corpuscles has been pointed out by Hayem (**5**, 728), Bizzozero (**3**, 357), and Lavdovsky (**2**, 64). They find that in different animals the size of the plaques always varies in the same ratio as that of the red corpuscles.

To these many interesting points of relationship between the plaques and red corpuscles, I can add that, in trying to get a preparation from which to make a photograph of the plaques, I tried ten different staining fluids on the blood after hardening in osmic acid, and *in every case* the plaques and red corpuscles stained proportionately.

In addition to the points of resemblance already given, Hayem (**10**, 565) also mentions a somewhat complicated process of drying, washing and staining in which the plaques deport themselves exactly like the red corpuscles.

The many prominent marks of relationship between the plaques and red corpuscles, together with the idea that they contained haemoglobin, prompted Hayem to advance his haematoblast theory: that the plaques are early stages in development of the red corpuscles, the gap between the largest plaques and smallest red corpuscles being filled by elements to which Hayem gives the name "globules nains." In support of his haematoblast theory, Hayem (**11**, 120) has found that in pathological conditions of the system where "new blood" is demanded, we always find the plaques in increased numbers— a fact which had previously been stated by Riess (**2**, 696).

The sources of error in all numerical determinations of the plaques are very great, on account of the tendency which these elements have to adhere to each other or to any foreign body with which they come in contact. I was early led to see that for this reason numerical determinations where the blood had to be measured, were practically of little value. The same point has been made by Schimmelbusch (**2**, 226–231), who, in addition to the results of his own observation, has shown that enormous variations exist in the results of the same observer, while different observers have obtained diametrically opposite results, in determining the number of plaques in certain pathological con-

ditions. This point was also noticed by Howlett (**1**, 224). I think the only way to obtain a reliable numerical determination of the plaques would be to prick the skin through a drop of osmic acid, examine a thin layer of this mixture, and count the relative number of plaques and red corpuscles. A separate determination would then have to be made by the usual methods for the red corpuscles, from which the number of plaques could be calculated. Even this method would not be free from error, for Hayem (**26**) has shown that enough plaques adhere to the edges of the wound to make an appreciable difference in the extravasated blood.

Probably the most suggestive observations in support of the haematoblast theory were made by Hayem (**4**, 330), who has found the plaques in the vaso-formative cells of the mesentery of newly-born kittens, where the young red blood corpuscles are in process of development, while both Hayem and Pouchet (**1**, 97) have described intermediate stages between the plaques and the red corpuscles.[1]

We cannot regard the haematoblast theory as proved, but we must acknowledge that the relationships between the plaques and the red corpuscles are most striking; and it seems as though this is the most plausible explanation afforded.

§ 3.

Micro Chemistry.

In studying the micro-chemical properties of the plaques, a combination of methods was used.[2]

With water the plaques swell up into large bladder-like structures, which consist of a very pale, hyaline sphere with granules collected at one point on its surface. The hyaline part is not easily distinguished in water without a very fine lens. By running iodine under the slips, the bladders may be studied to much better advantage. If the blood be so completely washed away that no fibrin will form, the bladders will begin to disappear

[1] The observation of Hlava just mentioned may also be taken in support of Hayem and Pouchet, the colored bodies which he distinguishes from the plaques, being plaques containing hæmoglobin, and on the way to form red corpuscles.

[2] Pp. 301–303.

after a day, although at the end of three days some of them may still be found, especially the smaller ones. (Plate, Figs. 8–12.)

Acetic acid (.1 per cent.) acts like water, only more powerfully.

Concentrated acetic acid readily dissolves the red corpuscles and plaques. The white corpuscles are gradually dissolved, the nuclei resisting the action of the acid the longest.

Concentrated solution of potassium hydrate quickly dissolves everything in the field except the granules of the coarsely granular leucocytes.

Solution of magnesium sulphate (27 per cent.). If this solution be used on the finger (p. 301), the plaques will remain without breaking down for a considerable length of time.

Cold.—One of the best means of studying the breaking down of the plaques is to observe them in .75 per cent. sodium chloride solution or Bizzozero's fluid at a reduced temperature—*e. g.* 6° C. This will retard the clotting so that the different stages may be conveniently observed.

A very low temperature—*e. g.*, —1 to +2.5° C.—will prevent coagulation. If a preparation be kept at this temperature for a few hours, and then the temperature be raised, the plaques will break down, but no fibrin will be formed. This is interesting, for, as Hayem (**5**, 708) has pointed out, there must be some deep-seated chemical change produced by the cold, which prevents the plaques from yielding that which appears to make them essential to the process of coagulation.

Concentrated solution of mercuric chloride preserves the plaques, but causes a precipitate in the blood which renders its use objectionable.

Of the different staining fluids, methyl violet, gentian violet, and strong fuchsin, stain the plaques readily and deeply. They should be used in very dilute solutions, especially the methyl violet.

Iodine, when run under the slip, appears to stain the plaques, and makes them stand out very satisfactorily. It is particularly adapted for studying the bladder stage. The coloration from iodine is not, however, a true stain, for the last traces of it may be washed away by water even after the iodine has been allowed to act for thirty-six hours. Bismarck brown, magenta, Kleinen-

berg's haematoxylin and aqueous haematoxylin, give very good, and for permanence the best, staining of the plaques, but a longer time is required than for methyl violet, gentian violet or fuchsin. Eosin will also stain them, but to get satisfactory results it is necessary to use a tolerably strong solution and allow it to stand from twelve to fourteen hours. Aniline blue-black, borax carmine, Frey's carmine and picro-carmine, do not stain the plaques even in forty-eight hours.

I also used a double staining fluid of carmine and indigo-carmine, recommended by Shakespeare and Norris,[1] which is claimed to stain green any cell which contains haemoglobin. Hoping to find another method of determining whether the plaques contained haemoglobin, I tried it several times, but without success.

§ 4.

Origin of the Plaques.

The question now arises: Are the plaques normal and independent elements of the blood, or have they been derived from the other elements of the blood, either normally or by the methods of preparation?

This is a subject on which the greatest diversity of opinion exists, all of the following views having been held, and experimental evidence adduced to support them:

1. They are haematoblasts, or young red corpuscles.
2. They are derived from the red corpuscles.
3. They are derived from the white corpuscles.
4. They are nuclei floating free in the blood.
5. They are fibrin.
6. They are globulin depositions produced by cooling of the blood.
7. They are independent elements.

While the red and white corpuscles were the only recognized histological constituents of the blood, it was most natural for observers to refer the plaques and granular masses to one of these elements, without considering the possibility of the plaques being independent elements themselves.

[1] Amer. Jour. Med. Science, Jan., 1877.

The theory that they are derived from the red corpuscles has received but little support. Boettscher (**1**), who believes the red corpuscles to be nucleated, thinks that the plaques are free nuclei from them. Ehrlich (**1**, 405) thinks that in anaemia the red corpuscles break down and give rise to the structures pictured by Riess.

Laker (**1**, 192) has made this particular point the object of special study, and he comes to the conclusion that the plaques are not derived from the breaking down of the red corpuscles, for he has produced this artificially in various ways; but of all the fragments which he examined, he did not find one that he would have mistaken for a plaque. The same subject has been investigated by Schimmelbusch (**2**, 216), with similar results.

The view that the plaques are derived from the leucocytes has met with much more general acceptance, and has been defended by observers of high authority.

Riess says (**1**, 224) that they are destruction-products of the white corpuscles, produced by an insufficient nutrition of the blood. In another article (**2**, 696) he says that in pathological conditions, when the number of leucocytes is increased, we find, as a rule, that the number of plaques is increased also.

Even more to the point than the observations of Riess are those of Halla (**2**, 376–378), who has shown that while the number of plaques and leucocytes respectively is not necessarily increased or diminished at the same time, yet in general the cases where the plaques are found in the greatest abundance are those in which the leucocytes are also most numerous, or closely following a condition where they had existed in great numbers.

As a matter of fact, the very cases in which we find this increase of leucocytes and plaques are generally those in which the number of red corpuscles is diminished. This fact was noticed by Riess (**2**, 696), and especially by Hayem (**11**, 120), who says that his view is supported by the results of over fifty observations. Thus we see that the points made by Riess and Halla, will serve as well to support Hayem's haematoblast theory, as the theory of the derivation of plaques from the leucocytes.

Pouchet (**2**, 136), from similarity of staining, thinks the plaques are derived from the protoplasm of the leucocytes; and Howlett, whose work was done after Hayem and Pouchet, but

about the same time as, and independently of, Bizzozero, is also of the opinion (**1**, 224) that the plaques are derived from the leucocytes, his reasons for this belief being: that they are absent from scrapings of lymphatic glands, spleen, and the medulla of bones in the dog; and that they resemble processes of leucocytes.

