THE STRUCTURE OF WHITE THROMBI.

BY

W. H. WELCH, M.D.,

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A year ago, upon an occasion similar to this, you had the pleasure of listening to Dr. J. Collins Warren's address upon the healing of arteries after ligature. As his researches were directed especially toward the later stages of the changes which follow injury of the blood-vessels, it will, perhaps, not be unacceptable if I call your attention to the histological structure of those plugs which often constitute the earliest alteration following such injury.

While all that pertains to the subject of thrombosis is of importance, recent investigations have lent especial interest to the study of the minute structure and the mode of formation of white thrombi.

Since Virchow's memorable publications[1] upon the subject of thrombosis, it has been generally believed that a thrombus is essentially a blood coagulum, and differs from an ordinary post-mortem clot only in the arrangement and the relative proportion of the constituent histological elements. The most important of the differences noted by Virchow are the characteristic lamination of thrombi, and their greater richness in white blood-corpuscles, and in granular material. These differences were believed to be sufficiently explained by the slow formation of thrombi from the circulating blood, in contrast with the rapid coagulation of blood at rest, and by secondary changes in the thrombus.

During the two decades following the publication of Virchow's researches on this subject, more attention was paid to the causes, to the effects, and to the metamorphoses of thrombi, more particularly to their so-called organization, than to the intimate structure of recently formed thrombi. Zahn's investigations of thrombosis, published in 1875, marked an epoch in the history of our subject.[2] Zahn had been preceded by Mantegazza,[3] who, in 1869, called attention to the rôle played by white blood-corpuscles in the formation of white thrombi, but the observations of the latter author had remained comparatively unknown.

Zahn emphasized the most important differences existing between thrombi formed from the blood in repose, the so-called red thrombi, and those developed from circulating blood, viz., the white and the mixed

[1] Virchow: Gesammelte Abhandlungen. Frankfurt a. M., 1856.
[2] Zahn: Virchow's Archiv, 1875, Bd. 62, p. 81.
[3] Mantegazza: Gaz. med. Lombarda, 1869.

thrombi. Whereas the former do not differ from an ordinary coagulum of blood, the latter, according to Zahn, originate from clumps of white corpuscles. Zahn observed microscopically in the mesenteric vessels of the living frog, the first formation of white thrombi out of white blood-corpuscles which accumulated in vessels at places which had been subjected to various injuries. The white corpuscles thus accumulated, if they were not detached by the circulation, rapidly disintegrated into a mass of granular material which Zahn considered to be granular fibrin. According to the widely accepted views of Zahn, therefore, a white thrombus at its inception consists essentially of white blood-corpuscles, which, after a short time, break up into a mass of granules identical with fibrin in their reactions.

The observations of Mantegazza and of Zahn were confirmed, in 1876, by Pitres,[1] who made corresponding observations of the living circulation in warm-blooded animals, whereas Zahn studied the circulation only in frogs. Pitres, however, did not, like Zahn, identify the granular material resulting from the disintegration of white blood-corpuscles with fibrin.

The rôle thus assigned to the white corpuscles in the formation of white thrombi certainly seemed to be at variance with Virchow's view that all thrombi are coagula. A reconciliation, however, was effected between the new observations and the old doctrine, chiefly through the investigations of Weigert.[2] This pathologist, adopting the views of A. Schmidt as to the part taken by white corpuscles in the spontaneous coagulation of the blood, assigned to these corpuscles essentially the same rôle in white thrombi. The coagulation necrosis of leucocytes in thrombi is a process differing, according to Weigert, morphologically, but not in essence, from the dissolution of white corpuscles and the formation of fibrillated fibrin in the ordinary coagulation of the blood. White thrombi, therefore, continued to be regarded as in the main genuine coagula.

The first opposition to the views of Zahn came from Hayem,[3] who, in 1878, attempted to prove that the coagulation of fibrin is a function of the small bodies, called by him hæmatoblasts, and subsequently, by Bizzozero, blood plates, the name now generally adopted. Osler, who was among the first to observe the existence of human thrombi composed almost exclusively of blood plates (or plâques, as he, accepting the

[1] Pitres: Arch. de Phys. norm. et path., 1876, p. 230.

[2] Weigert: Virchow's Archiv, Bd. 70, 1877, and Bd. 79, 1880. Fortschritte d. Medicin, 1883.

[3] Hayem: Recherches sur l'Anatomie norm. et path. du Sang. Paris, 1878. Comptes Rendus de l'Acad. d. Sc., 1882, 18 Juli.

suggestion of Kemp, prefers to call them), has presented fully in the last series of Cartwright Lectures, the existing state of our knowledge concerning these bodies.[1] In 1882, Hayem published his observations on the structure of thrombi. He found that the thrombi which are formed in wounds of arteries are made up of blood plates.

A few months later Bizzozero[2] described, with much detail, both the fibrin-forming properties of the blood plates, and their presence as the essential and primary constituent of white thrombi, in these respects confirming the opinions of Hayem. Bizzozero was the first to study the formation of thrombi from blood plates in the living circulation, using for this purpose the mesentery of warm-blooded animals.

In the following year, Hlava,[3] working under Weigert's direction, was unable to confirm the views of Hayem, and of Bizzozero, and upheld the doctrine of Zahn and of Weigert, that white thrombi, in their earliest formation, consist mainly of leucocytes, which subsequently undergo coagulation necrosis.

