Physiology. — *"On the Behaviour of Megakaryocytes in the Spleen of the young Kitten"*. By J. J. L. DE NEVE. (Communicated by Prof. J. BOEKE).

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I. Introduction.

It is a well known fact, though hardly any mention is made of it in the literature, that in the normal mammalian spleen the giant-cells (which have been named mega- or megalokaryocytes by W. H. HOWELL, 1890) of embryonic life persist after birth. Nay, several French investigators, notably JOLLY and ROSELLO (1909), and especially DE KERVILY (1912) have discovered that giant-cells are demonstrable in the normal full-grown spleen of most mammals.

They are, however, far more numerous in the embryonic than in the adult spleen. To find out the way in which this diminution in number takes place, has been the object of my research. I set myself the question: in what way and when do megakaryocytes disappear from the spleen of the cat. I presume to have found the answer to this question.

II. The Literature.

Giant-cells in the liver and in the umbilical vesicle have been oftener described than those in the embryonic spleen, because most investigators, writing about this subject, have studied the youngest stages of hematogenesis. Now the spleen is of comparatively late growth, when the bloodrelations are already so complicate as to present insuperable difficulties for a study of the formation of the blood. Besides, another difficulty is met with in the complicate structure- and tissue-relations that soon arise in the spleen itself. This, however, does not interfere with our study, since it regards only one special type of cells.

Assuming the giant-cells to perform some function, the disappearance of the cell must in one way or other be connected with this function. We, therefore, deemed it interesting to consider the views adopted by others.

VAN DER STRICHT is, among the first to discuss this point (1888 and

1891). He ascribed to the giant-cells a variety of functions, the principal of which was no doubt a phagocytic one. FLEMMING is more decided in his opinion, asserting that the giant-cells, wherever they appear, are pathological products, that are without a special function and even succumb. VON KOSTANECKI (1892) maintains that he has seen the giant-cells break up into smaller cells, and also admits a phagocytic function. On this point he is, as he himself says, at variance with RANVIER and KUBORN who hold the giant-cells to be "cellules vasoformatives". Afterwards this "vasoformative function" turned out to be merely a degeneration process. VON KOSTANECKI also refuses to agree with VAN DER STRICHT, who asserts that the nuclei of the phagocyted cells themselves tend to enlarge the nucleus of the giant-cells, and he, therefore, maintains that there is no reason for the conclusion that the giant-cells have been made up of different smaller cells. Finally the says about this function. "Meinen Erfahrungen nach muss ich aufs entschiedenste die Ansicht vertreten, dass die Riesenzellen der embryonalen Leber — fur andere blutbildende Organe der Saugetiere wird die Ansicht auch von manchen Autoren verfochten —, so auffallig und interessant sie in ihrer Form and in ihren Lebenserscheinungen auch sein mögen, bezuglich ihrer Funktion und ihres Verhaltnisses zum Vorgang der Blutbilding vollig nebensachlich und bedeutungslos sind".

SAXER (1896) discusses this point at greater length, saying that some appearances led him to suppose that the giant-cells could take up smaller cells as well as be broken up into others. He disputes FLEMMING's view that the giant-cells are only an "abgeartete und ausgeartete" form of cells. SAXER believes there is a certain relation to hematogenesis, but he leaves this point undecided. In the end he says: "Sodass es in der That unmöglich erscheint aus den verschiedenen Erscheinungsphasen einen einheitlichen Vorgang zu konstruieren".

MAXIMOW (1908) supposes that the giant-cells occur where erythropoësis and granulopoësis take place, though he never observed any splitting into smaller mononuclear cells.

As early as (1901) SOPHIE LIFSCHITZ had already pointed in her thesis to a parallelism between erythropoësis and the number of megakaryocytes in the spleen of the human fetus.

It appears, then, that many observers admit a relation to the formation of white and red bloodcorpuscles, but the most recent view is that of WRIGHT (1906) and OGATA (1912) that the giant-cells form and split up thrombocytes or bloodplatelets.

We see, then, that the theories regarding the fate of megakaryo-

cytes may be grouped under the following headings: 1o. degeneration; 2o. relation to erythropoësis; 3o. formation of mononuclear, white bloodcorpuscles; 4o. formation of thrombocytes.

III. *Material and Methods.*

Provided the material be good, giant-cells, when present, are always demonstrable by every proper staining method. To bring out details well the sections should not be made too thick, because the megakaryocytes are large bodies (some, indeed, measure 40μ or more). This is why some experimenters attach value only to sections of 3μ at the most. Some of them achieve the best results with paraffin-sections, but MAXIMOW e.g. asserts that only celloidin material, treated in his own complicated way yields results of any value at all. Others recommend the most various fixation-fluids and dyes.

