

Note on the formation of fibrine / by Mrs. Ernest Hart.

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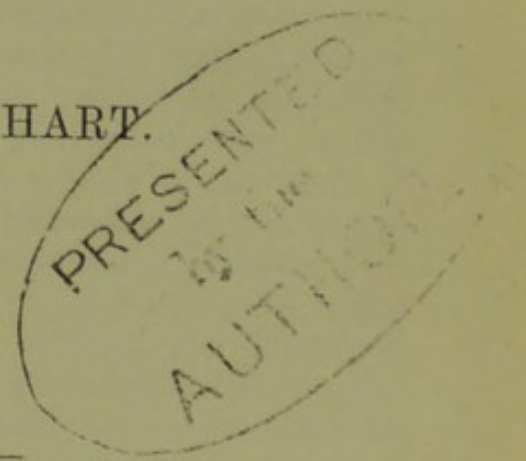
ON THE

FORMATION OF FIBRINE.



BY

MRS. ERNEST HART.



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NOTE *on the* FORMATION of FIBRINE. By Mrs. ERNEST HART.

(With Plate XXI.)

THE research, of which this paper is a record, was made in the laboratory of M. Ranvier, at the Collège de France, in the Autumn of 1879. It has remained so long unpublished because I was always in hopes of being able to make the work more complete and exhaustive. In presenting it now for publication and criticism, I do so with great diffidence, knowing that the work is incomplete; yet I am assured by competent authorities that, viewed in connection with other work on this subject now being carried on in various parts of the world, it may not be without utility and suggestiveness, as an addition to the data for the investigation of the production of fibrine.

At the conclusion of the little paper which I published in the 'London Medical Record' in January, 1880, on the Norris corpuscle, I stated my opinion that the colourless corpuscles described by Dr. Norris, and seen by him and others who follow his methods, "are red corpuscles that have undergone post mortem changes prior to taking part in the formation of the fibrine. On this subject I hope shortly to publish some further observations." It was in repeating Dr. Norris's work on the invisible corpuscles, and by means of the very ingenious methods which he has invented to obtain exceedingly fine films of blood, that I observed the appearances I am about to describe. It will perhaps be remembered that Dr. Norris's methods consist in what he calls "isolation" and "packing." The method of "isolation," which is that which I found so very useful, is as follows:—A perfectly smooth and level slide is chosen, and at some slight distance from the centre a small hole is drilled into which a metal eyelet is inserted, care being taken that the metal edge is not raised above the glass surface. A smooth and level thin cover-glass is now chosen and strapped on to the slide by means of a narrow piece of diachylon plaster, so that the free edge exactly overlaps the metal eyelet; a screw fitting the eyelet

is then inserted into the hole, and the method of procedure is as follows:—The tip of the finger is pricked and a drop of blood is placed, as rapidly as possible, at one of the free edges of the cover glass; the blood enters by capillary attraction, forming a delicate even layer between the two glass surfaces; the slide is then inverted over a shallow vessel containing a 2-per cent. solution of osmic acid, and the hinged cover glass is gently raised by passing the screw further through the eyelet. All the fluid particles, and the great majority of the corpuscles, flow immediately towards the hinged edge of the cover glass, leaving only a few red corpuscles, and here and there a white one, adhering to the glass surface; these are *instantaneously* fixed by the action of the osmic acid vapour—a fact originally pointed out by Professor Ray Lankester many years ago—indeed so perfect and complete is the fixing of the corpuscles on the glass by the action of the osmic acid vapour, that the glass may be immersed for a long time in water, and may even be dried roughly with a towel without displacing or injuring them. Among the corpuscles which have adhered to the glass surface, Dr. Norris discovers, by various means of staining, his invisible corpuscle. That it is there I do not deny, but that it is there because it previously existed in this condition in the blood in the living state is I think open to dispute; in fact, as I stated in my former paper, these colourless discs are in my opinion unstable red corpuscles, or corpuscles of low resistance, which have parted with their hæmoglobin, possibly simply by the fact of the withdrawal of the serum.