From the latter statement we would infer that Howlett regards the plaques as derived from the body, rather than the nuclei of the leucocyte, although he mentions that they stain like nuclei with the aniline colors.

Hayem (**4**, 49) at first appeared to advocate the view that the plaques were independent elements (éleménts particuliers, et à tous les dégrés de leur évolution, parfaitement distincts des leucocytes). In a later article (**9**, 198) he says: "The haematoblasts arise in the lymph; they are found in the protoplasm of the white corpuscles, from which they are set free before entering the blood, except in certain pathological conditions." He describes leucocytes found in the lymphatic glands, in which ready-formed plaques may be recognized. These elements possess a nucleus more or less hidden by the plaques. He does not mention the multinuclear leucocytes to which Hlava attaches so much importance, and it is possible that he had these under observation, regarding their nuclei as plaques. Löwit (**3**, 127) also has seen structures in the leucocytes, in warmed peptone blood, which he took to be plaques.

He (**3**, 125) believes that he has also seen hyaline drops exude from leucocytes, swim off in the plasma, and become plaques. He gives a figure illustrating this process, which I reproduce (Plate, Fig. 13, *c*), and from which I infer that what he has really seen is a plaque which is adhering to the edge of a leucocyte, and which has become a small bladder.[1] He has taken the hyaline part of the bladder for a plaque, while the granular part is lost against the leucocyte. Sometimes small bladders are formed, the hyaline part of which is little, if any, larger than a plaque (Plate, Figs. 11 and 12). He admits also, that this process takes place very quickly in blood drawn into salt solutions, and cannot always be seen, while it very rarely can be traced; which is well in accordance with such an inference.

Halla thinks the plaques are derived from leucocytes which

[1] See page 308.

break down in the circulation, because they are found to be most numerous at the same time, or immediately after the leucocytes are found in greatest numbers. He thinks they are nuclei of leucocytes because they resemble these structures more than the rest of the cell.

The most ardent supporter of the theory that the plaques are nuclei of leucocytes is Hlava. Hlava (**1**), after a severe criticism of Bizzozero's work, gives the results of some experiments of his own on plaques and the origin of fibrin, in which he is led to the conclusion that the plaques are nuclei of the leucocytes, which are set free when the latter break up, whether in the circulation or in extravasated blood. He adduces the following facts in support of his theory:

First (**1**, 403): He admits that he was unable to find any white corpuscle breaking down while in the circulation, but he has seen white corpuscles filled with plate-like structures. "Such white corpuscles have the greatest similarity to conglomerations of the true plaques, and stand perhaps in close relation to them."

Second: He divides the leucocytes into several varieties, and says that one definite variety of these—the multinuclear (see Plate, Fig. 14, *a*, *b*, *c*, *g*)—always breaks down much more readily than the others when the blood is shed. The total number of leucocytes is much smaller before coagulation than after, and the few which remain are nearly all of the uninuclear variety.

Third: He has observed (**1**, 404) that we find more plaques when we examine blood in salt solutions than when it is drawn into osmic acid. He gives as the reason for this that the leucocytes are set by the osmic acid, and therefore cannot break down, while a destruction of the multinuclear leucocytes takes place in salt solutions, giving rise to what have been taken for groups of plaques.

Fourth: He has also found (**1**, 408) that as the multinuclear leucocytes break down, their nuclei stain less deeply with gentian violet. The same may be noticed with regard to the breaking down of the plaques; moreover, the plaques and nuclei have about the same size and shape.

Fifth (**1**, 394): He quotes Rauschenbach that fibrin formed from a necrosis of the leucocytes has the same micro-chemical properties as the plaques; and from this he draws the conclusion

that the micro-chemical properties of the plaques and dead leucocytes are the same.

Sixth (**1**, 409): He explains the presence of plaques in the blood while yet in the vessels by supposing the breaking down of the leucocytes to be a perfectly normal occurrence in the circulation. To support this view he refers to the work of Schmidt, who, with Jackowicki, Birk and others, has found that free ferment exists in the blood, and is increased in amount during fever.

In regard to the first point that Hlava makes, I can say that I have seen the corpuscle which he describes (Plate, Fig. 14, *a*, *b*, *c*, *g*), but have always regarded it simply as a multinuclear leucocyte. It is certain that dilute acetic acid brings the small bodies in them into prominence, just as with a nucleus, while there is no such reaction as that shown when the plaques are treated with this reagent. Furthermore, I have not observed any biconcavity in the small bodies within the leucocytes, such as is seen in the plaques.

As to the second observation of Hlava, I must say that my observations, in several instances where my attention was directed particularly to this point, do not go to confirm it.

In the third point Hlava is correct in stating that we find fewer plaques in osmic acid than in dilute salt solutions, but I think the reason of this is, that in salt solutions the plaques adhere to the slide or cover-slip, while the other elements are drawn hither and thither, or even washed away entirely. Thus the plaques from the whole drop of blood will collect together on the slide or cover-slip, and appear very much more numerous in proportion to the other elements than they really are. This very fact is taken advantage of to get a great many plaques under observation in the same field.

To the fourth point, I can only say, that in all the preparations of clot which I have examined I have not seen half-a-dozen leucocytes broken down, but I have seen groups of plaques which presented exactly the appearance which we would expect to find in a leucocyte breaking down; and I am only able to feel

[1] See p. 302. When the blood is mixed in proper proportions with strong salt solution, which prevents them from adhering, their number is found to be about the same as in osmic acid.

confident that it was not a leucocyte from the fact that many of the latter could be distinguished, of all varieties, which showed no signs whatever of disintegration; and, more important still, I have followed the process of breaking down in masses the individual plaques of which *I had seen come together*, and these, when well broken down, would sometimes present the appearance so easily mistaken for a leucocyte.

Hlava's fifth argument will not hold good, because by necrosis the micro-chemical properties of the leucocytes may be changed entirely. Hlava himself regards coagulation as a necrosis of the white corpuscles; but the normal plaques and leucocytes do not have the same micro-chemical properties, as I shall show later.

Hlava's sixth point also cannot be said to be free from objection. In the first place, the plaques are biconcave, while the nuclei of the leucocytes have never been made out to be so. They also differ in micro-chemical properties, the nuclei resisting the action of concentrated acetic acid longer, and also staining more readily than the plaques with certain reagents. They are not found in the blood of ovipara, nor in the blood of invertebrates, although multinuclear leucocytes are found in the blood of these animals. The fact that ferment may exist in the blood, and even be increased in fever, does not prove that the leucocytes break down, for the plaques were present also, and it may have been derived from them.

Of the older writers, Simon and Andral have mentioned structures which were possibly plaques, in connection with the formation of fibrin. (See historical section.)

The chief supporter of the view that the plaques are depositions of fibrin is Ranvier (**1**, 215 and 217), who comes to this conclusion from a few micro-chemical resemblances which exist between them, one of which is that they are both stained with iodine. Lavdovsky (**2**, 65) and Halla (**1**, 222) think that the plaques are morphologically allied to fibrin, from the intimate connection which exists between the granular masses and the fibrinous network. Ranvier also makes this a strong point.

Now, I have shown (p. 309) that iodine does not give a true stain to the plaques, and the same may be said with regard to fibrin; but in preparations stained with Bismarck brown, or methyl violet especially, we have the remnants of the plaques deeply

stained, while the fibrinous threads radiating out from them are nearly colorless, and can be seen to run directly up to the mass, from which they can be more or less sharply distinguished. The same thing may be more clearly shown if haematoxylin be used instead of Bismarck brown or methyl violet. To do this, let the plaques and fibrin stain thoroughly, then remove the excess of haematoxylin by acid. The last traces of color will be removed from the fibrin while the plaques are still deeply stained.

These methods of staining do not show, beyond doubt, micro-chemical differences between the plaques and fibrin, for in the case of haematoxylin it is only natural that the stain should be removed from such delicate structures as the fibrin-threads before it is from the granular masses. In the case of methyl violet or Bismarck brown, the difference in staining between the granular masses and the fibrin-threads seems to be too great to be accounted for by their difference in size, yet I have never been able to wash the stain entirely away from the fibrin. The action of distilled water, however, shows a true micro-chemical difference between the plaques and fibrin. The plaques take on the bladder structure described, and gradually dissolve, while the fibrin remains unchanged.

