

**On the structure of the red-blood-corpuscule of oviparous vertebrata / by
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Publication/Creation

[London] : [publisher not identified], [1869], [©1869]

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ON THE
STRUCTURE
OF THE
RED BLOOD-CORPUSCLE OF OVIPAROUS
VERTEBRATA.

BY
WILLIAM S. SAVORY, F.R.S.

THE red blood-cell has been perhaps more frequently and fully examined than any other animal structure ; certainly none has evoked such various and even contradictory opinions of its nature. But without attempting here any history of these, it may be shortly said that amongst the conclusions now, and for a long time past, generally accepted, a chief one is that a fundamental distinction exists between the red corpuscle of Mammalia and that of the other vertebrate classes—that the red cell of the oviparous vertebrata possesses a nucleus which is not to be found in the corpuscle of the other class. This great distinction between the classes has of late years been over and over again laid down in the strongest and most unqualified terms.

But I venture to ask for a still further examination of this important subject.

As the oviparous red cell is commonly seen, there can be no doubt whatever about the existence of a "nucleus" in its interior. It is too striking an object to escape any eye; but I submit that its existence is due to the circumstances under which the corpuscle is seen, and the mode in which it is prepared for examination. I think it can be shown that the so-called nucleus is the result of the changes which the substance of the corpuscle undergoes after death (and which are usually hastened and exaggerated by exposure), and the disturbance to which it is subjected in being mounted for the microscope. When a drop of blood is prepared for examination, little or no attention is given to the few seconds, more or less, which are consumed in the manipulation. It is usually either pressed or spread out on the glass slip, and

often mixed with water or some other fluid. But it is possible to place blood-cells under the microscope for examination so quickly, and with such slight disturbance, that they may be satisfactorily examined before the nuclei have begun to form. They may then be shown to be absolutely structureless throughout; and, moreover, as the examination is continued the gradual formation of the nuclei can be traced. The chief points to be attended to are—to mount a drop of blood as quickly as possible, to avoid as much as possible any exposure to air, to avoid as much as practicable contact of any foreign substance with the drop, or any disturbance of it.

After many trials of various plans, I find that the following will often succeed sufficiently well. Having the microscope, and everything else which is required, conveniently arranged for immediate use, an assistant secures the animal which is to furnish the blood (say, a frog or a newt), in such a way that the operator may cleanly divide some superficial vessel, as the femoral or humeral artery. He then instantly touches the drop of blood which exudes with the under surface of the glass which is to be used as the cover, immediately places this very lightly upon the slide, and has the whole under the microscope with the least possible delay. Thus for several seconds the blood-cells may be seen without any trace of nuclei; then, as the observation is continued, these gradually, but at first very faintly, appear; and the study of their formation affords strong proof of their absence from the living cells.

The "nucleus" first appears as an indistinct shadowy substance, usually, but not always, about the centre of the cell. The outline of it can hardly, for some seconds, be defined; but it gradually grows more distinct. Often some small portion of the edge appears clear before the rest. At the same time the nucleus is seen to be paler than the surrounding substance. Synchronously with this change—and this is noteworthy—the outline of the corpuscle (the "cell-wall") becomes broader and darker. What was at first a mere edge of homogeneous substance, becomes at length a dark border sharply defined from the coloured matter within. Thus a corpuscle, at first absolutely structureless, homogeneous throughout, is seen gradually to be resolved into central substance or nucleus, external layer or cell-wall, and an intermediate, coloured though very transparent, substance. But—and this is significant—these changes are not always thus fully carried out. It not seldom happens that the nucleus does not appear as a central well-defined regularly oval mass. Sometimes it never forms so as to be clearly traced in outline, but remains as an irregular shapeless mass, in its greater portion very obscure. Sometimes only a small part, if any, of an edge can be recognized, most of it appearing to blend indefinitely with the rest of the cell-substance. Sometimes it happens that in many corpuscles the formation of a nucleus does not proceed even so far as this. No distinct separation of substance can anywhere be seen, but shadows, more or less deep, here and there indicate that there is greater aggregation of matter at some parts than at others. Occasionally some of the cells

present throughout a granular aspect. I have almost invariably observed, too, a relation between the distinctness of the nucleus and of the cell-wall. When the nucleus is well defined, the cell-wall is strongly marked; when one is confused, the other is usually fainter. This, however, does not apply to colour; on the contrary, when the nucleus is least coloured it contrasts most strongly with the surrounding cell. As a rule, the wall of the cell is more strongly marked than the nucleus.