Lubnitzky,[4] working under the direction of Langhans, published, in 1885, an interesting article, in which she claimed that the thrombi which are formed in arterial wounds, and which are the chief agent of nature in checking hemorrhage from this source, are composed primarily of blood-plates. The blood-plates, when thus accumulated, are, according to Lubnitzky, either identical with fibrin, or are quickly transformed into this substance.

The most thorough study hitherto made of the share taken by blood-plates in the formation of thrombi we owe to Eberth and Schimmelbusch.[5] These authors consider that sufficient proof of the existence of blood-plates in the normal circulation is afforded by the observation of the plates in the circulating blood of the mesenteric vessels of dogs and rabbits examined under physiological salt solution, with high magnifying powers. In opposition to Hayem and to Bizzozero, they deny that the plates have any share in the coagulation of fibrin, which they regard rather as a kind of crystallization in the plasma. The plates, when removed from the natural conditions of their existence, rapidly undergo a metamorphosis, called by Eberth and Schimmelbusch viscous metamorphosis, and characterized especially by the sticking of the plates to each other and to foreign substances. Under normal conditions the

[1] Osler: On Certain Problems in the Physiology of the Blood Corpuscles. The Medical News, April 3, 10, 17, 1886.

[2] Bizzozero: Virchow's Archiv, 1882, Bd. 90, p. 261.

[3] Hlava: Arch. f. exp. Path. u. Pharm., 1883, Bd. 17, p. 392.

[4] Lubnitzky: Arch. f. exp. Path. u. Pharm., 1885, Bd. 19, p. 185.

[5] Eberth u. Schimmelbusch: Virchow's Archiv, 1885, Bd. 101; 1886, Bd. 103, Bd. 105.

plates circulate with the red corpuscles in the axial blood current, but they make their appearance in the plasmatic zone when the rapidity of the circulation is diminished. A moderate slowing of the blood current is attended by the formation of the so-called border zones, or accumulation of white corpuscles in the plasmatic current, whereas a greater diminution of the velocity of the stream is characterized by fewer leucocytes, and more plates in the peripheral current. Other irregularities of the circulation, such as the little eddies produced by obstacles or projections in the stream, or by dilatations of its bed, may likewise throw the plates from the axis into the periphery of the stream. Mere slowing of the circulation is not attended by the formation of thrombi. In order to observe this formation, Eberth and Schimmelbusch subjected the living mesenteric vessels, chiefly of dogs, to various mechanical and chemical injuries. They then observed under the microscope, in many, but not in all instances, the accumulation of blood plates at the seat of injury. Here the plates became adherent to each other and to the wall of the vessel, in consequence of their viscous metamorphosis, and thus formed plugs which were often subsequently washed away, but which sometimes increased in size so as to obstruct completely the lumen of the vessel. Red and white corpuscles may be included in the mass of plates, but their presence is purely accidental, and they are not to be regarded as an essential constituent of the primary thrombus.

As the result of their microscopical observations of the formation of thrombi in living bloodvessels of warm-blooded animals, Eberth and Schimmelbusch, therefore, conclude that white thrombi are at first composed essentially of blood plates, and that the chief factors in the causation of such thrombi are slowing of the circulation or other irregularities in the current, and the viscous metamorphosis of the blood plates. This metamorphosis may be the result of various influences, such as contact with injured or diseased vascular walls and with foreign substances.

These conclusions as to the structure of white thrombi at their earliest formation Eberth and Schimmelbusch confirmed by the microscopical examination of sections of thrombi produced artificially by various injuries to the vessels. In experimental thrombi produced by mechanical injury of the vessels, as by wounds or by temporary ligation, they failed to find any fibrillated fibrin, whereas, in thrombi formed around foreign bodies introduced into the lumen of a bloodvessel, they observed some fibrin, situated usually between masses of plates, although even here they think it probable that fibrin is absent in the very earliest stages. They also detected fibrin, but in less amount, in thrombi produced by cauterization of the vessel walls.

While the investigations of Eberth and Schimmelbusch confirm the view of Hayem, Bizzozero, and Lubnitzky that white thrombi are made up primarily of an accumulation of blood plates and not of leucocytes as Zahn had led us to believe,[1] they are opposed in one important particular to the conclusions of the latter group of authors. They deny that the blood plates are in any way concerned in the generation of fibrin or are transformed into a substance resembling fibrin. They, therefore, deny that a white thrombus is primarily a coagulum, as has hitherto been unquestionably believed. They regard the process of thrombosis, here under consideration, as a *conglutination* of bodies pre-existent in the blood and not as a coagulation.

The arguments brought forward by the preceding investigators in favor of the existence of blood plates in large number in the normal circulation, convincing as they may seem, are nevertheless opposed by several observers. In view of the researches of Löwit,[2] this must for the present be considered as an open question.

Notwithstanding the brief period which has elapsed since the publication of Eberth and Schimmelbusch's researches upon thrombosis, their conclusions have already met with considerable opposition. It was hardly to be expected that such a radical overturning of accepted beliefs as these recent investigations involve should pass unchallenged.

While there is general agreement of opinion as to the important participation of blood plates in the composition of white thrombi, Eberth and Schimmelbusch's conception of the process of thrombosis as a conglutination of blood plates which have undergone a viscous metamorphosis is opposed by Hanau[3] on the ground that thrombi never have a viscid consistence. In support of the coagulative nature of the accumulation and metamorphoses of blood plates in white thrombi Hanau finds that plates as well as fibrin are transformed into hyaline, that a rim of hyaline forms around masses of plates, and that fibrin and plates often take the place one of the other in thrombi.