The theory that the plaques are precipitated globulins is held only by Löwit.[1] This observer believes (**3**, pp. 111, 114–117, 124–125) that the plaques are globulin depositions which at the body-temperature are normally in solution in the plasma, or contained in the leucocytes, but which are precipitated from the one by cooling, or extruded from the other when the blood is drawn. He claims to have produced plaques by precipitating separately both fibrinogen and paraglobulin (**3**, 124 and 125).

Löwit believes that the plaques do not exist in circulating blood, for the following reasons:

First (**3**, 85–93): Their number is found to vary when drawn into salt solutions of different strengths.

[1] Unfortunately, the numbers of the Sitzungsberichte of the Vienna Academy which contain Löwit's two first articles (**1** and **3**) were not in the University library at the time this article was written, and I was only able to find them after Schimmelbusch's later article (**2**) came to hand. The work of Löwit is rather out of the usual line, and I hope, in a future publication, to enter more closely upon his investigations and results than I shall be able to do here.

Second (**3**, 89): If blood be drawn into 25 per cent. salt solution, no plaques will be found.

Third (**3**, 107): The plaques in the blood of peptonized dogs are not so numerous when drawn as after standing for some time.

Fourth (**3**, 111): The plaques cannot exist in circulating blood because they dissolve in the plasma[1] at the body-temperature.

Fifth (**3**, 113): Plaques do not exist in the blood of cold-blooded animals. The element described by Hayem, Bizzozero and others, is the representative of a stage in the development of the white corpuscles.

With respect to the first reason, we should expect the number to vary in dilute salt solutions, on account of the adhesion of the plaques to the cover-slip.[2] Additional evidence in favor of the assumption that the plaques are present in the circulation, is derived from the fact that if a large drop of strong salt solution: 28 per cent. $MgSO_4$, 25 per cent. NaCl, etc., be put on the finger, and the finger pricked through the drop so as to get a small amount of blood in comparison to the drop of salt solution, the plaques are found isolated and floating freely, like the corpuscles, without showing any tendency to adhere. In this case the number of the plaques is about the same as when drawn into osmic acid.

In repeating the experiment on which Löwit bases his second reason, I can only say that although in some preparations the number of plaques was very small, I have never found a preparation in which they were entirely absent.

I have never worked with peptonized dog's blood, and therefore have no suggestions to offer, at present, in regard to the third point.

The fourth reason will be referred to with the work of Schimmelbusch.

The fifth point seems rather to be against the assumption that the plaques are globulin precipitations produced by cooling, for the blood of cold-blooded animals undoubtedly contains both paraglobulin and fibrinogen, as well as leucocytes.

Löwit grants that Bizzozero and others may have found

[1] His work was done with peptonized blood. [2] See p. 302.

plaques in the circulating blood, but says they were produced by cooling down the mesentery. He says if the mesentery had been surrounded by .75 per cent. NaCl solution at 37°–40° C., the plaques would not have been found.

Schimmelbusch combats the views of Löwit, supporting his position mainly on two observations:

1. In repeating Löwit's experiment with normal plasma, he was unable to confirm Löwit's observations with the peptonized plasma. His method of procedure was as follows (**2**, 212):

A piece of mesentery was cut out with a red-hot scalpel, so as to prevent hemorrhage, and the plaques in the capillaries were found to be quite homogeneous. The piece of mesentery was then heated up to 40° C., but the plaques refused to dissolve.

2. He has followed Bizzozero in examining the circulation in the mesentery of the rabbit, and has found the plaques circulating in the blood through the mesenteric vessels. In this experiment (**2**, 213–217) he has taken the precaution to meet Löwit's objection by keeping the mesentery of the animal immersed in a bath of .75 per cent. salt solution at the body-temperature. The mesentery is not drawn out until the animal is in position in the bath; so that at no time is there an opportunity for a precipitate to occur on account of cooling.

The last view to be considered is that the plaques exist in the circulating blood as normal, independent elements.

That they are not due to changes produced in other elements after the blood is drawn, is shown by pricking the finger through a drop of osmic acid, by which process all the elements of the blood are set immediately upon leaving the vessel. It is also pretty conclusive proof, that four competent observers—Bizzozero (**7**, 17), Lavdovsky (**2**, 64), Hlava (**1**, 403) and Schimmelbusch (**1**, 100)—have seen them circulating through the vessels in the mesentery, and Osler (**1**, **2** and **3**) has seen them in the uninjured vessels of the connective tissue of young rats. Pouchet (**2**, 136), prior to Bizzozero, made observations which should be mentioned here as proof of the existence of free plaques in the circulation, although he regards them as derived from the leucocytes, and as being haematoblasts. His observations were made on the mesentery of rabbits. By compressing a small vessel he was able to cause stasis; by then observing the different

leucocytes he found them with plaques adhering to their surface, as well as plaques free in the plasma. He concluded that the plaques had exuded from the leucocytes; but I think, from the light of more recent investigations, we may safely explain this as we did the observations of Löwit—viz.: that the plaques are normally free in the plasma, and that they are found adhering to the leucocytes by reason of their viscosity.

There is no doubt as to the existence of the plaques in the blood, and though recognizing their close resemblances to other structures also found there, we have not as yet sufficient evidence to believe them to be other than *an independent morphological element.*

This view is held by Max Schultze (**1**, 36), Osler (**1**, 144 and **2**, 530), Bizzozero (**7**, 17 and **3**, 348), Laker (**1**, 193), Lavdovsky (**2**, 64), Halla (**2**, 378) and Schimmelbusch (**1**, 100) and (**2**, 217).

§ 5.

Histology of Coagulation with Especial Reference to the Part Played by the Plaques.

In this section it is not my intention to dwell upon the different chemical theories of the coagulation of the blood; but I shall take up the histology of the formation of fibrin, and its relation to the histological elements of the blood, the plaques in particular.

The granular masses[1] already described as a stage of the breaking down of the plaques, have been found by many observers, and various opinions are held as to their origin.[2] Schmidt (**5**, 356; 562–567) says they come from peculiar forms intermediate between the leucocytes and the red corpuscles. He calls this intermediate form "Rothe Körnerkugeln." This opinion is also supported by Semner (**1**), whose work was done in Schmidt's laboratory, and published the following year. About the same time that Semner's work came out, Schmidt (**3**, 528 and 529) described the white corpuscles as breaking down, and giving the appearance of a mass of granules, which float off singly or in small groups in the surrounding plasma. This description differs from that given by Schmidt (**3**, 562) for the

[1] See page 295.

[2] The views of the older writers may be found in the historical section.

breaking down of the "Rothe Körnerkugeln," so that he evidently makes a distinction; but he thinks that both have to do with coagulation.

The view of Schmidt and Semner is adopted by Slevogt (**1**) and Feiertag (**1**), pupils of Schmidt.

Heyl (**1**, 35–38) has observed that in Bizzozero's fluid the number of granular masses increases as the number of leucocytes decreases; also that both granular masses and leucocytes stain with methyl violet. From this evidence, together with the assumption that the leucocytes break down during coagulation, he draws the conclusion that the granular masses are derived from leucocytes.

Riess (**1**, 245) and Hlava (**1**, 404–409) both maintain that the granular masses are chiefly formed from the leucocytes, and claim to have found intermediate stages.

Osler (**2**, 531) and Laker (**1**, 148) have each given special attention to this point, and both deny most emphatically that the granular masses are derived from leucocytes.

Löwit (**1**, 281) has studied the coagulation of lymph under the microscope, and has found nothing that could be taken for leucocyte detritus.

The most generally accepted view in regard to the granular masses is that they are formed by the plaques. This view is held by Max Schultze (**1**, 36 and 37), Osler (**2**, 530), Hayem (**5**, 675 and 704 and **16**, 215), Leube (**1**, 654), Bizzozero (**3**, 352 and **7**, 18), Davidson (**1**, 1028), Laker (**1**, 183), Halla (**1**, 217), Löwit (**1**, 297), and Schimmelbusch (**1**, 101 and **2**, 220).

Hlava believes that both plaques and leucocytes form granular masses. He thinks that the plaques break down only because they are parts of white corpuscles, and has failed to observe that the plaques break down sooner than the other white elements. Löwit thinks that the granular mass proper is made up of plaques, but that leucocytes are often enclosed.

I have succeeded, in the course of my investigations, in getting preparations in which the individual plaques were seen to unite and form the granular masses. By keeping these masses under observation, I was able, as they broke down, to follow the process continuously until after fibrin was formed.

The results of my observations are given in the introductory section.

In addition to this, experiments were made, in which specimens of blood were taken at different intervals after extravasation and hardened in osmic acid, so as to get the different stages in the formation of fibrin.

The results of my work all go to show that the breaking down of the plaques is intimately connected with the formation of fibrin.

