Observations on the development of the blood corpuscles in the chick, with the various changes which they present from their first appearance to their full development : with some remarks on these changes / by William Macleod.

To Dr Craigie with the Author's Most Respectful Compliments

OBSERVATIONS

ON THE

DEVELOPMENT OF THE BLOOD CORPUSCLES IN THE CHICK,

WITH THE

VARIOUS CHANGES WHICH THEY PRESENT FROM THEIR FIRST APPEARANCE TO THEIR FULL DEVELOPMENT;

WITH SOME REMARKS ON THESE CHANGES.

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(*Extracted from the Lond. and Edin. Med. Jour. for Sept.* 1842.

BALFOUR AND JACK, PRINTERS.

OBSERVATIONS ON THE DEVELOPMENT OF THE BLOOD CORPUSCLES IN THE CHICK.

Previous to 1838, physiologists were wholly unacquainted with any general law by which the development of structure could be explained. In that year, Schwann of Berlin induced, from the generalization of numerous isolated facts scattered through the various scientific journals, along with his own observations, the grand and universal principle, that all organized structures have their origin from cells.[1] Since that year, numerous microscopic observers have increased to a great extent the facts by which the above principle is supported.

The following observations are designed to show the manner of formation of one of the bodies belonging to the first class of Schwann's classification of tissues—the blood corpuscles—with the different changes which they present from their first appearance to their full development. These were made on the blood corpuscles of the chick. Before describing the appearances which they present in their process of development, I shall state shortly the manner in which these observations were conducted. To get the blood perfectly pure, I removed it from the heart of the chick, (this can be done so early as the third day,) so that none of the granules contained in the nutritious matter which surrounds the embryo could enter the blood. I diluted the blood thus removed with fresh filtered serum. To allow the appearances going on within the corpuscle to be seen more distinctly, I used weak acetic acid, which was the only reagent employed. I also examined the blood contained in the germinal membrane and allantois, diluting it with the fluid albumen contained in the

[1] This statement is not altogether true, as Raspail, in his *Organic Chemistry*, has the following sentence:—"Organization, on the contrary, is, as it were, a crystallization which gives a tendency to assume a vesicular form, and produces a cell capable of absorbing the gases necessary both for its vesicular development, and for the reproduction of others similar to it. This cell is a laboratory where gases are condensed into liquids, which in their turn are organized into textures. It is in short, an organized body."[1] And in one of his papers in reply to Dutrochet, published a short time subsequent to his *New System of Organic Chemistry*, is contained the following startling expression. "Give me a cell capable of producing others, and I will form an organised world." These are not his exact words, and I cannot at this moment lay my hands on the paper in which this remark is contained; but the idea, to which the above sentence gives birth, is the same as that contained in the original.

[1] Raspail's Chemistry, translated by Dr Wm. Henderson, p. 10.

egg; and the appearances which the blood corpuscles presented in all these situations, were the same, provided the two last structures were examined at the proper time; for if the examination is made very early, the oval appearance of the blood corpuscles will not be seen, as they do not take this form until after the fourth day.[1] By examining the blood in these different ways, I hoped to be able to remove all chance of fallacy.

I shall divide the following description into three stages. In the first, the different changes which the blood corpuscles present from their first appearance, until their central part has become granular. Second, from the gradual disappearance of this granular structure, to the flattening of the sides of the corpuscle. And, *thirdly*, the changes which they present whilst passing from the circular to the oval form.

First Stage.—On placing the blood removed in the manner above described under the microscope, a number of small granules are seen floating about in the field; these enlarge and become clearer in the centre; this enlargement goes on very rapidly, and when they have gained to about twice their original size, the central clear part becomes dull. This dulness slightly increases, and in a short time it is seen to be distinctly granular, whilst the borders are observed to be well defined, smooth, and clearer than the central part. The enlargement of these bodies, with the granular appearance of their centre, seems *not* to depend on the aggregation of granules round a central one, but on a property which they have *in* themselves of enlarging and of presenting that figure. In the field, a number of these objects are frequently seen close together, and many of them appear more granular than the rest. During all this time they are quite spherical, and are of good consistence, as they do not lose their form by considerable pressure.