Weigert[4] protests even more vigorously against the effort of Eberth

[1] Since the delivery of this address Eberth and Schimmelbusch have published the results of their repetition of Zahn's experiments on the mesenteric vessels of frogs, and they find that fusiform corpuscles, which they consider to correspond to the mammalian blood plates, are the main constituents of white thrombi artificially produced in these animals. Vide Virchow's Archiv, Bd. 108, 1887. Löwit, on the other hand, regards these fusiform corpuscles as a variety of the white corpuscles and not as the analogues of blood plates, and he confirms the original statements of Zahn regarding the formation of white thrombi in frogs. Archiv f. exp. Path. u. Pharm. Bd. 23, 1887.

[2] Löwit: Beiträge z. Lehre von d. Blutgerinnung, Sitzb. d. k Akad. d. Wiss. Wien, Bd. 89, Abth. iii. u. Bd. 90, Abth. iii, and Tageblatt d. 59ter Versaml Deutscher Naturforscher u. Aertze in Berlin, p 306, 1886.

[3] Hanau: Fortschritte der Medicin. No. 3, 1887

[4] Weigert: Tageblatt der 59ter Versamml. Deutscher Naturforscher u Aerzte in Berlin, p. 306, 1886.

and Schimmelbusch to remove a large class of thrombi from the category of coagula. He has made a careful examination of human white thrombi, and points out especially their richness in fibrillated fibrin, which he demonstrates by a new staining process, and the abundance of leucocytes. He is unable to identify these anatomical thrombi with the experimental thrombi of Eberth and Schimmelbusch, and argues that until some reconciliation is effected between the two we should continue to base our conception of the nature of thrombi upon the study of the anatomical thrombi. Eberth and Schimmelbusch reply that their studies have been directed to the very earliest stages of the process of thrombosis, whereas the thrombi examined by Weigert belonged to subsequent metamorphoses.[1]

It is apparent from the foregoing review of recent investigations concerning the nature and structure of thrombi that unanimity of opinion on this subject has not been reached. There is general agreement that the blood plates play an important rôle in the early formation of many thrombi. Further investigations are needed to determine whether or not the plates are present in the perfectly normal circulation. For a proper understanding of the process of thrombosis it is important to determine whether or not the blood plates when accumulated to form a thrombus, are products of coagulation or subsequently undergo any metamorphosis which can be called coagulation. To determine this the gross characters of the plate thrombi, such as their color and consistence, will serve as important criteria, as has been pointed out by Weigert. It is, of course, of capital importance to learn whether the experimental white thrombi differ in their nature from human thrombi as seems to be intimated by Weigert. Before far-reaching conclusions can be drawn it is necessary to demonstrate the identity of the experimental and of the anatomical process of thrombosis. The microscopical study of human thrombi certainly seems opposed to the opinion that fibrin and leucocytes are unessential constituents of white thrombi. So constant and so abundant are these elements in post-mortem white thrombi that pathological anatomists will not readily admit that their presence is accidental or unessential to our conception of the nature of the thrombotic process.

In view of the fundamental importance of the question last touched upon, I have undertaken some investigations, first, as to the structure of

[1] Schimmelbusch: Tageblatt d. 59ter Versamml. Deutscher Naturforscher in Berlin, p. 306, 1886. Eberth u. Schimmelbusch, Fortschritte der Medicin, No. 6, 1887. The paper of Löwit, on thrombosis, who is opposed in many important particulars to Eberth and Schimmelbusch, appeared after the delivery of this address. Arch. f. exp. Path. u. Pharmak. Bd. 22, 1887.

human white thrombi; and second, as to the structure of thrombi produced experimentally in animals by mechanical injury of the blood-vessels. In the study of experimental thrombi I have directed my attention, in the first place, to their constitution at their earliest formation, and especially to the presence or absence of fibrin and of leucocytes at this period. It has seemed to me that a control with reference to the latter point of the observations of Hayem, Bizzozero, Lubnitzky, and especially of Eberth and Schimmelbusch, notwithstanding the carefulness of these observations, might not be unwelcome. I have also studied the structure of experimental thrombi in their later stages. It is undoubtedly upon this point that our knowledge is the least complete, and it is to be expected that when this gap is filled up there will be less divergence of opinion as to the relation between the experimental and the human thrombi.

There will be found on exhibition under the microscopes sections of human marantic thrombi formed in various infectious and wasting diseases. Among others specimens are present from a case of widespread thrombosis following parturition. In this case there were fresh thrombi in the femoral and iliac veins, the inferior vena cava, the branches of the pulmonary artery, and the cerebral sinuses. The constituent elements of these thrombi are fibrillated fibrin, hyaline substance, red blood-corpuscles, leucocytes, fragmented nuclei, and granular material, of which a considerable part can be recognized as blood plates. The proportion of each of these elements in the composition of thrombi varies much in different cases, and it will be well to consider the share taken by each in the formation of thrombi.

There have been various opinions as to the nature of the granular material found in thrombi. Thus it has been regarded as produced by the breaking up of fibrillated fibrin (Virchow), as molecular or granular fibrin deposited as such from the blood—a view advocated by the majority of the older (Mandl, Addison) and by many recent authors—as granular fibrin formed by the necrosis of white corpuscles (Zahn), as the result of simple disintegration of white corpuscles (Pitres). At present, however, there can be no doubt that most of what has been called in thrombi granular fibrin, or the products of disintegration of leucocytes, consists of more or less altered blood plates. The acquisition of this knowledge is an important advance in pathology, whatever may be thought of the nature of the plates.