The granular masses formed by the plaques become centres from which the threads of fibrin radiate. The threads are also deposited freely in the field, and often as long, needle shaped bodies (Figs. 5, 6 and 7), but there is generally a thicker deposit of fibrin in the immediate vicinity of the granular masses, especially the large ones, than is noticeable elsewhere.

The plaques, either before or after breaking down, are not morphologically identical with fibrin, so that they do not contribute as such to the formation of the fibrinous network; the remnants which are seen enclosed by the threads of fibrin are held there mechanically, and are not an essential part of the reticulum.

That fibrin has a tendency to radiate from the granular masses as centres, has been observed by Riess (**1**, 744), Ranvier (**2**, 93), Hayem (**5**, 704 and 705), Leube (**1**, 654), Bizzozero (**7**, 19 and 20; **12**, 531), Davidson (**1**, 1028), Lavdovsky (**2**, 65) and Halla (**2**, 222).

Ranvier, Bizzozero, Davidson and Halla think there is a direct connection between the fibrin-threads and the granular masses, while Max Schultze (**1**, 38) is of the opinion that the threads pass over or through the masses, but do not proceed from them.[1] Halla (**1**, 218) has noticed that the fibrin-threads are deposited most thickly in the neighborhood of large masses of plaques. Fano (**2**, 394; **4**, 211) says that sometimes the plaques form the centres of radiation for the fibrin, but more frequently the leucocytes are such centres.

In many observations that I have made, I have found that when the plasma is very much diluted, but little fibrin is formed. Under these circumstances the field is comparatively clear, and it can then be seen that no fibrin proceeds from the leucocytes, but all comes from the plaques, or is deposited freely in the field (Plate, Fig. 6). When the clot is thicker, the fibrin is seen to

[1] From his figures one would judge the contrary.

radiate in many instances from large masses, of which it is impossible to decide positively, from their appearance, whether they are plaques or leucocytes (Plate, Figs. 5 and 7).

Sometimes a leucocyte will be covered more or less with plaques which can easily be distinguished at first, but which later would make it appear as if the leucocyte had broken down (Plate, Fig. 3). In some specimens of clot, I have seen leucocytes with only a few adherent plaques (Plate, Fig. 12). In such cases the fibrin has been seen to radiate typically from the plaques at the edge, while it bore no relation to the free margin of the leucocyte. In specimens hardened in osmic acid and stained with carmine this point can be made out very clearly. The leucocyte stains with the carmine, while the plaques and fibrin do not.

Hayem (**5**, 720) and Schimmelbusch (**2**, 242) have each pointed out that fibrin may be formed elsewhere than around the plaques. Schimmelbusch (**1**, 102; **2**, 236 and 242) believes that the fibrin does not show any preference for one or another of the histological elements of the blood. Bizzozero (**4**, 109) is of directly the opposite opinion, and states that fibrin is deposited around the plaques, and nowhere else.

When the formation of fibrin is abundant, there is a thick network of threads all over the field, and every spot is seen to be full of fibrin, whether it contains histological elements or not (Plate, Fig. 7); but when the formation of fibrin is scant, it will nearly always be noticed that the fibrin is deposited most thickly in the neighborhood of the masses of plaques (Plate I, Fig. 6). Even in preparations where the fibrin is sparingly formed, the threads are deposited elsewhere than around the granular masses, and occasionally, though rarely, I have found granular masses around which the fibrin did not appear to lie any more thickly than in the clear field. The fact that nearly always the fibrin is deposited most thickly around the granular masses, even radiating from them as centres, while interesting and significant, is not conclusive proof that the plaques are connected with coagulation; for the same adhesive property of the plaques which makes them adhere to each other, may also cause the threads of fibrin to stick fast as they separate out from the medium around them. This seems all the more probable when we consider that the fibrin as well as the plaques is sticky and adheres to the glass.

Preparations in which the clot is scanty are generally obtained from blood when diluted with a reagent which retards coagulation; and the fact that in these preparations the fibrin is deposited most thickly in the vicinity of the masses of plaques may be due to the plaques giving up something which produces or hastens the coagulation, and that in dilute solutions this substance is more plentiful in the neighborhood of the granular masses than elsewhere. More will be said of this later.

In a recent publication Laker has taken the view that the fibrin-threads are folds of a membrane which he calls the primary fibrin membrane. Laker (**2**, 156) finds that if a very thin layer of blood be gotten under the cover-slip, at the end of fifteen minutes no network will be visible, but the fibrin will be formed as a transparent membrane without differentiation. If now the cover-slip be pressed or moved, folds may be produced in the membrane which he believes to be identical with fibrin: first (**2**, 155), because their histological properties are the same, and secondly (**2**, 156), because with defibrinated blood he failed to get the membrane. There is no relation between these folds of the membrane and the cellular elements, the latter being found just as they were caught and held by the viscous substance of which the membrane is formed.

Reference has been made to such a formation of fibrin by Nasse,[1] Anderson[2] and Virchow.[3] Rauschenbach (**1**, 8 and 9) and Halla (**1**, 232) describe a membranous form of fibrin from the coagulation of fibrinogen and paraglobulin with ferment. This membrane is transparent and homogeneous, and can be plainly seen only by sundry folds in it.

The view of Laker is almost identical with that of Virchow,[3] who, as early as 1856, says: "The fibrin-coagulum is at first a homogeneous, structureless mass in which the appearance of fibres is given only by foldings on its surface or by splitting and rolling up from the edges. . . . . After the coagulation is complete we have a homogeneous jelly in which lighter cells are suspended. . . . . Any shaking or jarring, or movement of one

[1] Nasse, Das Blut, p. 40. Also Müller's Archiv 1841.—Quoted from Holzmann, Archiv für Anatomie und Physiologie, Phys. Abth., 1885, p. 220.

[2] Froriep's Notiz, 1844, p. 676.—Quoted from Holzmann, op. cit.

[3] Virchow, Gesammelte Abhandlung, 1856.—Quoted from Holzmann, op. cit.

part of the coagulum upon another, produces folds, which in a thick layer are confined to the surface, but in a membrane involve the whole thickness. The folds sometimes form asters and sometimes a reticulum; at other times they are parallel, but they always appear as fine, smooth lines. . . . . The cellular elements are [simply mechanically] retained by the clot."

Rindfleisch and Hermann have also mentioned a homogeneous stage in the coagulation of the blood which precedes the formation of fibrin-threads. Rindfleisch (**1**, 159) describes numerous splits and cavities which are seen as the threads of fibrin are formed by consolidation (festwerden) from the homogeneous jelly, while Hermann (**1**, Vol. 1, p. 253) thinks the threads are formed by a process closely resembling crystallization.

Schimmelbusch (**1**, 102; **2**, 236) insists very emphatically that the formation of threads of fibrin is a true crystallization process, and does not believe there is a previous stage, either homogeneous or granular. Ranvier (**1**, 217) regards the plaques as fibrinous, and says that when the blood begins to clot, processes run out from them which form the groundwork (premières travées) of the reticulum; this then develops by depositions of fibrin, just as a crystal of copper sulphate will grow in a solution of that salt.

Hayem (**5**, 719) does not believe in the fibrinous character of the plaques, but nevertheless compares the formation of fibrin to a "sort of crystallization starting from small crystals already formed."

I have often noticed the membrane which Laker has observed, and I have also noticed that folds of this membrane, when fine, cannot be distinguished from threads of fibrin; but I do not think that all fibrin-threads can be regarded as folds of such a membrane. The fibrin-threads appear to be separated out from a homogeneous mass in which they lie, but when two threads are seen to cross, they can often be seen to be distinctly marked off from each other, the one lying beneath the other, and not connected as they would be if both were folds of the same membrane. Furthermore, formations of fibrin have often been obtained in which no traces of a membrane are to be found, if such ever existed. This is generally the case in specimens of blood considerably diluted.

I think it most probable that what Laker describes as a mem-

brane is a layer of the homogeneous substance described by Virchow, Rindfleisch and Hermann, which is essentially of the same composition as fibrin, and from which the fibrin-threads are formed by a process very closely resembling crystallization, if not identical with it.

If a specimen of blood be drawn into a beaker and allowed to clot, it will first be seen to "set" into a firm jelly, in which the larger fibrin-cords develop later, and by their contraction squeeze out the serum. Now, the consolidation (festwerden) of this colloid mass into *threads*, with the formation of splits and cavities (Spalten und Lücken) between them, as described by Rindfleisch, may be looked upon as "a process closely resembling Crystallization," as Hermann defines it. The definite form which the fibrin-threads take, especially the typical needle-shaped form of the threads when deposited isolated in scanty clot, speaks strongly in favor of a crystallization; while the subsequent toughening and contraction of the threads show a clear resemblance to the coagulation of certain proteids, notably myosin.