Second Stage.—The central portion becomes gradually less opaque, and gradually ceases to appear granular, the external portion at the same time separating in some degree from the central part. This part I shall subsequently call nucleus. The blood corpuscle in this stage of development has the appearance of a slightly flattened round cell, formed of a somewhat delicate but elastic membrane, with a nucleus in the centre. At this time, a number of these bodies, being close to each other in the field, present a yellowish colour. This colour seems to me to reside in the space between the nucleus and membrane. Of this, however, I

[1] The time of this appearance, however, varies much in different chicks, as also does the time of the appearance of the different structures and organs. This is not the effect of any external cause, as it is observed when the eggs are put into the hatching house at the same time, and removed together after having lain there for a certain period, and when consequently all external circumstances must have been the same to each and all of them.

am not certain. During the time that the elastic membrane is separating from the nucleus, the intervening space becomes flattened. This flattening goes on to such an extent, as to leave a depression between the nucleus and border. The edges of the corpuscles are flat, appearing as if they had been cut off abruptly. The convexity of the nucleus, the flattened edges of the corpuscle, and the concavity intervening between these two parts, are distinctly seen when any of these bodies, at this stage of development, are observed turning upon themselves. During all these changes the blood corpuscles are perfectly circular.

Third Stage.—One side of the corpuscle is seen gradually to elongate itself, until it has gained a pear-shaped appearance; the opposite side then elongates itself in the same manner, and to the same degree. At the same time that the envelope is becoming elongated, the nucleus undergoes a similar change, so that every part of the corpuscle becomes narrower than before, with the exception of the middle, which remains of the same size. During these changes, the concavity which was observed to exist in the second stage, in the space which intervened between the nucleus and border, is now seen gradually to disappear; at the same time that this change is going on, the borders, which were at that time like those of a guinea-piece, have now become rounded, so that there is a gentle convex declivity, (if I may be allowed so to express myself,) between the nucleus and borders. The nucleus, during all the above changes, remains convex, and never presents a granular appearance subsequent to the first, and beginning of the second stages. I have never seen any opening in the centre of the nucleus, the envelope appearing to pass uninterruptedly over its surface. The envelope itself continues gradually to increase in strength from its first appearance, to the complete formation of the corpuscle.

It is stated in the above remarks, that the cytoblast of the blood corpuscle is seen to enlarge, and its central clear part to become granular, and that these two changes seem to depend on a process *inherent* in the body itself. This notion of the manner of formation of the above appearances, differs from that of most observers on this subject; for it is stated by them that it is caused by the joining together of numerous nucleoli contained in the blood. That this statement is not correct, the following circumstances clearly prove. At the time of the enlargement of the cytoblast, or cell-germ, its circumference is seen to be *not* scolloped, but smooth and regular. Now, this it could *not* be, if the enlargement depended on the aggregation of nucleoli. Again, at the time of the granular appearance of the central part, the nucleus is seen to be surrounded by a thin but distinct membrane, and that this membrane separates somewhat from the nucleus, *during the time that body is becoming granular.* These circum-

stances hinder us from believing that the granular appearance is caused by the junction of several independent granules to each other, whilst *they entirely agree with the statements above made.* This appearance is exactly similar to that discovered by Prévost and Dumas in the ova of frogs, and by Martin Barry in the germinal spot of the ovum, during the development of these bodies. I do not state this merely from the plates of Martin Barry on this subject, but from the structure itself. I saw those granular or disc-like bodies so correctly described and illustrated by him, in the ova of a bitch, whilst assisting Dr Allen Thomson during last summer. Here I may state, that those divisions seen in the ovum were observed by Dr Allen Thomson a year previous to the publication of Martin Barry's papers in the *Philosophical Transactions*, of which he made drawings, which he has now in his possession by him. I do not state this circumstance with the most distant intention of withdrawing from Martin Barry any of the credit which is due to him as the original discoverer. My design is to show, that there can scarcely remain any doubt as to the correctness of the *description* of the disc-like bodies seen in the developing ovum, as given by Martin Barry, when the same appearance has been seen by one of the best and most careful observers of the present day, and this the more especially, when these were made previous to the publication of the observations which they support. Dr Allen Thomson also observed, and made drawings of the granular appearance which the centre of the blood corpuscles present, in some stages of their development, some years since, when engaged in observing the development of the vascular layer in the embryo chick. With these I was wholly unacquainted, until after I had shown to Dr Thomson the observations above described, when he exhibited to me these drawings, representing correctly the granular appearance of the nucleus in its first stage of development.