Blood plates seem to be a constant constituent of fresh marantic thrombi. The plates are often present in such thrombi in as recognizable form and arrangement as in recent experimental thrombi. I have

found thrombi, particularly some endocardial vegetations and parietal arterial thrombi, which at first glance appear to be composed of nothing but plates; but careful examination in such cases has always revealed the presence also of fibrillated fibrin and leucocytes. In the majority of cases, however, the part of the thrombus composed of plates is less extensive then that made up of fibrin and leucocytes. Frequently the plates are arranged in masses between which lie the fibrin and leucocytes. Such masses of plates, which are more frequently situated in the interior of the thrombus than adjacent to the vessel-wall, are often enveloped in a rim of dense material resembling fibrin. In sections stained with hæmatoxylin and eosin the areas occupied by the plates can often be recognized with a low power by the various manner in which the different constituents of the thrombus stain.

All of the granular material in thrombi cannot be demonstrated to be composed of plates, but it is probable that most of this formless granular matter is the result of the disintegration and metamorphosis of the plates. That some of the granules are produced by the disintegration of leucocytes is probable, for it is not difficult to demonstrate the destruction of leucocytes in many thrombi. I believe also that a granular precipitate in thrombi is sometimes caused by the hardening agents.

As regards fibrin, I can confirm the recent statements of Weigert as to the abundance and the constant presence of this substance in all marantic thrombi, except in softened foci where it is absent. Some thrombi are composed almost wholly of fibrin. The fibrin may assume various forms, such as the form of a delicate network, or of coarse interlacing or parallel bands, or of irregular masses, or of the so-called canalized fibrin. In sufficiently thin sections, such as can be made from specimens imbedded in paraffine, there is generally no difficulty in demonstrating in thrombi a rich network of fibrin even without the aid of Weigert's special stain for this purpose. Leaving out of question, therefore, the nature of the blood-plates there can be no doubt that human thrombi, as we meet them at autopsies, are genuine coagula, save in the foci of so-called puriform softening.

Hyaline material appears to be formed both out of fibrin and out of blood plates. Thrombi composed wholly of hyaline I have found in the liver of a cat in which a few drops of croton-oil had been injected, in hemorrhagic infarctions of the lungs, and in corroding ulcers of the duodenum and of the stomach. Hyaline is an inconstant constituent of thrombi, but its presence is not rare.

The accumulation of leucocytes in human white thrombi is so well known that there would be no necessity of emphasizing it here, were it

not that the recent study of experimental thrombi has led to a revision of the doctrine that white thrombi are composed primarily of masses of white blood-corpuscles. While it is true, as has already been mentioned, that there are thrombi which are composed almost entirely of plates, or of fibrin, or of hyaline, or of these substances in combination, this is the exception, and in the vast majority of fresh thrombi leucocytes are present in large number. In inflammatory thrombi leucocytes may be so abundant as to obscure all other constituents. Usually the leucocytes in marantic thrombi are not scattered uniformly throughout the thrombus, but are massed together in clumps; these clumps of leucocytes, unlike the clumps of plates, are generally pervaded by a network of fibrin.

It is not at all infrequent to find in old thrombi leucocytes and even masses of them which are devoid of nuclei. In undergoing this necrosis the nuclei of the white blood-corpuscles may be broken up into fragments which can be recognized as small irregular particles which assume a nuclear staining, but this nuclear fragmentation does not seem to be the rule. Generally the necrotic leucocytes can be recognized simply by their form, without any trace of nuclei.

Red corpuscles are present in variable numbers in marantic thrombi. They cannot be regarded as an essential constituent of the thrombus. I find in many marantic thrombi the so-called shadows of the red blood-corpuscles, which can be easily overlooked unless especial attention be given to searching for them.

In properly prepared sections it is not rare to find colonies of micrococci even in thrombi not connected with pyæmic processes, especially in marantic thrombi from cases of typhoid fever or other infectious diseases.

From the foregoing summary of the histological characters of human white thrombi, it is apparent that any satisfactory explanation of the process of thrombosis must account for the presence of blood plates, of fibrin, and of leucocytes, for these are essential constituents of thrombi. The valuable investigations by Eberth and Schimmelbusch of experimental thrombi have led them to regard the blood plates as the sole primary elements in these plugs. Further investigations are needed to determine the rôle played by fibrin and white blood-corpuscles in the formation of experimental thrombi.

My experiments upon the production of thrombi have been made mostly on dogs. The vessels selected have been the femoral artery, the femoral vein, and the jugular vein, in the majority of cases the femoral vessels. Various methods were employed to produce thrombi, such as

the application of caustics, the introduction of foreign bodies, and various mechanical injuries. I have given the most attention to the thrombi resulting from mechanical injuries, for it is admitted by Eberth and Schimmelbusch that the thrombi following the application of caustics, and those formed around foreign substances, contain, in an early stage, if not at their beginning, fibrin as well as blood plates. These authors assert that "coagulation is a process which plays only a modest rôle in the circulating blood, whereas here the most prominent and frequent phenomenon is conglutination, which, indeed, is solely concerned in the practically most important form of thrombosis, viz., that following simple mechanical injury of the vessel-wall, in whatever way this may have been produced."[1]

The following two modes of producing mechanically thrombi have given good results. One method is to tie a stout ligature tightly and suddenly around the vessel and at once cut the ligature loose. In this way the intima and a part of the media are usually torn. It is only when great force is used that the vessel is ruptured. The ligature leaves a whitish ring around the vessel at the seat of its application. If, as often happens, the walls of the vessel remain stuck together after removal of the ligature, then moulding the vessel slightly between the fingers will restore the lumen, which now presents a fusiform dilatation at the seat of injury. The adventitia in this situation often becomes infiltrated with blood.