In fact, it appears that we have in blood an interesting process which may be regarded as intermediate, in a certain sense, between a true crystallization on the one hand, and the coagulation of certain proteids, such as myosin, etc., on the other.

From the foregoing, I think it is evident that there is no histological connection between the plaques and fibrin, so that if the plaques are involved at all in coagulation, the connection must be a chemical one; that is, the plaques must give up something to the plasma at the same time that they break down.

Hayem (**5**, 704 and 705), Bizzozero (**4**, 101–103; **7**, 19), Lavdovsky (**2**, 65), Halla (**1**, 222; **2**, 378) and Ferraro (**1**, 295) have pointed out that *fibrin is formed pari passu with the breaking down of the plaques;* and both Hayem and Bizzozero have shown that *reagents or conditions which retard the breaking down of the plaques, retard to* PRECISELY THE SAME EXTENT *the formation of fibrin;* while reagents which PRESERVE THE PLAQUES PREVENT THE FORMATION OF FIBRIN ALTOGETHER.

I think the strongest evidence we have of a connection between the plaques and the clotting of the blood is derived from these facts, and all my observations thus far completed confirm them in every respect.

Schimmelbusch (**2**, 238 and 239) notices the observations of Hayem and Bizzozero, that reagents which affect the breaking down of the plaques also affect coagulation. He does not attach much importance to this, but says that the reagents employed may possibly prevent coagulation, even if the plaques broke down. He admits, however, that there is more weight to be attached to the action of cold and warmth respectively slowing or hastening the breaking down of the plaques.

Schimmelbusch (**1**, 102 and 103; **2**, 242) does not believe that there is any connection between the plaques and coagulation. The chief objections which he urges against this assumption may be given briefly as follows:

1. He refers to the experiment of Bizzozero (**2**, 308; **4**, 103), in which this observer found that in the blood of animals after death the plaques retained their original form as long as the blood remained fluid, while in blood that had clotted the plaques were found more or less broken down. Schimmelbusch (**2**, 309) made several experiments in connection with this question, and was led to deny the parallelism between the breaking down of the plaques and the clotting of the blood. He opened the hearts of cats, dogs and man, at intervals of from one to twelve hours after death, and took a small bit of fibrin, which he transferred to .75 per cent. NaCl solution, or better, to osmic acid. Upon teasing out this fibrin, he found a great number of plaques, free and intact, lying on and between the threads of fibrin. On the other hand, he found that in the hepatic vein the blood was slow to clot, but the plaques changed rapidly. The plaques, however, were star-shaped, and less distorted than in arterial blood, owing to the absence of fibrin. From these observations he draws the conclusion that there is not an inseparable connection between the breaking down of the plaques and the coagulation of the blood.

This work of Schimmelbusch is exceedingly interesting, as yielding positive results which tend to disprove the relation between the plaques and coagulation, so clearly indicated by the work of Hayem and Bizzozero. I do not think, however, that they can be regarded as *conclusive* proof to the contrary, as I hope presently to show.

To Bizzozero's observations that solutions of neutral salts

would retard the breaking down of the plaques, and also the formation of fibrin, Schimmelbusch (**2**, 238) objects that even if plaques broke down, the salt might still prevent coagulation by acting chemically on what was set free. Now, it seems to me that this same objection may be urged against the conclusiveness of Schimmelbusch's own observations on the hepatic blood. We know that a number of ferments and other substances can be extracted from the liver after death, and it is not impossible that some of these may act in the manner described.[1]

The fact that well-preserved plaques are found enclosed in fibrin taken from the heart some time after death, cannot be regarded as conclusive proof that the plaques are not connected with the formation of clot, unless we knew definitely what they were supposed to yield, and at the same time could be sure that the plaques are the only source from which the substance produced could be derived. We should also have to know positively that *all* the plaques were well preserved, and that *none* of them had broken down.

A striking illustration of this came up in my own work a short time ago. In a series of transfusion experiments recently made in this laboratory by Professor Martin and Mr. J. P. Campbell, peptone was tried to render the blood incoagulable. It was found to work very well for dogs, but was without effect on cats. Thinking that this might throw some light on the subject of the relation of the plaques to coagulation, I took some specimens of peptonized cat's blood for examination. The blood was drawn from the carotid artery, through a glass canula and a piece of rubber tube about six inches in length. Some specimens were taken and hardened in osmic acid with as little loss of time as possible. These were found to be the same as perfectly normal blood. What was my astonishment, however, upon examining specimens from blood which had stood about two minutes, to find a beautiful typical fibrin-reticulum, with hundreds of plaques well preserved and enclosed in the network![2] I naturally

[1] Haycraft thinks he has found such a ferment in the buccal glands of the medicinal leech, which prevents the action of fibrin-ferment. (See Proceedings of the Royal Society, London, Vol. 36, p. 478.)

[2] Preparations of peptone blood were obtained in the following manner: When it was desired to harden a specimen of blood as soon as possible after being drawn, one surface of a cover-slip was touched to the stream of blood as it flowed from

thought I had found a proof that the blood would clot without the plaques breaking down; but upon looking into the matter, I found that the tube was not clean, but that blood had been drawn through it shortly before I took my specimens, and that the walls were filled with pieces of adherent clot. Another cat was peptonized, and some specimens of blood drawn through a tube already used in the carotid, while others were drawn through a fresh tube in the femoral.

The blood drawn through the dirty tube was found to clot a little more quickly than that drawn through the clean tube. In blood drawn through the clean tube, coagulation went hand in hand with the breaking down of the plaques. From blood taken through the dirty tube, specimens could be gotten in which there was a copious formation of fibrin, but in which *nearly all* the plaques were well preserved. I could always find some broken-down plaques, but whether these were brought from the clot on the sides of the tube, or whether they broke down as the blood flowed through, of course cannot be decided definitely.

The difference in time between the clotting of blood drawn through the dirty tube, and that of the blood drawn through the clean tube, though slight, was noticeable, and this, together with the fact that when coagulation first sets in, the plaques are found preserved in the specimen from the dirty tube, while they are broken down in the specimen from the clean tube, would seem to show that *in passing through the dirty tube the blood took up something which brought about coagulation before the plaques broke down, but which is also formed later by the plaques when they break down.*

From what we know of coagulation at the present time, it seems probable that the agent in fibrin-formation that would be most apt to conduct itself in this way is the *ferment.*

Hayem (**5**, 720) and Bizzozero (**2**, 320) each think that the

the tube; a stream of .75 per cent. NaCl solution from a wash-bottle was then directed against it immediately, so as to wash off the excess of red corpuscles, and the slip was dropped into a watch-glass of 1 per cent. osmic acid.

To obtain specimens of the blood after any desired interval of time, the blood was drawn into watch-glasses and a cover-slip laid on its surface (the cover-slip will float, so that only the under surface comes in contact). At the end of the desired interval the cover-slip was lifted off with a pair of forceps, washed, and hardened as above.

part played by the plaques is to furnish something essential to the coagulation, and both agree that ferment is in all probability the agent in question. Bizzozero (**2**, 319; **4**, 111; **6**, 278; **13**, 353) attempts to prove this by his well-known experiment of whipping blood with threads, by which operation he gets them full of adherent plaques and some leucocytes. The threads are carefully washed in .75 per cent. NaCl solution, and then added to a liquid coagulable with ferment. A clot is formed sooner or later, according as there are many or few plaques adhering to the threads (**4**, 111). To eliminate the objection that the adherent leucocytes had caused the clot, as well as to disprove the theory that the leucocytes yield fibrin-ferment, he took specimens of the same coagulable liquid, and to them he added pieces of spleen, lymphatic gland, red marrow, etc., all of which contain leucocytes, and got no coagulation; from which he concludes that the leucocytes do not yield ferment, and therefore the ferment must have come from the plaques adhering to the threads.

Rauschenbach (**1**, 76–95) enters into a detailed and lengthy criticism of Bizzozero's work, in which he raises two objections to the experiment just described: First, that the threads may have absorbed ferment which was not washed out, and this it was that produced coagulation; secondly, he says that the protoplastic fluid used by Bizzozero[1] is only a test for *free* ferment. He thinks the ferment is combined in some way with the leucocytes, and that the $MgSO_4$ prevents it from being set free.