It will of course be said that the nuclei are present all the while, but are at first concealed by the surrounding substance—the contents of the cell. Thus the fact has been accounted for, that the nuclei are not so obvious at first as they subsequently become. But I think a careful comparison of cells will show that those in which a nucleus may be traced are not more transparent than others which are structureless; and, moreover, when one cell overlaps another, the lower one is seen through the upper clearly enough to show that the substance of these cells is sufficiently transparent to allow of a nucleus being discerned if it exists. When a nucleus is fully formed, it hides that portion of the outline of a cell which lies beneath it. How is it, then, if the nucleus is present from the first, that the portion of the cell over which it subsequently appears is, for a while, plainly seen?

The success of the observation is of course influenced by numerous circumstances. The rate at which the nuclei form in the corpuscles varies in different animals. I have usually found that in the common frog they are more prone to form than in many other animals—quicker than in most fishes, or even than in some birds. But this does not seem always to depend upon their larger size; for in the common newt the cells, which are larger than those of the frog, remain, as I have noticed, for a longer period without any appearance of nuclei. But even in the frog it can be satisfactorily demonstrated that the corpuscle is structureless.

I have found, too, that the observation succeeds best with the blood of animals which are healthy and vigorous. Thus the first observations upon fresh animals are usually the most satisfactory. After they have been repeatedly wounded or have lost much blood, the cells are more prone to undergo the changes which result in the production of nuclei.

Again, the formation of nuclei may be hastened, and their appearance rendered more distinct at last, by various reagents. Acids and many other reagents are well known to have this effect. The addition of a small quantity of water acts in the same way, but less energetically. It hastens the appearance of an indistinct nucleus, but interferes with the formation of a well-defined mass, so that, after the addition of water, neither the outline of the cell nor of the nucleus becomes so strongly marked as it often does without it. Exposure to air also promotes their formation; indeed, as a rule, the nuclei form best under simple exposure. Any disturbance of the drop, as by moving the point of a needle in it, certainly hastens the change; and perhaps it is influenced by temperature.

Sometimes, when the drop of blood has been skilfully mounted, the majority of cells will remain for a long while without any trace of nucleus; but, again, in almost every specimen, the nucleus in some few of the cells, particularly in those nearest the edges, begins to appear so rapidly that it is hardly possible to run over the whole field without finding some cells with an equivocal appearance.

It would follow, of course, from these observations that, if the living blood were examined in the vessels, the corpuscle would show no trace of any distinction of parts; and this is so. Indeed, in my earlier observations*, before I had learnt to mount a drop of blood for observation in a satisfactory manner, I examined, at some length, blood in the vessels of the most transparent parts I could select; and several observations on the web and lung of the frog and elsewhere were satisfactory. But still, when the cells were thus somewhat obscured by intervening membrane, one could not generally feel sure that the observation was so clear and complete, but that a faintly marked nucleus might escape detection. While, therefore, the result of observations on blood-cells in the vessels fully accords with the description I have given, I do not think that the demonstration of the fact, that while living they have no nucleus, can be made so plain and unequivocal as when they are removed from the vessels.

The question naturally arises, Why, then, does not a nucleus form in the mammalian corpuscle? But while it is accepted that the great majority of these corpuscles exhibit no nuclei after death, excellent observers still affirm their occasional existence; and I am convinced that an indistinct, imperfectly formed "nucleus" is often seen; and the shadowy substance seen in many of the smaller oviparous cells after they have been mounted for some time is very like that seen under similar circumstances in some of the corpuscles of Mammalia. Many, too, affirm that these corpuscles do not exhibit that distinction of wall and contents which is generally described. It appears to me that this difference of opinion depends on the changes they are prone to undergo. How far the absence of a distinctly defined "nucleus" after death depends on their smaller size I am not prepared to say.

Many questions of course follow. For example, how far is this separation of the substance of a homogeneous† corpuscle into nucleus, cell-membrane, and contents to be compared to the coagulation of the blood? and how do the agents which are known to influence the one process affect the other? A still further and more important question is, How are these changes in the corpuscles, and in the blood around them, related? But in this paper I propose to go no further than the statement that the

* Made many years ago. Other observers have been unable to detect a nucleus in the living cells within the vessels.

† By the word homogeneous I do not mean to affirm that the substance of the corpuscle is of equal consistence throughout. The central may be the softest part of it. But I regard the corpuscle, in its whole substance, as "having the same nature."

red corpuscle of all vertebrata is, in its natural state, structureless. When living, no distinction of parts can be recognized; and the existence of a nucleus in the red corpuscles of ovipara is due to changes after death, or removal from the vessels.

I cannot conclude this paper without acknowledging the great help have received in this investigation from Mr. Howard Marsh, Demonstrator of Microscopical Anatomy at St. Bartholomew's Hospital.