In course of time the various fixatives have been abandoned, ZENKER's fluid being now used by most researchers, either in its original composition, or slightly modified.

The dye now generally adopted in staining methods is hematoxylin (EHRLICH)-eosin and in special cases iron-hematoxylin (HEIDENHAIN)-eosin, besides the various ROMANOWSKI-variations.

It seemed to me to be best to try these various methods, in order to select the one best suited to my purpose.

IV. *Personal Observations.*

The animal. I experimented upon cat-embryos, young kittens and adult specimens. In view of the problem I wished to solve and the facts known, I confined myself chiefly to young kittens.

Tissue. Of those kittens which were killed with chloroform and opened immediately (still warm), the spleen, the liver, and the bone-marrow of the femur were excised and put in different fixation-fluids.

Fixation. With cats ZENKER's fluid at ± 37° C. gave most satisfactory results. It yields beautifully fixed preparations and causes the least shrivelling.

Embedding. Initially the embedding was done in paraffin as well as in celloidin, but when the latter method proved ineffective, it was abandoned and only paraffin was used.

Sections. These were cut, at a venture, 7μ thick, which proved quite effectual, though I also cut some of 3μ, which, however, opened up no fresh point of departure.

Staining. After trying various staining methods subsequent to different fixations, I achieved the best results after ZENKER fixation

with the iron-hematoxylin (HEIDENHAIN)-eosin stain. I proceeded as follows:

1. xylol; 2. alcohol 100 %; 3. alcohol 96 %; 4. alcohol 70 %; 5. water; 6. for six hours in 2½ % iron-alum used as mordant; 7. washing in water; 8. allowing to overstain in iron-hematoxylin after HEIDENHAIN; 9. washing in water; 10. differentiating in iron-alum; 11. washing and examining under the microscope; repeating 10 and 11 till the differentiation is sufficient and after this 12 washing for

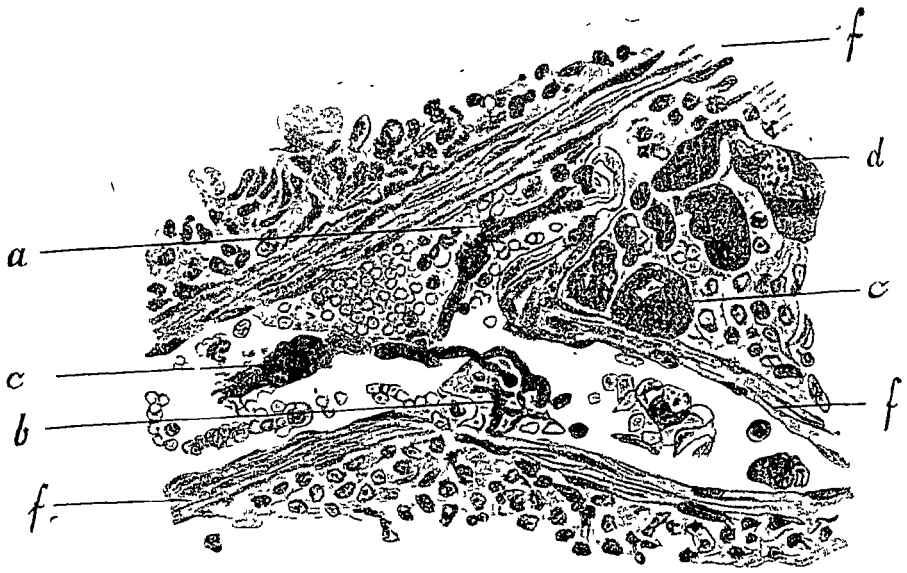

Fig. 1. Spleen of cat, 2 weeks. ZENKER. Iron-hematoxylin-eosin, ± 250× natural size. *a.* megakaryocyte degenerating in a vein with only slightly stainable protoplasm-loop. *b.* dissolving protoplasm-filaments. *c.* pyknotic nucleus of a megakaryocyte, some protoplasm still surrounding it. *d.* megakaryocyte protruding into the vein. *e.* normal megakaryocyte against the vessel-wall. *f.* vessel-wall.

one hour in tapwater. Lastly after treatment with the successive alcohols, the usual after-staining with eosin.

To secure a good set of animals I killed some young kittens of the same litter, one of a day's lifetime, the following after 1, 2, 3 and 4 weeks. To fill up the number I took another kitten, 46 days old, from another litter. Afterwards, of course, also some control-animals.