The first objection is undoubtedly valid. By granting the second, we exclude the leucocytes adhering to the threads from a share in the formation of ferment, if any is formed by what does adhere to the threads. It then remains to be explained why the time of coagulation should depend upon the number of plaques, and the experiment remains of value as offering strong support to the theory that ferment may be derived from the plaques.

[1] Bizzozero used a fluid recommended by Schmidt. It is made by drawing blood into a 28 per cent. solution of $MgSO_4$ in the proportion—blood, $MgSO_4$ :: 1 : 3. The salt solution should be at 0°. When the blood is drawn into it, a constant, but not violent, stirring is kept up until the two are well mixed. The mixture is then allowed to stand until the cellular elements have settled. The plasma is then drawn off from the top layer and filtered at 0°, and this is then the protoplastic liquid.

The question of the formation of thrombosis is not without interest as bearing upon the question of coagulation.

Before the plaques were well known, there was a great consensus of opinion among physiologists and pathologists that the "white thrombus" which is first formed around a lesion in the vascular wall was composed of leucocytes. Within the last four years this question has received the special attention of Osler (**4**; **2**, 531), Bizzozero (**7**, 19; **3**, 358; **4**, 117), Hayem (**22**, 654 and 655), Ferraro (**1**, 298) and Lubnitzky (**1**, 207), all of whom find that the white thrombus is not composed of leucocytes, as formerly supposed, but of plaques.

In connection with my work on the plaques, I have taken up the subject of thrombosis, and hope to have my results ready for publication in the near future. I may say now, however, that my work thus far completed tends to confirm the results of the later observers just mentioned, and attribute the formation of the white thrombus to an agglomeration of plaques around a lesion in the vascular wall, or a foreign body introduced into the vessel.

From the foregoing it would appear pretty evident that there is an intimate connection between the breaking down of the plaques and the coagulation of blood. On the other hand, some evidence has been adduced to show that coagulation may sometimes take place without the plaques.

Lymph will clot, and Fano and Löwit (**1**, 295) state that it contains no plaques. Hayem (**9**), on the contrary, maintains that the lymph does contain plaques, and, in fact, that the plaques in the blood have their origin in the lymph. I have not yet studied the coagulation of lymph, so that I cannot say at present whether the clotting of lymph is similar to that of blood, or whether differences exist that will throw light on the relation of the plaques to the coagulation of the blood.

Löwit (**1**, 293) also claims to have obtained specimens of blood in 25 per cent. NaCl solution in which there were no plaques, but from which he could get a clot. I have repeated this experiment of Löwit, but with different results.[1]

The strongest evidence yet brought forward against the supposition that the plaques are necessarily connected with the coagu-

[1] See p. 317.

lation of the blood, is derived from an experiment of Löwit (**1**, 297). This observer found that if rabbit's blood be mixed with 28 per cent. $MgSO_4$ solution, and allowed to stand, the whole operation being conducted at 0°, the corpuscles will soon settle, and plasma from the top layer may be obtained in which there are numerous isolated plaques, a few red corpuscles, and no leucocytes. On adding water the plasma will not clot, although the plaques in it are numerous, whereas if fibrin ferment be added it soon forms a firm jelly.

I have not had an opportunity, since consulting Löwit's article, to repeat this experiment.[1] Hayem (**5**, 708) has shown that plaques which have been subjected to temperatures of 0° C. and under for a considerable length of time, lose their power to bring about coagulation when the temperature is raised. This I have found to be true, at least for a thin film of blood and .75 per cent. NaCl solution, in an ordinary specimen for microscopic examination. It may be that the action of $MgSO_4$ is somewhat similar, and produces deep-seated chemical changes in the plaques that destroy their power to yield the agent active in producing coagulation.

From a careful consideration of both sides of the question, it is clear that much can be said for and against the theory that the plaques take part in the coagulation of the blood. But the fact that the breaking down of the plaques and coagulation go hand in hand in blood as nearly normal as it is possible to observe it, can hardly be without significance, and speaks strongly in favor of a connection between the plaques and coagulation as it normally takes place. The evidence on the other side is mostly derived from experiments involving the use of strong salt solutions and other very abnormal conditions, which detracts from its conclusiveness with regard to normal blood.

Three other methods of formation of fibrin have been given by different observers, besides those already considered:

1. From the red corpuscles.
2. From the leucocytes, the leucocytes playing the part ascribed in this paper to the plaques.
3. From the leucocytes by a process of necrosis.

Each of these will be taken up in turn.

[1] See footnote, p. 316.

The work of Virchow,[1] Hoppe-Seyler,[2] Van der Horst,[3] Heynsius,[4] Mantegazza,[5] Landois,[6] Semner,[7] Dogiel,[8] Hart[9] and Hayem (**24**, 62), all goes to show that fibrin may be obtained from the red corpuscles, and Dogiel thinks it possible that there is a relationship between the stromas of the red corpuscles and fibrinogen.

The methods employed in most of the above work place the corpuscles under conditions which do not exist in the ordinary process of coagulation; and, besides, it is now not claimed that red corpuscles are exclusively the source of fibrin, so that it is sufficient to mention this work as a matter of interest, but which need not be further discussed, as the red corpuscles do not enter the field as rivals of the plaques, in any theory of coagulation. There are two observations, however, which should be mentioned here, viz.: those of Landois and Hart.

Landois[10] and Hart[10] claim to have observed the stroma of the red corpuscles changed directly into fibrin. This was likely due to currents, as Landois admits. In speaking of the red corpuscles he says: "At first we can recognize the contour of individual corpuscles, *but as soon as a current is set up*[11] in the surrounding liquid, the masses of stromas are carried hither and thither, by which process the adhering stromas are drawn out into tough threads, the cell-contour being at the same time lost." He attributes the rapid clotting which follows the mixing of blood of animals of different species to this process.

In the case of thrombi in the vessels, Landois says: "When once the stroma-fibrin is formed, the plasma-fibrin can be deposited around it, as around a foreign body."

The next theory to be considered is, that the leucocytes play the part in coagulation which in this paper has been ascribed to the plaques.

[1] Virchow, Gesammelte Abhandlungen, 1856.
[2] Landois' Lehrbuch der Physiologie des Menschen, 4th edition, 1885, p. 57.
[3] Over de Eiwitachtige stoffen van het bloed, Leyden, 1868.
[4] Pflüger's Archiv, Vol. II, pp. 29–49; also, Vol. III, pp. 414–424.
[5] Centralblatt für die Medicinischen Wissenschaften, 1868, p. 292.
[6] Centralblatt für die Medicinischen Wissenschaften, 1874, pp. 420–422.
[7] Ueber die Faserstoffbildung im Amphibien und Vogelblut, etc., Dorpat, 1874.
[8] Centralblatt für die Medicinischen Wissenschaften, 1875.
[9] Quarterly Journal Microscopical Science, 1882, p. 255.
[10] Op. cit.
[11] The italics are mine.

The belief that the leucocytes break down during coagulation, thus furnishing ferment, has always gone hand in hand with Schmidt's theory of the origin of fibrin from three fibrin factors; and as Schmidt's theory for a long time met with almost universal acceptance, it is natural that the theory of the breaking down of the leucocytes should have gained a firm hold on the minds of physiologists and pathologists. It is therefore not strange that often in work on subjects involving the question of coagulation of the blood, the leucocytes have been said to break down, not because the attention of the observer was directed to this point, but because it was a generally accepted fact. This will account for a large amount of literature that may be quoted apparently in support of the theory of the breaking down of the leucocytes as part of coagulation.

This question has been the subject of many researches, however, and has been approached in many different ways, with almost as many varying results.

Schmidt, Hoffman and Heyl have found that there were fewer leucocytes in blood after coagulation than before, and from this draw the conclusion that the leucocytes break down during the process. Heyl says there is a loss of 70 per cent. of the leucocytes, while Schmidt estimates the loss at 90 per cent.

These results were obtained for defibrinated blood, and the obvious objection has been raised by Bizzozero, Laker and others that whipping the blood would injure the leucocytes, and hence these figures would not hold good for normal clot. Heyl (**1**, 30) himself recognizes the fact that leucocytes are destroyed by whipping, for he shows that if blood be shaken instead of whipped, the loss of leucocytes is considerably smaller. He also points out that by shaking a solution containing fibrinogen, paraglobulin and fibrin-ferment, the time of coagulation is not affected, while if the same be tried with blood, it hastens coagulation. He therefore concludes that the leucocytes are broken down by shaking, and that the reduced time of clotting is due to this.

