On Bone-Marrow in Hookworm Disease (*)

by

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(With plates LXXXII–XCVIII)

From the very beginning of our investigations intended to ascertain the mode of action of the Ancylostoma duodenale and the Necator americanus in their harmful effect on the organism, we considered as being one of the most important signs, the morphologic and functional state of the organ producing haemoglobin, classically the element mostly attacked in this disease and from the deficiency of which all the main symptoms are recurrent.

Since the first works on the pathogenesis of anaemia in anchylostomiasis, one of the most controverted facts was, without any doubt, the functional state of the bone-marrow. Whilst some authors have described its aspect as being frankly regenerative, there were others who sustained the existence of a blood image resembling that observed in pernicious or aplastic anaemia, consequently without any evidence whatever of medullar reaction. Now it is known that this divergency was induced by the intercurrence of other diseases, mainly malaria which, generally, is met with in the same countries where anchylostomiasis is ravaging, for there exist the same epidemiological conditions.

The functional reaction of marrow in various anaemiae is brought into evidence by the appearance of cells of new formation in the peripheral blood. Thus in malaria, in anaemia due to acute haemorrhage, in haemolytic icterus and others, we observe an aspect which indicates an intense regeneration of red blood cells, as there are to be seen, polychromatric red cells, red cells with nuclear remainders and a reticulocytosis which in haemolytic icterus may reach 70 %. In anchylostomiasis these regenerative cells are not observed and the reticulocytes seldom reach the ratio of 6 %, in the majority of cases this being between 2 and 4 %, even in the last stages of anaemia.

In hookworm disease there is a hypofunction of marrow.

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Our main object has always been the observation of pure cases, and indeed we always met with identical results in respect to the appearance of peripheric blood. The same care was likewise exercised in our study of marrow, always selecting records of necropsies showing changes produced by uncinariotic anaemia.

The morphology appearance of marrow, while indicating the regenerative power, is a fact which is fundamental in any pathogenic interpretation of the anaemiant process. The toxic or the haemorrhagic theory as well indicates a primary or secondary aplasia of this organ, i.e. a great decrease or even the absence of ancestral cells of the red cell. For the explanation of the anaemiant mechanism a detailed study of marrow in a number of cases which allow an acceptable inference, imposes itself from the very beginning.

In spite of the evident importance of the matter, this morphologic appearance however did not yet constitute an object of numerous and detailed investigations, and is still, up to the present, an incognito in the pathology of this verminosis, the contrary being the case with its functional aspect.

The authors who have dealt with this matter, have done so in a superficial manner and in a reduced number of cases. Rogers (1) informed regarding the red coloration of marrow from femur diaphysis; Ferguson (2) noted red coloration, vascularization, abundance of cellular tissue and multiplication of nucleated red cells. After our first paper (3) we verified that, nearly at the same period, the Italian author Fieschi (4), in a detailed paper, had described the marrow from two pure cases of anchylostomiasis. As this is the only work with documentary evidence, we shall further on transcribe the observations and conclusions of this author. In one of the cases, marrow was removed from sternum for biopsy, and the other was a post-mortem examination without indication of the bone from which the material had been removed. In the fatal case he observed grey coloration, numerous eosinophilous myelocytes without any anomaly in their maturation, numerous «plasmazellen», little reticular hyperplasia, very rare haemoostioblasts, very numerous erythroblasts to a large extent basophilous, a few erythroblasts with their nuclei in pyknosis, megakaryocytes considerably increased in number.

In the case of biopsy he observed numerous eosinophiles myelocytes, megakaryocytes not increased in number, very numerous erythroblasts forming nests. Some of these cells presented a basophilous protoplasm, and others a polychromatophilous one. He observed numerous «plasmazellen», rare reticular cells and haemoostioblasts, rare macrophagi containing yellow pigment, and absence of megaloblasts. The author states
that, the sternum marrow being active (which statement leads us to suppose that the marrow of the fatal case had also been removed from sternum), his conclusions have not an absolute character; nevertheless, he points out that Tibaldi has observed persistency of red marrow in long bones from fatal cases.

From this histo-pathological study and various considerations of other kinds, the author concludes that anchylostomatic anaemia is induced by a myelotoxic pathogenic mechanism. In spite of this, he lays stress upon the facts hardly accountable by a toxic action, believing in an individual factor, for he says: «The individual factor which plays its rôle in the determinism of anaemia and which is not yet known (a constitutional or an acquired factor) supplies the condition for the production of a myelopathy...».

In two cases of sternum marrow we met with a noteworthy decrease of elements of the red series in an advanced evolutionary stage, just as described by Fieschi. In one of these cases marrow from femur diaphysis presented the aspect observed in the other 23 cases, i.e. a remarkable predominance of orthochromatic erythroblasts (normoblasts).