For what end is this appearance designed? It appears to me to be the cause of the enlargement of the nucleus, and of nothing else. This opinion is different from that held by Martin Barry, for he states,[1] "that the corpuscles of the blood are generated by a process essentially the same as that described in one of my former memoirs, as giving origin to those cells which are the immediate successors of the germinal vesicle or original parent cell;" and in the last paragraph of this paper are contained the following statements:—"On a former occasion, I showed that the blood corpuscles are not formed, as supposed by some observers, out of the granules of the yolk. The facts recorded in the foregoing memoir leave little doubt, I think, that these corpuscles—not only in the embryo, but at all periods of life—are

[1] Phil. Trans. 1840, p. 204.

descendants of the two cells constituting the foundation of the new being in the ovum. If so, it is not requisite to seek the origin of these corpuscles in the organised parenchymatous substance of the body, or in the globules of the chyle, the only two sources," it has been said, "in which it was possible for them to arise." I am inclined to believe these statements to be incorrect, and that from the two following reasons: namely, that the granular appearance of the nucleus is *only* seen while that body is *developing* itself, this shape wholly disappearing when it has become fully formed; and from the fact that I have *never* seen these disc-like bodies, of which the nucleus, in its process of development, is composed, *separate* from each other, or *escape* from within the envelope of the blood corpuscle. If these three last statements be correct, it is clear that Martin Barry's assertion as to the origin of the blood corpuscle is erroneous.

In the foregoing observations it is said, that the membrane separates gradually from the nucleus. This separation does *not* take place in the manner generally described, namely, by a delicate transparent vesicle arising on the surface of each granule or cytoblast, in the form of a flat segment or sphere, like a watch-glass on a watch; but by the separation of a membrane or vesicle round the *whole* circumference of the cytoblast at the *same* time. This separation goes on gradually, and to the same extent round the whole surface of the cell-germ in the first and second stages; but in the third, one of its sides juts out, and then the other, until it has attained the oval shape of the full-grown blood corpuscle of the chick. Struck with the great resemblance between the envelope of the blood corpuscle in its physical appearances, at its full development, as also in the different changes towards that end, with the basement or primary[1] membrane of the ducts of the Wolffian bodies, with the sarcolemma of the muscular tissue, with neurilema of the nervous structure, &c. &c., I was led to examine into the structure and properties of these parts more minutely; and although my observations are not yet sufficiently extensive to cause me to deduce from them with certainty any definite principle, yet so far as I have gone, I am led to believe that *every* organised substance in the animal kingdom is immediately surrounded by a pellucid and elastic membrane, having *no* fibres, vessels, or nerves in its structure,—that it is acted on in the same manner, and to the same extent, in all situations, by acetic acid—and that in this membrane *resides* the property of elimina-

[1] I give these two terms out of justness to Mr Bowman and Mr Goodsir; as Mr Bowman, in his description of the mucous membrane, gave the first term to that structure, lying immediately beneath the epithelium; whilst Mr Goodsir, about the same time, and without any knowledge of these observations, gave the latter term to the same structure, seen in the uriniferous ducts of the kidney.

ting or forming (?)[1] from the blood the various structures in which reside the *inherent* properties of the different tissues, as likewise the secretions and excretions. If this general statement be correct, then the proper name for this structure, in *all* circumstances, as also in *whatever shape* it may present itself, will be the *eliminative* or *formative* membrane.

In the foregoing pages, it is observed, that we suspect the colouring matter of the blood to reside in the space between the nucleus and capsule. The data on which this suspicion rests are as follow. At the time that the capsule closely surrounds the cytoblast, this body seems to be wholly deficient of any colouring matter, for let ever so many of these bodies, at this stage of development, be gathered together, they never present a yellow or reddish appearance, while as soon as that membrane or vesicle separates itself from the cell-germ to any extent, and a number of these bodies being at the same time close together, this yellow colour is seen. Moreover, this colour appears to take somewhat of a reddish hue, as the separation extends. From these facts, we are fully warranted to suspect, if not more than suspect, that the colouring matter resides in the situation above stated.