These observations of Heyl show pretty clearly that numerical results obtained from blood which has been shaken or whipped for defibrination, will not hold good for blood under normal conditions. It is obvious that Heyl's observation on the acceleration

of clotting by shaking does not prove that the *leucocytes* have anything to do with it, for he had the plaques and the leucocytes together in the blood, and from the extreme vulnerability of the plaques, we should expect *them* to be broken down by shaking or whipping. This objection may be raised to the work of Feiertag (**1**), who made comparative observations on the number of leucocytes and plaques before and after coagulation. To prove a connection between the leucocytes and coagulation, Hlava (**1**, 396 and 397) refers to work done by Heyl (**1**, 47), who, following v. Samson Himmelstjerna and Hoffman, injected septic matter into the veins of animals, and studied the relation of the increased number of leucocytes to the yield of fibrin. It is quite evident that these results throw no light on the subject, as here also the plaques as well as the leucocytes were involved. Laker makes a good point also: that if 71 per cent. of the leucocytes really did break down during coagulation, *some* of them should be caught in the act. Schmidt (**3**, 528) says that he has found all stages in the breaking down of the leucocytes, but admits that he has never seen progressive changes in one individual, while Rauschenbach compares the breaking down of the leucocytes to an explosion (Der Vorgang verlauft . . . . in fast explosiver Weise.)—a view also adopted by Groth (**1**, 69 and 70).

Hlava (**1**, 410) says he has seen leucocytes arrested in different stages of disintegration, but like Schmidt does not claim to have observed changes in the same leucocyte.

A view directly opposite to this is taken by Bizzozero (**7**, 20), Laker (**1**, 198), Lavdovsky (**2**, 65) and Löwit (**1**, 277, 280–283, 302). Bizzozero and Laker are of the opinion that the leucocytes undergo no change whatever during coagulation. Lavdovsky says, "The breaking down of the leucocytes, if it occurs at all, is of no importance in clotting, and can scarcely be perceived."

Bizzozero, Laker and Löwit have all seen the leucocytes in blood after coagulation, and Bizzozero and Laker have kept leucocytes under observation for several days without finding any disintegration. My experience has been the same. Hlava (**1**, 408, 409, 416) and Rauschenbach (**1**, 12 and 13) have advanced a theory, based on their direct observations, which will account for this—viz.: that there are different varieties of leucocytes, and that one variety breaks down readily, while others do not.

My observations on this point have already been given (pp. 313, 314), and, as is seen, do not confirm it. The results of my work on this subject receive confirmation from the work of Löwit on the lymph. Löwit has counted the leucocytes in a given field before and after coagulation, and finds their number to be the same. Löwit thinks that the leucocytes participate in coagulation by emitting something which dissolves in the plasma; but from work both on blood and lymph, he comes to the conclusion that they do not break down.

This view, according to Bizzozero (**4**, 99), was first held by Mantegazza (1871), and I have seen no mention of any other advocate of it up to the time of Löwit.

The following observations of Löwit have led him to the belief that the leucocytes take part in coagulation:

He has found (**1**, 290) that if lymph containing leucocytes be added to hydrocele or ascites-fluid, it will cause coagulation. If, however, the lymph had been previously filtered through glass wool, and the leucocytes thus removed without breaking down, no clot would result from the addition of the lymph-plasma.

He claims (**1**, 293) to get blood in which there are no plaques by drawing it into 25 per cent. NaCl solution. On diluting this mixture it will clot if leucocytes be present, otherwise not.

He has observed (**1**, 301) that if blood be drawn into vessels surrounded with ice, and the different histological elements allowed to settle, they will be found in layers, some of which are particularly rich in one kind of corpuscles, some in another. If now a portion be taken from the layer which is richest in leucocytes, it will be found to produce coagulation more readily than specimens from layers poorer in leucocytes. These results are very striking, and would almost carry conviction on their face, but Löwit also mentions (**1**, 300) that the layer in which leucocytes are most plentiful also contains many masses of plaques, which, of course, detracts from the decisiveness of the experiment.

Rauschenbach has succeeded in extracting ferment from leucocytes obtained from a number of sources, but his methods involved rubbing them up into a pulp, treating them with distilled water, salt solutions, the serum of blood from species other than their own, etc. These results are all interesting and suggestive, but do not show that ferment is derived from the

leucocytes as a normal part of the coagulation. It is well known that peptone injected into the veins of some animals prevents the blood from clotting. Such blood has been studied for information regarding coagulation chiefly by Fano and Wooldridge, and many points of great interest are suggested by their work. I may say, however, that from this work there has been derived no positive proof that the leucocytes furnish ferment in ordinary coagulation. The ferment has always been obtained by the action of distilled water, a continued stream of carbon dioxide, etc.—conditions which are not present in the normal clotting of the blood.

There is great need of more thorough microscopic investigation combined with the chemical; and as I propose, in continuing my work on coagulation, to take up a series of investigations in this field, I shall leave a detailed discussion of this subject for a future publication.

We are now in a position to decide between the relative claims of the plaques and of the leucocytes to the function of yielding ferment, or being otherwise concerned in the coagulation of the blood. It can by no means be said that it is *impossible* to get ferment from the leucocytes. The results of many observers are strongly in favor of the assumption that, under certain circumstances, the ferment can be so derived. Rauschenbach (**1**), Wooldridge (**6**, 417), Lea Green[1] and Holzmann,[2] claim to have extracted ferment from sources other than the cellular elements of the blood. Bizzozero (**2**, 321), while claiming for the plaques the power of furnishing ferment, expressly states that he does not attribute the origin of ferment exclusively to these elements. The point which I wish to emphasize is this: that whatever may occur in leucocytes subjected to abnormal conditions—from chemical reagents, crushing, concussion, from whipping of the blood, etc.—in the coagulation of the blood *as it normally occurs* there is no *histological* evidence that the leucocytes take part, while the contrary is true of the plaques.

I may here add that I have seen apparently abnormal leucocytes in many specimens of clot. I do not, however, regard this as proof that the leucocytes break down during coagulation, but

[1] Journal of Physiology, Vol. IV, pp. 380–386.

[2] Holzmann, op. cit.

think it more likely that the leucocyte was in that condition when the blood was drawn, or that, possibly, alterations were produced by its being caught in the contracting fibrin-threads. In specimens where the coagulation was studied in Bizzozero's fluid, or at a reduced temperature, where the *process is so retarded that its individual steps can be distinguished*, I have never seen anything that could be taken for PROGRESSIVE CHANGES *in the* LEUCOCYTES, *while such changes in the* PLAQUES *are most striking.*

The last theory to be considered is that fibrin is formed by a necrosis of the white corpuscles.

This view is foreshadowed in Beale (**1** and **2**), and was next taken up by Weigert, whose object was to point out the interesting generalization that a necrosis of structured cells produced a substance resembling fibrin. He would gladly include blood-fibrin in this generalization, deriving it from a necrosis of the leucocytes, but he is forced to admit (**2**, 92) that "Fibringerinnung" is "ganz isolirt," and to speak (**1**, 469) of the substance derived from the leucocytes as "fibrinähnlich."

The later supporters of this view are Wooldridge and Hlava.

Wooldridge believes that in ordinary coagulation of the blood there are two processes, one of which involves substances in solution, the other the cell-substance of the leucocytes. His view is briefly given by himself (**2**, 418) as follows:

"There are two essential processes in the coagulation of the blood, one of which has been, hitherto, entirely wrongly appreciated or overlooked. This latter process is that the 'dead' plasma converts the white corpuscles directly into fibrin. At the same time, however, that this occurs, a substance is liberated from the cells which converts the fibrinogen also into fibrin. This is the other process. The substance which is liberated from the cells is fibrin-ferment."

The view of Hlava (**1**, 414) is very similar to that of Wooldridge. He believes that fibrin is produced directly from a necrosis of the leucocytes. For convenience of description he divides the formation of fibrin into four stages:

1. The agglomeration of the leucocytes.
2. The breaking down of these (the protoplasm breaking down before the nuclei).

3. The death of the nuclei (which according to Hlava are plaques).

4. The solidification of the fibrin.

At the third stage the ferment is set free, which causes the coagulation of the fibrinogen and paraglobulin. The fibrin is first granular, then fibrillar.

I think the most likely explanation of these results has already been given—viz.: that groups of plaques may have been mistaken for leucocytes, and that in many experiments he used a concentrated solution of mercuric chloride, which produces a precipitate of proteids in the plasma that obscures the whole field and vitiates the accuracy of any observations.

Rauschenbach (**1**, 52) has given attention to the formation of fibrin in the presence of many leucocytes, and his conclusion is that the fibrin formed by adding different kinds of leucocytes to plasma is true fibrin produced by the spontaneous coagulation of the plasma, the apparent differences being due only to the detritus of the leucocytes, and other foreign matter mechanically retained.