Another method which I have employed, is to push into a branch of the femoral artery or femoral vein one of the hooked instruments or gouges employed by dentists and called excavators, and then, after pressing the hooked extremity forward into the main trunk to scrape the inner wall of the vessel to any extent desired. The stem of the instrument, if necessary, can be filed down so as to render its introduction easier. After the withdrawal of the instrument the opened branch of the vessel is secured by two ligatures. Of the various shapes which the working extremities of these instruments possess, those with a small cup-shaped gouge bent at right angles to the handle (spoon excavator) I have found particularly suitable.

The animals experimented upon have been tied down and anæsthetized, usually with morphine and chloral, or morphine and ether, or morphine alone.

It is important, when the vessel is removed from the body, that four ligatures should be applied, two above and two below the seat of injury,

[1] Eberth and Schimmelbusch. Virchow's Archiv, Bd. 105, p. 459.

and that any collateral branches included between the pairs of ligatures should also be tied. These ligatures should be applied with as little disturbance of the vessel as possible. If the vessel be cut out without the preliminary application of ligatures, the contents of the vessel are partly discharged, and in this way the thrombus may be lost or its position changed.

Various hardening fluids were employed, such as corrosive sublimate, alcohol, Müller's fluid, picric acid, osmic acid, and Flemming's solution. Of these, warm saturated solutions of corrosive sublimate are decidedly the best. This fluid preserves the blood plates and other elements almost perfectly and admits satisfactory subsequent staining of the specimens.

The procedure adopted in hardening in corrosive sublimate is the following: A clear, saturated aqueous solution containing some undissolved sublimate at the bottom is heated to 40° C., and in this is suspended the specimen to be hardened. After a few minutes I have usually cut away the ligatures at the ends of the vessel, as there is now no danger of the escape of the contents. The vessel containing the sublimate solution and specimen is kept in a thermostat at a temperature of 40° for one to two hours. The specimen is then washed in water having a temperature of 40° and afterward placed in a mixture of half alcohol and half water, and kept in this mixture at a temperature of 40° for a number of hours, often over night. This prolonged washing is to remove crystals which otherwise are present in large number. Even after this treatment sometimes peculiar crystals are present, which, however, do not materially interfere with the study of the sections. The addition of a little iodine to the washing fluid, as suggested to me by Prof. Gaule, assists in removing the crystals. From the fifty per cent. alcohol the specimen is transferred to strong, and finally to absolute alcohol. I have made use almost exclusively of paraffine as an imbedding medium, as much thinner sections can be obtained in this way than by imbedding in celloidine. Satisfactory results can be obtained by staining the specimens *en masse* in hæmatoxylin and in eosin, but, as a rule, the sections have been stained after causing them to adhere to the slide. When serial sections were desired, they were cut in the form of ribbons. Gaule's method of making the sections adhere to the slide by placing upon each section laid dry upon the slide a drop or two of forty or fifty per cent. alcohol, and after ten minutes putting the slides in a thermostat at a temperature of 40° and keeping them there for two hours, is the simplest and best with which I am acquainted. After this treatment the sections are so firmly adherent that all the manipulations of staining and preparing the sections for mounting can be carried on without fear of their detachment.

In sections stained with hæmatoxylin and eosin the plates have a violet tint, and when in masses can be readily recognized with a low power.

I wish first to direct your attention to the macroscopic and the microscopic appearances of fresh experimental plate thrombi. Such a thrombus may be conveniently produced by tying forcibly a coarse string around the femoral artery of a dog and then at once cutting the string loose in the manner already described. After the time desired for the production of the thrombus has elapsed, the injured part of the artery inclosed between two pairs of ligatures is to be removed and the artery is to be carefully slit up with a pair of delicate scissors.

Let us examine an artery treated in this way which contains a thrombus formed within five minutes. There will be found, closely adherent to the torn inner wall of the vessel, a parietal thrombus which at this period does not usually extend in a longitudinal direction much beyond the ring of lacerated tissue. The thrombus can be readily distinguished by its color from the blood which envelops it and which can be washed away with salt solution. The thrombus projects irregularly into the lumen of the vessel, the projecting part being made up usually of round or irregular masses which are connected together.

The thrombus has a homogeneous, grayish, translucent appearance, comparable to particles of boiled sago, and resembling, therefore, the color of the Malpighian bodies in a waxy spleen. When bits of the thrombus are pressed into thin layers between the slide and the cover-glass they present a bluish transparency almost glass-like. The epithet hyaline can be appropriately applied to the naked-eye appearance of the thrombus.