Therefore, the greater part of bone-marrow is extremely regenerated, and there is not any myelotoxic process likely to prevent the evolution of the cells of the red series until the stage of normoblasts.

This dissimilitude between two marrow regions affords a new documentary evidence for the necessity of the observation of different spots in order to appreciate a myelopathy. Besides, Fieschi himself says: «Villa, lately, called attention to the possibility of great differences between one region and another and of diagnostical mistakes, with regard to such a disease as is pernicious anaemia; this remark proves true more thoroughly for this secondary haemopathy, in which occasional factors (age, constitution, acquired factors) assume a rather great importance in its determinism and in the distribution of the changes ».

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In our studies, the material was removed from fatal cases of anchylostomiasis, without the intercurrence of any other disease which might have exerted its influence on blood or the haematopoietic organs. In one single case marrow was removed from the living body by puncture of sternum. The slides were stained by May Grünwald's and overstained by Giemsa's. The material for sections with paraffin was fixated by Zenker's with 10% of formol for 6 to 12 hours, then included and stained by Giemsa-eosin with Ellermann's method (5). Every observation has its
anatomo-pathological diagnosis and the number of post-mortem examination in accordance with the catalogue of the Section of Pathological Anatomy of the Oswalido Cruz Institute. The clinical, hematological and therapeutical findings were gathered from the patient’s charts, made in various hospitals.

We gave here minute therapeutical data in order to show that in these fatal cases, iron administration, when given, was considered in secondary plan, what is in perfect agreement to the treatment method until now used for this helminthiasis.

Our extensive photographic demonstration is due to the absence of the same in previous works, and to prove the constancy of the results here described.

OBSERVATIONS

Case 1. Age: 64 years.

*Microscopical examination of marrow from femur diaphysis:* In one part of the preparation there are seen numerous fatty cells among which a parenchyma very poor in cells, presenting an intense congestion. In the other part, as may be seen, the fatty cells present a smaller diameter and are met with in a smaller quantity. There is also congestion, and the parenchyma is found to be very developed with abundance of cells. Megakaryocytes are to be found easily. Numerous eosinophiles myelocytes. Very numerous erythroblastic foci in which mitotic images and polychromatic erythroblasts are to be seen commonly. There is an evident predominance of erythroblasts with nucleus in pyknosis or in karyorrhexis (Fig. 1).

Case 2. Age: 41 years.

*Microscopical examination:* Nearly complete disappearance of fatty cells. Parenchyma very rich in cells and presenting small spots strongly congested where the cells are very numerous. Apparently normal number of megakaryocytes. Very numerous eosinophiles myelocytes. Numerous erythroblasts, scattered or in foci. Predominance of orthochromatic erythroblasts. Rare polychromatic erythroblasts. Mitotic images are to be seen in these cells. Some erythroblasts with nucleus in rhaxis (Fig. 2).

Case 3. Age: 54 years.

*Microscopical examination:* Very abundant parenchyma full of cells mainly of the red series. Abundant megakaryocytes. Numerous eosinophiles myelocytes. Predominance of erythroblasts with nucleus in pyknosis. Very rare mitotic images and rare erythroblasts in karyorrhexis (Fig. 3).
Case 4.

Microscopical examination: Rare fatty cells. Abundant parenchyma rich in cells. Congestion. Megakaryocytes are to be seen frequently. The white and red series are very developed. Very numerous eosinophiles myelocytes. Polychromatic erythroblasts are to be seen with a certain frequency. Numerous erythroblasts with pyknotic nucleus (Figs. 4, 5 and 6).

Case 5. Age: 60 years.

Microscopical examination: In this preparation a central zone of parenchyma is to be seen with small abundance of myeloid cells, with numerous fatty cells. Around it, a zone of intense regeneration, very congested, is to be seen. Rare megakaryocytes. Eosinophiles myelocytes are not very frequent. Numerous polychromatic erythroblasts. Predominance of erythroblasts with nucleus in pyknosis and mainly in rhesis (Fig. 7).

Case 6. Age: 68 years.

Microscopical examination: Rare fatty cells. Very abundant parenchyma rich in cells. Intense congestion. Apparently normal number of megakaryocytes. Numerous eosinophiles myelocytes. Mitotic images in erythroblasts with nucleus in pyknosis (Fig. 8).

Case 7. Age: 52 years.

Microscopical examination: Parenchyma rich in myeloid cells. Rare fatty cells. Numerous megakaryocytes. Eosinophiles myelocytes are not numerous. Great development of the red series. Erythroblasts scattered, but without forming foci, with clear predominance of erythroblasts with nucleus in rhesis (Fig. 9).