When studying a preparation from a one-day-old kitten we see in the spleen numerous giant-cells, mostly in groups of 2, 3 or 4, and often close to the trabeculae, so also close to the large vessels that enter the trabeculae. This typical arrangement is even

more conspicuous in a preparation from a kitten of 8 days. Here a slight alteration in the appearance can also be noted. Whereas the giant-cells were on the first day comparatively quiescent and the compound nuclei formed beautiful rings and horseshoes, on the 8th day a general mobility is discernible. The number of giant-cells is, indeed, about as large as before, but the nuclei present fewer ring- and horseshoe-shapes and stain rather more deeply than their surroundings. This statement does not apply to every individual cell. Likewise the cytoplasm often stains more intensely with eosin.

Fig. 2. Spleen of cat, 1 week. ZENKER-Ironhematoxylin-eosin, 950 × natural size.
a. megakaryocyte in a mitotic stage. *b*. wall of a vein.

One single mitosis is also noteworthy. (Fig. 2). Here the cell-body is stained more basophilic and granular. In a similar preparation of a kitten of two weeks I was struck, on cursory inspection, by the location of a giant-cell in a large vein. (Fig. 1). This discovery gives a clue to the whole process as in nearly every large vein appeared several cells similarly disposed. It also soon became clear how they got there, when I encountered cells protruding crosswise through the vessel-wall (Fig. 3 *a* and *b*). On closer investigation the following process can be deduced from the various preparations: A giant-cell displaying great amoeboid activity (SCHRIDDE 1905) sends out, when about to vanish, pseudopods in all directions, which process is attended with all the typical features of degeneration. The nucleus shrinks, gets rounded, presents no longer a distinct appearance, gets much more deeply stained, in a word: pyknotic. The protoplasm of this cell-body at first stains slightly deeper with eosin, rather brownish, but in a more developed stage even the cytoplasm is no longer stainable, only the blurred periphery being visible. (Fig. 4 *c* and *d*). The cytoplasm of the cell now protrudes through the vessel-wall either

actively or passively and the nucleus is carried along with it. On watching the process closely we are under the impression that it is rather a passive act than a manifestation of vital activity. The

Fig. 3. Spleen of cat. 2 weeks. ZENKER Iron-hematoxylin-eosin. ± 580 × natural size. *a*. megakaryocyte with a long protoplasm-filament in the lumen of the vein, no longer stainable at the extremity. Nucleus pyknotic; still lying in the spleen-pulpa. *b* like *a*, but the shrivelled nucleus lying just in the lumen of the vessel. *c*. degenerating megakaryocyte lying entirely in the vein. *d*. megakaryocyte with easily stainable cytoplasm, without filaments, but with markedly pyknotic nucleus. *e*. vessel-wall.

filaments of the protoplasm (they can no longer be called pseudopods) extend invariably in the same direction in the same bloodvessel (Fig. 3 *a* and *b*). It would seem then that the protoplasm is carried along by the blood-stream, when once it has entered into the lumen of the vessel. Now it is still a subject of dispute whether the vessel-wall in the spleen is closed or whether there are openings in it. When assuming the latter, the limp, inert, degenerating giant-cells, lying close to the vessel-wall, are supposed to be pressed through the openings in the vessel-wall, when the spleen contracts. This squares with the appearances presented by the megakaryocytes in these preparations.

For, if it were an active diapedesis we should see a pseudopod, after it had once protruded through the bloodvessel, lodged against the opposite side of that vessel; now this we do not see anywhere. Contrariwise we see that a protoplasm-process is gradually prolonged in the lumen of the vessel and dissolves. If sometimes a whole cell is seen in the lumen of the vessel, all sorts of irregular protoplasmic strands are seen to form (Fig. 1 *a* and *b*), while the detached

pyknotic nuclei are occasionally noted in the lumen entirely deprived of cytoplasm. I take it, therefore, that here we have to do with an unmistakable degeneration.

Further research revealed similar degenerations already in the first week. Also in the spleen-pulpa groups are found here and there presenting an appearance exactly like those afforded by the degenerating giant-cells in the lumen or in the wall of the vessel. The process, then, does not depend entirely on the protrusion into the vessels, which lends additional support to the conception that this protrusion is of a passive, not of an active nature.