The consistence of the thrombus is soft, the weight of the cover-glass sufficing to make bits of the thrombus spread out into thin layers. In attempting to tease apart portions of the thrombus, it is found that this does not break up into little granules, as would be the case if the blood-plates which compose it had undergone no changes and were simply in apposition to each other; but, on the other hand, the thrombus possesses considerable cohesion, and in breaking it into fragments with teasing needles fine sticky threads can be drawn out a short distance which break apart, or, if the tension be removed, retract. Portions of the thrombus pressed between the fingers present a sticky, gelatinous consistence. In view of Hanau's objection, already mentioned, to Eberth and Schimmelbusch's designation of the change by which the plates adhere to each other as a viscous metamorphosis, it is to be emphasized that fresh plate thrombi have a somewhat viscid consistence, which becomes more marked in the course of an hour after the removal of the

thrombus. I cannot, therefore, accept Hanau's objection, so far as this point is concerned, as valid.

If parts of the fresh thrombus be teased in physiological salt solution or in Bizzozero's methyl-violet salt solution, or in Hayem's solution, there will be seen masses of blood plates and a large number of free plates floating in the liquid. The plates appear as pale, well differentiated, round or somewhat irregular bodies varying in size, the average being about one-quarter the diameter of a red blood-corpuscle. Masses of plates resemble colonies of large micrococci. They can be made to assume feeble tints with a variety of coloring agents, but I have not been able to give them, in their fresh state, a sharp, elective staining. In water the plates become paler and somewhat swollen; in very dilute acetic acid they become darker in color and more distinct, in strong acetic acid they disappear from view.

By tapping with a needle upon the cover-glass it can be seen that the individuals composing the masses of plates adhere to each other. Such masses may be readily flattened out and compressed. The plates, especially when in masses, may be drawn out lengthwise by currents of fluid or by pressure. The remarkable viscidity of the plates can be demonstrated by placing a bit of filter paper at the edge of the cover-glass and thus causing currents in the fluid which fail to draw along even the isolated plates.

In order to see the regular and characteristic appearance of the fresh plates when arranged in masses, it is necessary that they should not be subjected to any pressure. When masses of plates are compressed even by the weight of the cover-glass they often appear to be made up wholly or in part of pale lines instead of coarse granules. This appearance of lines or threads seems to be due to an elongation and coalescence of the plates. These lines are often arranged with considerable regularity. They might be mistaken for threads of fibrin. A similar appearance of threads produced by coalescence of the plates is sometimes seen in hardened specimens where the threads are often varicose. Whether this appearance is due to the action of the hardening agent or to some other influence, such as the force of the circulating blood, must be left unsettled.

Although the plates make up the great mass of the thrombus leucocytes are present even at this early stage (during the first five minutes), and rapidly increase in number, so that at the end of half an hour the thrombus usually contains them in abundance. My investigations have not led me to assign so insignificant a rôle to the leucocytes in experimental thrombi as is done by Eberth and Schimmelbusch. I agree with

these authors in finding that thrombi produced mechanically in the manner mentioned consist in their inception essentially of blood plates. At the end of five minutes the leucocytes may be so scanty as not to attract attention. Usually, however, by this time clumps of leucocytes as well as scattered leucocytes are present here and there in the thrombus, and, as already mentioned, their number continues to increase. I have found them at the end of six hours, in mechanical thrombi, as numerous as in many human marantic thrombi. It is true that much diversity exists as regards the number of leucocytes even in thrombi of the same age, still it is the rule that white corpuscles, while they do not collect so rapidly or in such number as the blood plates, do accumulate and form a constituent part of experimental mechanical thrombi. In order to study the situation of the leucocytes sections of hardened specimens are necessary, but portions of fresh thrombi teased apart and treated with dilute acetic acid are favorable for ascertaining their number.

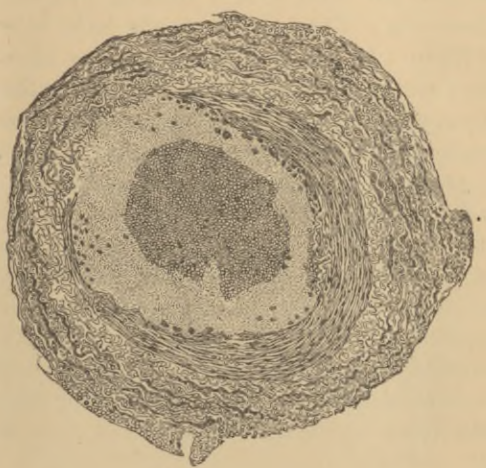
In searching for fibrin in fresh thrombi, it is important not to mistake for fibrin the threads produced by compression of the clumps of plates in the manner already mentioned. If these flattened-out masses of plates be washed with water, or, better still, with dilute Lugol's solution, there may be produced an appearance of interlacing and of parallel threads, which bear considerable resemblance to fibrin, but which are paler and which do not project beyond the margins of the clumps. Unmistakable fibrin in the form of fibrils, however, is to be found in experimental mechanical thrombi, but, so far as my observations go, not in the earliest stage of their formation. I have found fibrin in thrombi at the end of five minutes; frequently at the end of fifteen minutes, and usually at the end of half an hour. Fibrin is often found in so much larger amount in the fresh thrombi than in sections of the hardened thrombi that it is probable that it is formed in part after the removal of the vessel. As will be mentioned subsequently, fibrin can be demonstrated, also, in the hardened specimens, although not in the youngest thrombi. I am not able, therefore, to agree with Eberth and Schimmelbusch in denying altogether the presence of fibrillated fibrin in experimental thrombi produced by mechanical injury of the vessel, although our observations are in accord regarding the youngest thrombi.