In continuing these investigations and in repeating this experiment of "isolation" a great number of times, I began to observe that the appearances changed according to the length of time which elapsed between the spreading of the layer of blood between the two glass surfaces and the moment when the cover glass was raised; and thus discovered that a whole series of phenomena could be traced, leading from the pale or colourless corpuscle up to the complete formation of networks or bands of fibrine. In developing this method of working I found that the staining reagents recommended by Dr. Norris were not sufficiently powerful to bring out all the details that could be observed on the glass surfaces, and after many trials I found that a highly concentrated solution of nitrate of rosanilin in absolute alcohol was the best staining reagent to use. The method I adopted was to detach the cover glass from the slide after the corpuscles had been fixed by the osmic acid vapour, and to examine both the surfaces of the cover glass and the slide under the microscope, to see which presented the most perfect preparations. Having made a selection I deposited a drop of

the concentrated solution of nitrate of rosanilin on ^{the} ~~to~~ glass and allowed it to remain for a few moments, then washed it off with a fine jet of distilled water. The red, pale and colourless corpuscles, with their ramifications and the most delicate fibrils of fibrine, then become visible under a high power. These preparations may be mounted dry and will keep for a great length of time. If the process be performed as rapidly as the dexterity gained by an oft repeated experiment will allow, the appearances presented in fig. 1 will be seen. In this it will be observed that the circular appearance of the corpuscles is perfectly preserved, and that every shade of colour may be found, from the normal red corpuscles down to the colourless Norris corpuscle, which only takes the faintest tint of pink. If, however, the glass surfaces be allowed to remain in contact for a moment, the colourless corpuscles are found to have lost their globular form and to have become pyriform or elongated, as shown in fig. 2. On leaving the glass surfaces still longer in contact, these pale corpuscles are observed to undergo a remarkable change, they send out long processes or tails, which bifurcate and divaricate in every direction. Fig. 3 gives some specimens of these branching cells carefully drawn with the camera lucida; they were, it is true, not all obtained from the same plate, but have been grouped together for convenience. Fig. 4 gives perhaps a more remarkable specimen of these branching corpuscles. All the former specimens are from human blood, but figs. 4 and 5 are from rabbit's blood. On allowing a still longer interval to elapse, so that it is more than probable that coagulation would occur in a film of blood lying between two glass surfaces, and on separating these surfaces, perfect specimens of fibrine may be obtained after staining. These are represented in figs. 6 and 7. On now searching the field the pale corpuscles, which could formerly almost always be discovered, are nowhere to be found, and the conclusion is forced upon one that the branching corpuscles have developed or broken down into fibrinous threads. Small granules (D., fig. 6) are, however, found from which threads of fibrine appear to spring. These granules are described in M. Ranvier's "Traité Technique d'Histologie," as the centres of fibrine formation. They appear to me to be all that is left of the pale corpuscles, whose intermediate transformations have not before been recognised, but may, I believe, perhaps be identified with the appearances and changes which I have described and figured. I repeated these experiments a great number of times, with the object of arriving at the exact moment when I should be able to verify this hypothesis, and trace the fibres of fibrine into one or more of the pale corpuscles, and in fig. 8, which is drawn faith-

fully from the preparation, this departure of the fibrils of fibrine from the pale corpuscles is, I think, demonstrated.

In the well-known and generally accepted theory of A. Schmidt as to the formation of fibrine, it is assumed that the red corpuscles part with fibrino-plastin before the formation of fibrine can be accomplished. In the course of my research this theory seemed to me to be often capable of physical demonstration by the appearances the red corpuscles sometimes present, for they occasionally appear to be in the act of discharging part of their contents. This appearance is shown in fig. 9, E. The crescentic corpuscles, which are also figured (D., fig. 9), seem to show a loss of substance. They were very frequently found both in human and in rabbit's blood. Of these crescentic corpuscles I am unable to give any satisfactory explanation.

It may be objected that the tails of the colourless and pale corpuscles are produced by currents of the serum. This objection was present to my mind the whole time that these experiments were being performed, and great care was taken to ascertain if the processes pointed in the direction taken by the fluid. In fig. 5 it will be noted, however, that the tails of the corpuscles point in opposite directions, and their position does not seem to me at all to justify the hypothesis that they may be produced by eddies; also it will be observed that in fig. 5 both ends of the corpuscles are sending out processes. In fig. 3, moreover, it may be seen that the processes do not take the direction of any possible current, but that they regularly divide and ramify like the branches of a tree. In fig. 10 two pale corpuscles (C) also will be seen sending out branches, which cross and lie over one another; this one fact militates strongly against the explanation by currents.

Again, it may be objected that the processes sent out by the corpuscles and the bands and fibres represented in figs. 6 and 7 are not true fibrine. This is, I allow, a weighty objection.