The appearances above described disagree in many points with those of Martin Barry, the latest writer on this subject. It is not my intention in the following remarks which I am about to make on his description of the blood corpuscle, to examine into the correctness of the opinion as to the immediate formation of various structures from these bodies. My design is to endeavour to prove that many of the appearances which he describes as belonging to the blood corpuscle in its natural state are incorrect. He states, at page 203 of the *Philosophical Transactions* for 1841, that "in all the blood corpuscles now referred to, which it will be seen are those of the four classes of vertebrated animals, including the two divisions of both reptiles and fishes, the nucleus has a cavity or a depression;" and further on he says, that "the corpuscle of the blood in certain states exhibits an orifice by means of which there is a communication with the exterior of the corpuscle and the cavity in its nucleus." In support of the above statement, Martin Barry brings forth the observations of De Torre, published in the *Philosophical Transactions* for 1766, p. 252, and before bringing these forward he premises them with the following sentence, "So little importance is attached in the present day to the observations of De Torre, that in a historical account of the blood corpuscle, I believe it is usual to pass them

[1] I use the mark of interrogation after the word "forming," because we have *no* facts to prove that any organised structure has the power of changing chemically the ingredients of the blood. We have facts to prove that they *afford* circumstances for allowing changes to go on *in* the blood itself. We have *none* to show that these structures are the *immediate* cause of these changes.

by as not deserving of notice,[1] probably from an idea that his optical instruments were too imperfect; but I do not hesitate here to transcribe a passage from the observations of De Torre, although far from vouching for their entire accuracy." The following is that which he describes. "When any of the globules (blood corpuscles) happened to move with the serum in the most perfect focus, I could with great clearness distinguish the exterior and interior circumference of the ring of which each globule (corpuscle) consisted, the interior one being bounded by a black line or shade next the perforation exactly resembling that which bounded the exterior one. In such globules (corpuscles) I could easily observe the ring articulated, the transverse lines at the joints being very distinguishable; the figures of the articulations were various, in some they were roundish, so that the rings appeared like a bead necklace, in others cylindrical, and of some length. The numbers of which the whole was composed seemed uncertain, varying from two or three to six or seven, many of the rings were broken, either by some confinement of the talcs (talk) or by beating against each other, which I saw them continually do, and by these accidents the joints of the rings were detached, and wandered about separately in great numbers, and indeed they seemed separable with as much ease as if they had been united by mere contact only. Some of the rings were broken into semicircles, others into greater or less portions, others again divided into their constituent articulations, which, in some places, float about singly, or in others form, by their mutual attraction, a lateral union, like the pipes of an organ. I must observe, also, that these separated parts seem to be hollow and transparent, and like inflated bladders, and would easily yield and change their figures, stretching or contracting from round to oval and cylindrical, and *vice versa*, or any lateral pressure, in crowding them along with the serum, brought a constraint on them. Although the articulation was not distinguishable in every globule, I think it was so in the greater part of them, and it is natural to imagine that the rest were articulated likewise, though they might not pass at the proper distance for its being distinguished." The reason for transcribing the above long extract is, that we might have before us the principal statements of De Torre, whilst criticising his observations. The first thing that must strike every person who knows the history of the blood-corpuscle, and who reads the above quotation, is astonishment at finding that any one, at this time of day, quotes the above statements of De Torre, believing that the greater part of them are correct. This astonishment must be increased ten-

[1] This is not correct; Bostock, in his *Physiology*, mentions De Torre's observations succinctly but correctly.

fold when Martin Barry is known to be the believer. I have no hesitation in stating, that the appearances described by De Torre, when referred to the blood-corpuscles in their natural state, are erroneous. The basis on which the above assertion is founded, are the following facts, taken from De Torre's paper, when compared with the acknowledged physical properties and appearances of these bodies in the present day. He states, in the paper above referred to, "that many of the rings were broken, either by some confinement of the talc, or by beating against each other, which I saw them continually do; and by these accidents the joints of the rings were detached, and wandered about separately in great numbers, and indeed they appear separable with as much ease as if they had been united by mere contact only." From this statement it seems very evident that it was *not* the blood-corpuscle in its natural state which he saw; as these bodies then possess a good deal of strength and elasticity. That they have these properties, is very evident from the various forms which we see them undergo, whilst circulating in the capillary vessels, in consequence of them infringing on the walls of those tubes, and on each other, and on their immediate return to their original figure, when the causes on which these changes depended have been removed. Now, bodies which appear to *separate* with as much ease as if they had been united by contact only, could *not* possess these properties; the very circumstances of the case hinder it. De Torre's statement may be, and is true, in some degree, if confined to the blood in some unhealthy states, or as the effect of some re-agent; but the whole tenor of the above extract clearly shows, that such is not the case, as they lead us to believe that he always imagined the blood corpuscles to be perforated and articulated. Again, De Torre states, near to the end of the paper so often referred to, "I omitted to speak of the size of the globules in this observation, nor indeed can I, from so various an appearance, form any judgment thereof." Now, the blood corpuscles in their full growth are *not* very various in size, at least their difference is *never* so great as to cause any one to give out such a statement as the above. These appearances described by De Torre seem to me to depend on the three following causes: 1st, On the imperfection of his instruments; 2d, On his using water in making his observations on the blood; and, 3d, From the affinity which the blood corpuscles on their parts have for each other. We have no facts to prove the first cause, but I think we have a right to suspect observations of such minuteness as these were, the more especially as they differ from the observations of all other observers, when made with "rude and smoked lenses," for it must always be remembered that it is habit only that makes the expert microscopist, not genius or great talent—these are