Case 8. Age: 60 years.

Microscopical examination: Parenchyma is found to be constituted mainly by cells of the white series, fatty cells are very rare. Megakaryocytes not numerous. Eosinophiles myelocytes in a particulary accentuated number. Neutrophiles myelocytes abounding. In the red series, not very developed, orthochromatic erythroblasts with pyknotic nucleus predominate. In these cells rare images of karyorrhexis are to be seen. Numerous mitotic images in the elements which are less developed (Fig. 10).

Case 9. Age: 45 years.

Microscopical examination: Very rare fatty cells. Extremely rare eosinophiles myelocytes. Megakaryocytes in normal number. Extremely rare basophiles erythroblasts. Predominance of erythroblasts with nucleus in pyknosis are to be found in a very great number (Fig. 11).
Case 10. Age: 32 years.

Microscopical examination: Numerous fatty cells intermingled with parenchyma rich in haemoformative cells. Abundant megakaryocytes. Numerous eosinophiles myelocytes. Megakaryocytes abounding. The presence of basophiles and polychromatic erythroblasts is easy to be observed, in the latter numerous elements in course of mitosis are to be met with. Numerous neutrophiles myelocytes. Erythroblasts with nucleus in pyknosis are easy to be observed, for these cells clearly predominate in relation with those of the haemoglobinie series (Figs. 12, 13, 14).

Case 11. Age: 25 years.

Microscopical examination: Parenchyma rich in cells. Small number of fatty cells. Apparently increased number of megakaryocytes. Very numerous eosinophiles myelocytes. Mitotic images in erythroblasts are easy to be observed. Marked predominance of orthochromatic erythroblasts with nucleus in rhexis (Fig. 15).

Case 12. Age: 48 years.

Microscopical examination: Abundant parenchyma rich in cells. Presence of fatty cells. Normal number of megakaryocytes are easy to be met with. Among the cells of the red series orthochromatic erythroblasts with nucleus in pyknosis predominate (Fig. 16).

Case 13. Age: 17 years.

Microscopical examination: Fatty cells in a very reduced number. Parenchyma very rich in cells. Numerous megakaryocytes. Numerous eosinophiles myelocytes. Numerous mitotic images in erythroblasts. Predominance of erythroblasts in karyorrhexis (Fig. 17).


Microscopical examination (femur marrow): Abundant parenchyma. A few fatty cells. Abundant megakaryocytes. Eosinophiles myelocytes in a very remarkable number. Among the elements of the red series there is a clear predominance of orthochromatic erythroblasts in karyorrhexis (Fig. 20).

Tibia marrow (diaphysis): Abundant parenchyma rich in cells. Presence of fatty cells in a small number. Very abundant megakaryocytes. Extremely numerous eosinophiles myelocytes. Numerous foci of erythroblasts, in which erythroblasts in karyorrhexis with haemoglobin laden protoplasm preponderate (Fig. 19).

Marrow from humerus diaphysis: Very abundant parenchyma. Rare fatty cells. Abundant megakaryocytes. Extremely numerous eosinophiles
myelocytes. Remarkable predominance of erythroblasts in karyorrhexis (Fig. 18).

Examination of the slide of femur marrow (diaphysis): Rare fatty cells. Apparently normal number of megakaryocytes. Some reticulo-cells. Numerous "plasmazellen". Eosinophiles myelocytes are frequent. Numerous basophiles erythroblasts, and orthochromatic with nucleus in pyknosis.

Examination of sternum marrow: Very rare fatty cells. Frequent megakaryocytes. Eosinophiles myelocytes are met with easily. Frequent "plasmazellen". Rare reticulo-cells. In the red series abundant elements in all evolitional stages are to be met with, orthochromatic erythroblasts with nucleus in rhesis being relatively frequent.

Case 15. Age: 14 years.

Microscopical examination: Very rare fatty cells. Abundant parenchyma intensely congested. Very numerous megakaryocytes. Great number of neutrophiles and eosinophiles myelocytes. Polychromatic erythroblasts are easy to be met with.

There is a clear predominance of orthochromatic erythroblasts with nucleus presenting rhesis image (Fig. 21).

Case 16. Age: 12 years.

Examination of the slide of marrow from femur diaphysis: Small number of fatty cells. Rare megakaryocytes. Numerous eosinophiles myelocytes. Very rare basophiles and polychromatic erythroblasts. Numerous orthochromatic erythroblasts with nucleus at initial stage of pyknosis. Elements in karyorrhexis are not to be observed. Some reticulo-cells. Very rare "plasmazellen". The elements which predominate numerically are those of the white series.

Examination of the slide of sternum marrow: The same appearance as above, a greater number of eosinophiles myelocytes is to be seen.

Case 17. Age: 3 years.