I have dwelt thus at length upon the appearances of the fresh experimental thrombi because I have been unable to find any description of these appearances. With the exception of a brief allusion by Lubnitzky to sections of frozen thrombi, all the previous observations seem to have been made either upon the mode of formation of these thrombi in the living circulation or upon sections of hardened specimens. As has been

suggested by Weigert, and as is apparent from the foregoing description, a knowledge of the gross appearances of the plate thrombi is important in forming a judgment as to their nature. Sections are, of course, necessary to enable us to study more carefully the constituents of the thrombi and particularly their arrangement.

As already mentioned, I have made use chiefly of corrosive sublimate as a hardening agent, of paraffine as an imbedding medium, and of hæmatoxylin and eosin as staining agents.[1] In sections prepared in this way the plates can be seen with a distinctness and uniformity in shape that leave nothing to be desired. I am led to believe that most of the appearances which have been described as changes in the plates occurring during the first half hour (Lubnitzky and others), are due to imperfect methods of hardening. Eberth and Schimmelbusch recognize this fact in their preparations.

FIG. 1.



Section of dog's femoral artery containing thrombus formed in four minutes after forcible ligation of the vessel. Zeiss A. ocular 1.

Plate thrombi can be recognized in sections as well as in the fresh state by their peculiar translucence. I can only confirm the statements of Eberth and Schimmelbusch as to the composition of the experimental thrombi in their earliest formation. They are made up of blood plates. To the torn and partly detached internal elastic lamella as well as to the

[1] Since the delivery of this address I have also made use of Weigert's new method of staining fibrin on specimens hardened in alcohol.

lacerated media masses of plates are attached, which extend into the lumen of the vessel. Upon sections clumps of plates often appear to lie free in the lumen surrounded by blood, but subsequent sections show the connection of these clumps with others or with parts of the vascular wall. The thrombus often forms a complete ring around the torn inner wall of the vessel (Fig. 1). Not every injured part of the internal wall of the vessel is covered with a thrombus. One is often surprised to find such parts, even when severely lacerated, entirely free from thrombi or with only a thin layer of plates, recognized with a high magnifying power. The mass of plates composing the thrombus does not always appear uniform, but often, especially in older thrombi, there are lighter and darker portions, due apparently to greater density in the number of plates in some places than in others.

An appearance mentioned by Hanau is of interest, namely, the presence of a dark band around the margin of masses or islands of plates. Similar dense lines can often be seen running irregularly through sections of the thrombus. These denser bands have been interpreted as hyaline or as fibrin. It is not easy to determine their exact nature. They look like fibrin in many cases, but it is possible that they are produced by coalescence of the plates as the result of pressure or traction from the circulating blood in a manner similar to the lines which can be artifically produced in masses of fresh plates by pressure in the manner already described. Plates are not confined to the interior of the vessel, but when the internal elastic lamella and the media have been ruptured they often find their way in masses into the layers of the torn media or even into the adventitia. It is interesting to note the absence of any transition, as a rule, between the thrombus and the blood. The plates are just as dense usually at the margin of the thrombus as in the interior, and immediately adjacent to the peripheral plates come the red blood-corpuscles where the blood was still circulating before the vessel was removed.

Leucocytes are not generally present in large number in thrombi during the first five minutes of their formation. If serial sections be examined, here and there clumps of white corpuscles can be found even at this early stage. There are often more white corpuscles mingled with the plate masses in the coats of the vessel than in the thrombus proper. Leucocytes, scanty at first, continue to accumulate in larger and larger number, until they form a prominent part of the thrombus. I have found them in great abundance at the end of half an hour, although sometimes at this period their number is small. The leucocytes are generally arranged in clumps between masses of plates, although some

are usually scattered in among the plates. It is probable that the clumps of leucocytes are deposited in that form directly from the circulating blood. There is reason to believe that the leucocytes may also wander into the thrombus, for in sublimate specimens elongated nuclei, such as are seen in undoubtedly wandering white corpuscles in the wall of the vessel, can also be occasionally detected in the masses of plates. Moreover, the number of leucocytes within these masses increases with time. In successfully prepared sections the protoplasm can be seen around the nuclei of the white corpuscles, so that I do not agree with Lubnitzky that this has become merged with the plates. Sometimes the leucocytes are surrounded with a clear zone as if they lay in little spaces within the mass of plates, but this appearance is probably due to the action of the hardening fluid. Both uninuclear and multinuclear white corpuscles are present, but the latter predominate, and in the later stages many of the nuclei often appear much broken up.

FIG. 2.

From section of half-hour thrombus produced by forcible ligature of femoral artery of dog (see text). Shows masses of blood plates containing bands of fibrin and leucocytes. Zeiss D. ocular 3.

Although I have not seen any appearances which indicate that the white corpuscles disintegrate into granules, still non-nucleated white corpuscles can sometimes be detected, so that a necrosis or death of these corpuscles may take place within the thrombus. This does not seem, however, to be a common or extensive process.

As has already been stated, fibrillated fibrin is present in experi-

mental thrombi produced by mechanical injury of the vessel. It is not, however, found in the youngest thrombi, and the date of its appearance varies in different cases. I have found it in hardened specimens at the end of five minutes, but this is exceptional. It is not uncommon to find it at the end of fifteen minutes. I exhibit under the microscope sections of a thrombus of one-half hour's duration, in which there is a considerable amount of distinct fibrillated fibrin (Fig. 2). The amount of fibrin increases with the age of the thrombus, and in thrombi of twenty-four hours duration fibrin makes up usually a large part of the thrombus.