required to *use* the observations made, not to make them. In support of the second cause, I am unable to bring forth immediate facts to prove that water was used, but I think the following statements are sufficient to make us believe that such was the case. At the time De Torre wrote his paper, it was not known that water could change the appearance of the blood-corpuscles, at least we have no statement, as far as I am aware, to that effect. Now, as these bodies cannot be examined properly without the serum in which they are contained being diluted, either with more serum or some other fluid; and as water is always at hand, whilst serum is not; and, moreover, as many of the appearances described by De Torre may be seen on diluting the blood with water, we have every reason to believe that this fluid was used for that purpose. That the blood-corpuscles and their parts have an attraction or affinity for each other, almost all microscopic observers grant. To this property of the blood corpuscles do we owe almost all the errors contained in Sir Everard Home's description of the formation of the different organic structures. De Torre himself observed this property, and to it he indeed refers many of the appearances which he saw. He says, "and the others formed by their *mutual attraction*, a lateral union, like the pipes of an organ." I have frequently seen the blood-corpuscle, in its several stages of development, form a great number of the appearances which De Torre describes, with many others, "*and they all separated as easily from each other as if united simply by contact.*" These three causes seem to me fully to account for the different appearances of the blood-corpuscle described by De Torre,—appearances described by no other author. From the foregoing arguments, I think we are at full liberty to conclude that De Torre, in his paper published in the *Philosophical Transactions* for 1755, has *not* described the blood corpuscle in its natural state, and therefore that the above paper, for the truth which it contains in relation to this point, may be returned, and that without any disadvantage, to its former oblivion; and when it is again produced, it may be only to please the fancy of some laborious, but useless, book-antiquarian. In relation to the observations of Martin Barry, above quoted, concerning the cavity in the nucleus and of the opening in the envelope, I can only say, that I have never seen them in the blood corpuscles of the chick, in any stage of their development, and I have examined these bodies for days and weeks together. They always appeared to me to be very decidedly convex in the centre, and the envelope or vesicle seemed to pass continuously over them.

Note.—The statements made by M. Donné as to the changes which the blood corpuscles of mammalia present in their development, seem to agree somewhat with the observations just recorded. But the *manner* by which he states that these are produced is quite at variance with that given above; for he says that two or three

of the "globulines," (granules) unite together, and form the "white globules," which he describes as colourless, irregular in their contour, and having a granular aspect. This appears to be my second stage, and he says, that after three or four of the "globulines" have united together, and whilst circulating with the blood, they receive an albuminous envelope; whilst it is stated above that the granular appearance is *not* formed by a process of *affinity*, but by one *innate* in itself. As it is not at all likely that the process by which their appearance is produced will differ in the least degree in any species of animal, and as the process which I have described is agreeable to that first observed by Prévost and Dumas,[1] and afterwards supported and extended by Von Baer[2] and others in the ova of frogs, and by Martin Barry,[3] and Allen Thomson in the ova of mammalia; and, moreover, as we have no positive proof that a cytoblast ever enlarges by the aggregation of two or more granules, I will not, perhaps, be thought presumptuous, when I say that it seems probable, in consequence of the very great difficulty which attends the examination of the development of the blood corpuscles in mammalia, that M. Donné is not correct as to the cause which he assigns for the production of the granular appearance and enlargement of these bodies.

1 Ann. de Sc. Nat. t. ii., 110. 2 Müller's Archives, 1834, 481. 3 Phil. Trans., 1840-1841.