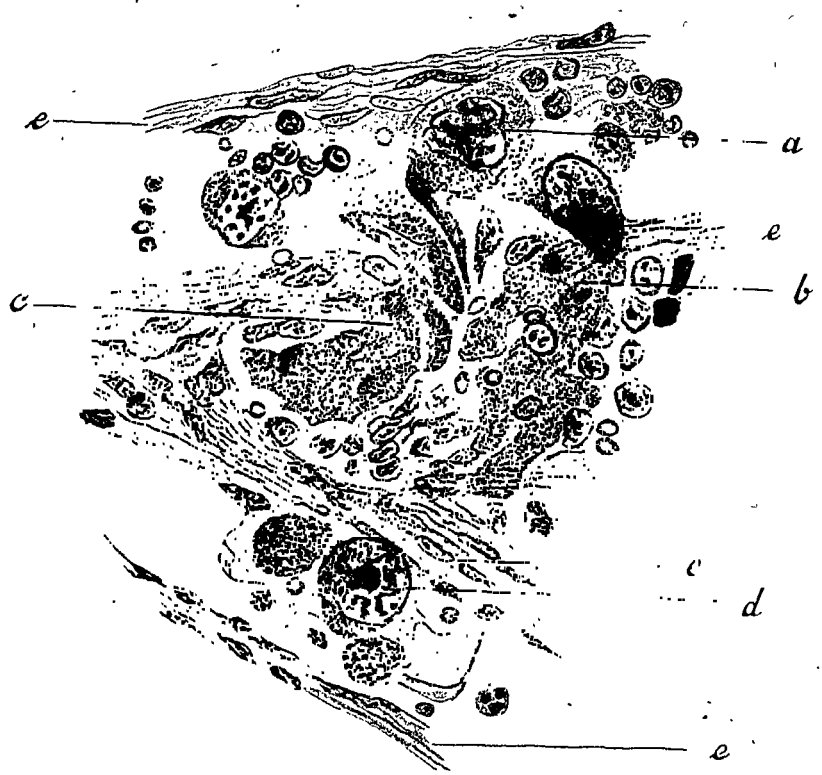

Fig. 4. Like fig. 3. 570 × natural size.
a. megakaryocyte with pyknotic nucleus, reaching only with a narrow point the vessel-wall. *b* and *c* degenerating megakaryocytes, especially *c* is poorly stained. *d.* degenerating megakaryocyte. *e.* vessel-wall.

Not in one instance did I see in the lumen of the vessel a normal giant-cell with a beautifully horseshoe-shaped nucleus, which confirms our conception of the whole process. Now if we observe the preparations from a kitten of three weeks, we note a considerable decrease in the number of giant-cells. This does not surprise us in the least, considering the extensive dissolution. In the fourth week only

few giant-cells are distinguishable, for the greater part, to all appearance, normal. Generally the number is not larger than DE KERVILY (1912) assigns for the spleen of the normal adult cat. He gives the numbers 2 per cm^2 to 20 mm^2, so it seems to be rather fluctuating.

Quite the same may be noted in the spleen of a kitten 46 days old. The number of the giant-cells seems to be rather smaller in this case. Anyhow the appearance does not differ from that of an adult cat's spleen. It is evident, then, that the giant-cells do not disappear completely, as indeed DE KERVILY has been able to demonstrate for the spleen of the majority of adult mammalia. In the Leyden laboratory I also have been enabled to detect giant-cells in all sorts of mammals except man.

The answer to the original question was now found: the giant-cells disappear from the cat's spleen through degeneration and dissolution, especially in the 2nd week after birth and in the large veins.

This does not fit in with WRIGHT's view. As early as 1906 WRIGHT described a process of extrusion also in the spleen of the kitten, but this process he holds to be a formation of blood-platelets. The process described by me is certainly not formation of bloodplatelets but a degeneration. May it be possible that WRIGHT has observed what I have seen, but that he gives a different interpretation? The process described by me leaves no room for another explanation.

WRIGHT's description is about as follows:

The megakaryocytes form pseudopods, which they send out into the small capillaries. These pseudopods are stained less intensely at the margin and have a granulated appearance. Small pieces are now constricted off, they are the thrombocytes. WRIGHT does not mention the age of the kitten upon which he experimented. Of this process he gives fourteen microphotographs, which are anything but clear, but still they resemble my findings too closely to conclude that they are widely different from WRIGHT's. He obtains these appearances by means of a special staining method of his own device with the exclusion of all others. As far as I know, OGATA (1912) is the only one who corroborates WRIGHT's histological findings, in spite of SCHRIDDE's failure (1907) to demonstrate a similar process in man. This, OGATA asserts, was because SCHRIDDE was obliged to work with post mortem material, whereas WRIGHT and himself were enabled to work with "lebenswarm fixierten Präparaten". As to the latter I was in the same condition. In this connection it seems strange that OGATA could not get good preparations when using WRIGHT's method, and succeeded when applying the SCHRIDDE-azure II-eosin method. Further-

more the drawings of OGATA also show that the process observed in the bone-marrow bears no resemblance whatever to WRIGHT's. In other respects their assertions are also clashing. Let us, however, dismiss OGATA's process from further discussion, considering that this worker confined himself to bone-marrow, and let us give our attention more particularly to WRIGHT's publication.