The fibrin appears in islands and bands between the masses of plates, and often extends in coarse fibres into the surrounding blood. The network is usually coarse, but fine threads are also present. After a time the clumps of white and of red corpuscles included in the thrombus are pervaded by a network of fibrin, whereas, this is absent in the dense clumps of plates. I have the impression that there is, in general, a relation between the number of leucocytes and the amount of fibrin, although the former appear in considerable quantity before the latter.

Inasmuch as in older thrombi (twenty-four to forty-eight hours) fibrin and leucocytes compose a large part of the thrombus, whereas, at its inception the thrombus is made up almost entirely of blood-plates, one is tempted to believe that the plates may be transformed into fibrin, but of this transformation I can bring no positive proof. The plate-masses, after a time, lose their regular granular appearance and appear darker in color and more homogeneous, but typical plates may be found in large number in thrombi forty-eight hours old.

It is apparent from the foregoing description, that experimental thrombi acquire with time all of the characteristics of human thrombi. The suspicion which has been raised that they represent a distinct class of thrombi, from the study of which we can draw no conclusion as to the formation of human thrombi, is unjustifiable. It is another question whether we are to suppose that all human white thrombi are formed in the manner described. Although I have not succeeded in producing permanent leucocytic thrombi experimentally, still there is every reason to believe that some human thrombi are composed from the beginning essentially of leucocytes. In observations which I have made recently for another purpose, of the living circulation in the mesentery of dogs, I have observed the formation of small parietal thrombi composed of white corpuscles, but these have invariably been washed away after a short time.

We may, it seems to me, think of the mode of formation of the experimental thrombi, which we have studied, and doubtless also of many human thrombi as follows: Given suitable conditions, such as alteration

of the vessel wall, slowing and irregularity of the circulation, the first constituents of the thrombus to accumulate are the blood plates. But although the plates collect at first in larger number and more rapidly, the leucocytes do not long remain absent, and in the course of time they are present in such quantity that they must be considered an essential constituent of the completed thrombus. At first the conditions for the coagulation of fibrin are not present, but with the increasing accumulation of leucocytes these conditions appear and fibrillated fibrin is deposited. It is in harmony with the current ideas concerning the cause of the coagulation of fibrin, to suppose that at first the fibrin ferment is absent, and that this is subsequently furnished by the leucocytes. The absence of fibrin in the early thrombi composed wholly of plates, is an argument additional to the evidence brought forward by Löwit and others, that the plates do not furnish the fibrin ferment. It is apparently only after the leucocytes have been included for a time in the thrombus that they die or undergo some alteration in their constitution which leads to the formation of the fibrin ferment. The final result is a plug composed of plates, leucocytes, and fibrin, and included red blood-corpuscles.

It seems to me an error to base our conception of the nature of a thrombus exclusively upon the constitution of the thrombus in its inception. While admitting that the thrombus is at first composed wholly of blood plates, we do not, as a matter of fact, meet with human thrombi in this early stage, or at least, only under exceptional circumstances. Our ideas as to the constitution of thrombi are based upon the examinations of the completed plugs which contain fibrin and leucocytes as well as plates. The study of the experimental thrombi enables us to form a clearer conception of the mode of formation of the thrombus, but does not necessitate any radical change in our ideas as to what constitutes a thrombus.

The question as to whether a thrombus is a coagulum or not, is, of course, open to discussion only regarding the plate thrombi in their earliest formation. Whether or not we are to regard the plate thrombi before fibrin has made its appearance as coagula, is a question which is not likely to be settled until we acquire more definite information as to the origin and nature of the blood plates. There is nothing in the gross appearances of these plate thrombi which would prevent us from considering them as soft, gelatinous coagula. Wooldridge, Löwit, and others believe that the plates are allied to fibrin but are not identical with it. I purposely avoid entering into any discussion here as to their existence in the normal circulation, for this is a point which must still

be regarded as *sub judice*, and which is not likely to be settled by the experimental study of thrombi.

The attempt of Eberth and Schimmelbusch to draw a sharp distinction between thrombi formed by conglutination and thrombi formed by coagulation, seems to me unwarranted. In the first place the process which they designate as conglutination may be, so far as we at present know, a form of coagulation. In the second place, whatever we may think as to the nature of the process of conglutination, the preceding investigations have demonstrated the transformation of conglutination thrombi into undoubted coagulation thrombi.

As regards the relation between changes in the walls of the vessels and thrombosis, I have reached the same conclusion as that expressed by von Recklinghausen, Eberth and Schimmelbusch, and others, that Cohnheim's views on this point were too exclusive. It is possible to produce experimentally severe injury of the internal coats of bloodvessels without any resulting thrombus. Among many positive results I have also in my notes the records of not a few negative results which have followed injury of the walls of the vessels by caustics, by forcible application of rough clamps, by scraping the interior of the vessel, etc. As is urged by these writers as well as by Weigert and others, slowing of the circulation and irregularities of the circulation produced by abnormalities in the lumen of the bloodvessels, are factors no less important in the production of thrombi than alterations in the vessel walls.

There is much which speaks for the correctness of the view advocated by Köhler, Hanau, and others, that some thrombi are caused by fermentative changes in the blood. Cases such as the one already mentioned, of extensive thrombosis of a large number of the bloodvessels throughout the body, are most naturally interpreted as examples of fermentation thrombosis.