As the preparations are all fixed by osmic acid vapour they are, therefore, incapable of being tested by chemical reagents for fibrine. To eliminate this error I may, however, state that I defibrinated fresh rabbit's blood and treated the defibrinated blood in the way already described, with the result of finding many pale corpuscles mixed with the red, (the pale corpuscle being, I imagine, the very first step in the formation of fibrine), but extremely few transparent corpuscles, a few crescents, and no fibrils. I also treated the serum of blood-clot in the same way, and found neither transparent corpuscles nor fibrils. To be certain, also, that the transparent branching corpuscles were not accidental productions, minute drops of serum coagulated on the glass by the action of the osmic

acid vapour, I repeated these experiments with pure white of egg, mixed with distilled water, till it had the consistency of blood, and also with fresh peritoneal and pericardial fluid, and failed to obtain any appearances resembling the pale or the branching corpuscles.

These preparations have been, during the last two years, inspected by many critical histologists and physiologists, who have expressed favorable opinions of the interest of the results, incomplete as they are. I have, however, long entertained the hope that I might have been able before this to have carried the research further, and to have demonstrated the same appearances in the blood by other methods, and under conditions which would have decided beyond a doubt whether, as I at present venture to opine, these branching corpuscles are the fibrine factors of the blood or not. If my opinions are correct and my work is confirmed, it is unnecessary to indicate the great interest which would attach to such an explanation of the formation of fibrine, and the light it would throw on much which has hitherto been obscure and doubtful in the phenomenon of coagulation, both within the body, and in blood withdrawn from the body. Other engagements have prevented me from carrying on the inquiry, and I do not see any immediate prospect of being able to resume it. In publishing this note now, I am sensible of the incompleteness of the observations, but I am led to believe that they may be useful in the present state of this subject, as furnishing material for suggestion and for further inquiry. The drawings were all made by my own hand by the camera lucida, and with the utmost desire to ensure fidelity of outline and correctness of colour, my object being to elucidate facts, not to enforce a theory.

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EXPLANATION OF PLATE XXI,

Illustrating Mrs. Ernest Hart's 'Note on the Formation of Fibrine.'

FIG. 1.—Red blood-corpuscles, isolated and fixed with osmic acid vapour, and stained with nitrate of rosanilin in absolute alcohol. The darkest-stained corpuscles are red corpuscles in their normal condition; those which are of a pale pink tint are the invisible corpuscles of Norris. Human blood.

FIG. 2.—Red blood-corpuscles, treated in the same way. A, normal red corpuscle; B, pale corpuscle; C, transparent corpuscles, beginning to send out processes. Human blood.

FIG. 3.—Pale and transparent corpuscles, sending out ramifications. A, A, A, the branches are proceeding from one point of the corpuscle only; B, B, corpuscles generally breaking down, and sending out branches in different directions. Human blood.

FIG. 4.—Group of transparent corpuscles, sending out bifurcating branches. Rabbit's blood.

FIG. 5.—Preparation of rabbit's blood, treated by "isolation," in which groups of transparent corpuscles are seen sending out tails, which in many instances are seen to bifurcate in opposite directions. A, A, normal red corpuscles; B, B, B, transparent tailed corpuscles, those at the lower edge of the drawing are observed to be sending out branches from both ends of the corpuscle.

FIG. 6.—Network of fibrine, entangling and distorting red corpuscles in its meshes. A, A, red corpuscles; B, multinucleated white corpuscle; C, pale corpuscle; D, granules; E, fibrils of fibrine. Human blood.

FIG. 7.—A band of threads of fibrine. A, A, normal red corpuscles; B, groups of granules; C, C, red corpuscles, divided by the band of fibrine. Human blood.

FIG. 8.—Preparation of human blood, showing the part the transparent corpuscles take in the formation of fibrine. A, A, normal red corpuscles; B, B, transparent corpuscles, from which threads of fibrine are seen to proceed; C, hæmotoblast of Hayem.

FIG. 9.—Preparation showing crescentic corpuscles, and corpuscles in the act of discharging their contents. A, normal red corpuscle; B, pale corpuscles; C, crescentic corpuscles; D, crescentic corpuscle with its circular rim complete; E, E, corpuscles discharging their contents.

FIG. 10.—Preparation showing the various changes of the red blood-corpuscles. A, normal red corpuscle; B, pale corpuscles; C, transparent corpuscles, three of which are seen to be sending out bifurcating branches; D, hæmotoblasts of Hayem.