SUMMARY.

The conclusions arrived at in this paper may be given briefly, in summary, as follows:

1. In addition to the red corpuscles and leucocytes, the blood normally contains a third histological element, the *plaques*.

2. Although strong resemblances exist between the plaques and other histological elements of the blood, there is not yet sufficient evidence to establish a *genetic* connection. We are therefore obliged, for the present at least, to regard the plaques as independent elements.

3. When the blood is drawn, the plaques break down almost immediately. This is not true of any other element of the blood.

4. The breaking down of the plaques is intimately connected, in its time-relations at least, with the clotting of the blood.

5. The connection between the breaking down of the plaques and the coagulation of the blood is not histological, but chemical—*i. e.*, the plaques appear to give up a soluble substance which is active in coagulation.

6. The active agent in question is most probably *fibrin-ferment*.

7. Fibrin is deposited histologically independent of any of the cellular elements of the blood.

8. When the clot is very scant, fibrin is deposited as long, needle-shaped, crystal-like bodies.

In conclusion, I would express my thanks to Prof. Martin for encouragement and valuable suggestions throughout my work, and also to Dr. J. R. Duggan, who kindly assisted me in photographing the plaques, and to whose skill and experience in micro-photography I feel my success in this direction has largely been due.

BIBLIOGRAPHY.

As the subject treated in this paper is comparatively recent, and as no attempt has yet been made to collect the bibliography, which is now becoming voluminous, I have been advised to publish the bibliography as here appended.

The numbering of the articles does not in every case correspond with the order in which they appeared, but as an attempt to rearrange the numbers so as to get the articles in the proper order of publication would involve not only considerable labor, but also a great risk of getting the references confused, I have thought it advisable not to make any change.

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DESCRIPTION OF PHOTOGRAPH.

The photograph was taken from a specimen of dog's blood prepared as follows: A cannula was introduced into the femoral artery and the blood allowed to stream freely through. A cover-slip was then held in a pair of forceps and touched to the stream of blood; without losing a moment a stream of .75 per cent. NaCl solution was directed obliquely against the slip, which was then instantly dropped into a watch-glass of 1 per cent. ~~arsenic~~ osmic acid. After about twenty

minutes it was taken out, washed and left all night in a 1 per cent. aqueous solution of Bismarck brown. It was then washed in water and mounted in a saturated solution of potassium acetate.

The photograph is of especial interest as being the first ever obtained, and showing at once the following points:

1. The form of the plaques (circular and oval).

2. Their biconcavity (appearing as a light shade in the centre of the plaque).

3. Their variation in size.

4. Their tendency to adhere to each other and to the cover-slip within a few seconds. Their adherence to the cover-slip is shown by the red corpuscles in the field. These appear as circular discs with a bright centre and a halo around their periphery. This is due to their being out of focus, the objective being focussed on the plaques adhering to the slip, while the red corpuscles lie in the liquid between the slip and the slide.

5. They are not fragments of disintegrated leucocytes, but are distinct histological elements.

DESCRIPTION OF FIGURES, PLATE XIX.

FIGURE 1.—Drawn from an unstained specimen prepared as described for photograph. The lenses used were Zeiss $\frac{1}{18}$ homogeneous immersion obj., oc. 4 (x = 1450), with Abbe condenser. *a*, Plaques seen full on face, slightly or not at all altered. *a'*, Plaques slightly altered in outline, showing the first changes toward breaking down. *b*, Plaque seen on edge. *b'*, Plaque seen partly on edge and partly on surface. *c*, Group of three plaques, each seen in different position. *d*, Plaque fixed at one end and drawn out by currents. This is quite a common appearance. The large, round figure to the right is a red corpuscle drawn to scale for comparison in size with the plaques.

FIG. 2.—Group of plaques enclosing three red corpuscles. The plaques have now become granular and of irregular outline. This is the stage in which the plaques are most commonly found when examined in .75 per cent. NaCl solution or Bizzozero's fluid. Drawn with Zeiss $\frac{1}{18}$ homogen. im., oc. 2 (x = 790), with Abbe condenser.

FIG. 3.—Same as Fig. 2, except that the plaques are more broken down, and farther on the way to form a typical granular mass. It will be observed that the plaques are not only jagged and granular, but they have begun to fuse together and lose their individual out-

line. The group includes a red corpuscle and a tetranuclear leucocyte. Lenses same as in Fig. 2.

FIG. 4.—Typical granular mass. The plaques are so far fused together that, for the most part, their individual identity is lost. Lenses same as Figs. 2 and 3.

FIG. 5.—Typical granular mass with fibrin-threads radiating from it, and needle-shaped threads (crystalloids) deposited freely in the field.

FIG. 6.—Drawn from specimen of scanty clot in diluted blood. Shows how fibrin is deposited freely in the field, as well as in connection with the granular masses.

FIG. 7.—Thick formation of fibrin. Shows fibrin-threads branching all over the field, enclosing granular masses, red corpuscles and a trinuclear leucocyte. Drawn from a specimen stained with methyl violet.

FIG. 8.—Single plaques showing the "bladder formation" from the action of water. The granules seen in the light part of the "bladders" are due to the method of preparing the plate. The dark part should be granular, as represented; the light part should be perfectly homogeneous.

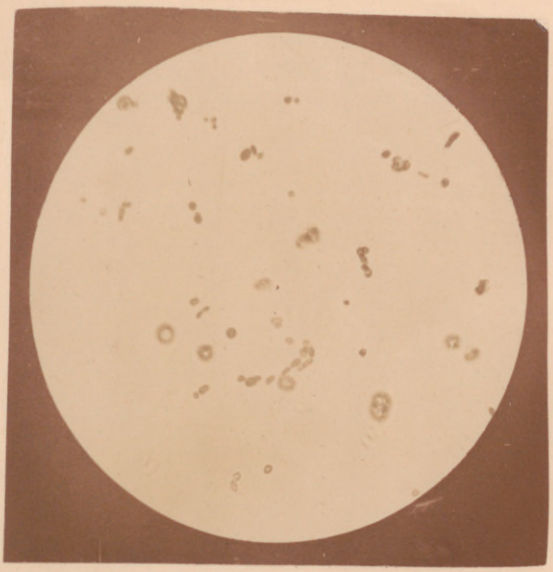
FIGS. 9 and 10.—Plaques which had come together and afterwards swollen up from the action of iodine solution (aqueous). The peculiar shape was likely produced by pressure on the cover-glass.

FIG. 11.—Same as Figs. 9 and 10. Drawn particularly for comparison with Fig. 13, *q. v.*

FIG. 12.—Leucocyte with adherent plaques which have swollen up and formed "bladders." From a specimen stained with methyl violet.

FIG. 13.—After Löwit. Löwit gives the figure (especially *c*) to illustrate the extrusion of plaques from the body of leucocytes, and I reproduce his drawing to compare with Figs. 11 and 12. (See text.)

FIG. 14.—Leucocytes. *a*, *b*, *c* [and *g* (?)], multinuclear leucocytes supposed by Rauschenbach and Hlava to break down during coagulation, while the uninuclear variety *d* do not break down. Notice the resemblance of the smaller nuclei in *a*, *b*, *c* and *g* to the plaques in Fig. 2.



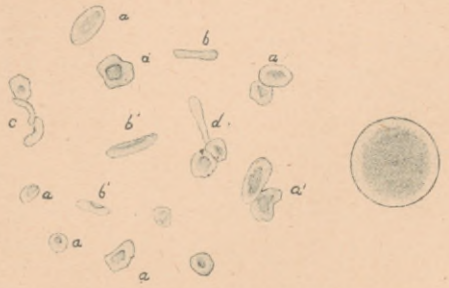


Fig. 1.

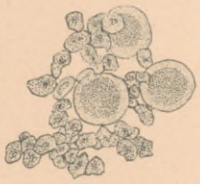


Fig. 2.

Fig. 3.

Fig. 4.

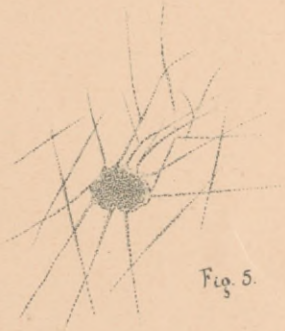


Fig. 5.

Fig. 6

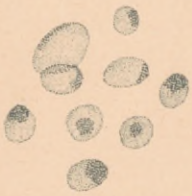


Fig. 8.

Fig. 9.

Fig. 10.

Fig. 7

Fig. 11.

Fig. 12.

Fig. 13

Fig. 14.

A. HOEN & CO. Lith.