In the sections examined by myself, and they are many, I have never been able to detect a process in agreement with WRIGHT's conception. WRIGHT may be mistaken. He may have drawn a far-reaching conclusion from a superficial similarity. I do not think it improbable. The process described by me is so perfectly evident that anyone who examines the spleen of a kitten of two weeks, must see it. I, therefore, believe that he has also observed it, but I am positive that it is not a formation of thrombocytes. This assertion is based on the following considerations:

First and foremost the process is demonstrable with any proper fixation- and staining method, also with those that do not show thrombocytes in any portion of the preparation. Protoplasm filaments are, indeed, vaguely discernible as well as those that are still properly stained. Moreover the thickness of 3 μ, which WRIGHT prescribes for the sections, is as little imperative as his special staining method. As stated above I took the trouble to cut sections of 3 μ, but they could not alter my opinion. The diameter of the protoplasm filaments also is many times larger than that of a thrombocyte.

Did WRIGHT overlook the extrusion of entire degenerating cells just in the large veins (not in the capillaries)? Again, how does WRIGHT account for the thrombocyte-formation when it should appear that also in mammalia the thrombocytes are nucleated cells? This question has not been solved as yet. H. G. LANGEMEYER (1916) e.g. again arrives at this conclusion. In that case the thrombocytes, which, according to OGATA, originate only from the marginal zone (HEIDENHAIN 1907), could not possibly contain any karyoplasm. And would it be likely that the giant-cells ever continue to form thrombocytes? How then does WRIGHT account for those detached pyknotic nuclei that occur too frequently to be considered as mere casual phenomena? They are lying in the vessels as well as in the spleen pulpa.

It is true, WRIGHT has reserved some clinical adhesion latterly, e.g. from HAL DOWNEY, BUNTING (1909), SELLING (1910) and above all from E. FRANK (1915). But histologically this support is not well-founded, and besides highly debatable. It is out of place here to enter into further discussion on this point.

Now about the giant-cells in the cat's liver. These are quite similar to those of the spleen but at birth they are less abundant. After birth they disappear gradually, so that only few remain in the 4th week. However, never did I find an instance of degeneration as is encountered in the spleen.

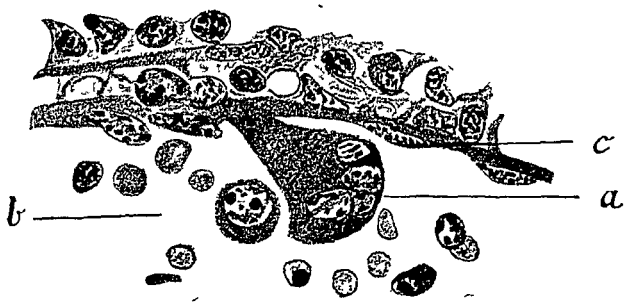

Fig. 5. Embryo guinea big 9 cm. long. Spleen nearly 1000 × natural size. *a*. megakaryocyte lying in the lumen of the vessel, with a process still in the wall. *b*. lumen of the vessel. *c*. vessel wall.

Because I would not limit my research to one species of animal. I also studied the guinea-pig in quite the same way as I did the cat. The result was nearly the same. In the guinea-pig the critical moment does not occur after, but before birth. This, no doubt, is explained by the fact that the cavia has already before its birth reached a stage of development that is attained by the cat only much later. The extrusion-process showed itself most distinctly in the veins of an embryo 9 cm long (from head to caudal bend) (Fig. 5,*a*) I would have studied earlier stages of suitable caviae, had any been at my disposal. The maximal number of giant-cells of the spleen is smaller in the guinea-pig than in the cat. In guinea-pigs I did not detect such a typical degeneration accompanying the extrusion as in cats. My failure in finding the most appropriate stage is perhaps responsible for this. In a cavia of 17 days the number of giant-cells has already diminished to a number not larger than is assigned by De Kervily for the adult cavia, viz. 3 per 25 mm^2.

SUMMARY.

1. In the cat's spleen a process is most distinctly demonstrable in the second week after birth, in which the megakaryocytes protrude into the large veins and break up into pieces through degeneration.

2. This process is not a formation of thrombocytes.

3. A similar process takes place in guinea-pigs shortly before birth.

I would take this opportunity of thanking Dr. A. B. Droogleever

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