Microscopical examination of marrow: In spite of the macroscopic appearance denoting an aplastic marrow, an intense regeneration mainly of elements of the white series is to be seen, a fact which accounts for the greyish yellow colour observed. Abundant parenchyma rich in cells. Extremely great number of eosinophiles myelocytes. Very rare megakaryocytes. Numerous erythroblasts with nucleus in pyknosis or in rhesis, with clear predominance of the former (Figs. 23, 24).
Case 18. Age: 21 years.

*Microscopical examination*: Parenchyma rich in haemoformative cells. Numerous eosinophiles myelocytes. Rare megakaryocytes. Numerous erythroblastic foci with predominance of the stage in which the nucleus is to be encountered in pyknosis. Polychromatic erythroblasts, and erythroblasts presenting karyorrhexis, are easy to be seen (Fig. 25).

Case 19. Age: 59 years.

*Microscopical examination*: Numerous fatty cells among which a parenchyma rich in cells is to be observed. Rare megakaryocytes. Numerous neutrophiles and eosinophiles myelocytes. Presence of erythroblasts at all evolitional stages with predominance of cells with nucleus in rhesis. In this preparation erythroblastic foci are not to be seen so abundantly as in the other cases; in this section the myelocytic reaction predominates. In another preparate numerous erythroblastic foci have been observed (Fig. 26).

Case 20. Age: 26 years.

*Microscopical examination*: Abundant parenchyma rich in cells. Fatty cells are present. Normal number of megakaryocytes. Numerous neutrophiles myelocytes. In the erythroblastic foci, cells in karyorrhexis (orthochromatic erythroblasts) predominate. Numerous eosinophiles myelocytes (Fig. 27).

Examination of the slide: Presence of numerous fatty cells. Eosinophiles myelocytes are easy to be met with. Rare megakaryocytes. Very numerous neutrophiles myelocytes. Presence of various myeloblasts. The elements of the red series are rare, most likely this being due to an artifice in the preparation of the slide. The elements observed are always laden with haemoglobin. There is neither structural nor numeric modification «plasmazellen» and reticulo cells.

Case 21. Age: 10 years.

*Microscopical examination*: Very numerous fatty cells. Parenchyma little developed and poor in cells. Numerous reticulo cells, at times in the innermost part of the parenchyma, are to be seen with nucleus losing its spindle-shape and becoming oval. Numerous mitotic images in myeloblasts and basophiles erythroblasts. Numerous eosinophiles myelocytes. Erythroblasts with nucleus in pyknosis are easily met with.

Examination of the slide of femur marrow: Fatty cells are not numerous. Rare megakaryocytes. Numerous eosinophiles myelocytes. Exceptionally, basophiles or polychromatophiles erythroblasts are met with. Predominance of orthochromatic erythroblasts in karyorrhexis (Fig. 28).
The aplastic appearance seen in the slide has not to be considered as a fact of great importance, for apart from the red colour macroscopically observed, a great richness in noble cells was seen in the slide, both of the white as well as of the red series. Thanks to the numerous mitotic images, in this case we are clearly dealing with a medullar region in course of regeneration.

Examination of the slide of sternum marrow: Eosinophiles myelocytes are seen in a greater number than in the femur marrow. The red series presents quite a different aspect, for orthochromatic erythroblasts are but rare, while basophiles and polychromatic erythroblasts are numerous (Fig. 29).

Case 22. Age: 11 years.
Microscopical examination of marrow: Rare fatty cells. Very numerous eosinophiles myelocytes. Megakaryocytes in normal quantity. Basophiles erythroblasts are easily met with. Mitotic images in erythroblasts. Clear predominance of erythroblasts with nucleus in rhesis (Fig. 30).

Case 23. Age: 23 years.

Case 24. Age 9 years.
Examination of the slide of marrow from femur diaphysis: Extremely rare fatty cells. Megakaryocytes are common. Very numerous eosinophiles myelocytes. «Plasmazellen» are easy to be found. Very rare reticulo cells. Cells of the red series in great number, constituted almost exclusively by orthochromatic erythroblasts with nucleus in pyknosis or already in rhesis. There is a clear predominance of these latter elements (Fig. 34).

Examination of the slide of marrow removed from a rib: the same picture as above. Basophiles erythroblasts are met with more easily; the great majority however is constituted by cells with haemoglobin and presenting a nucleus in rhesis (Fig. 33):

Case 25. Age 41 years.—Biopsia.
Microscopical examination of sternum marrow in section: Very rare fatty cells. Very abundant parenchyma rich in cells. Megakaryocytes are easily met with. Very numerous eosinophiles myelocytes. Numerous meta-
myelocytes and eosinophiles leucocytes. Rare elements of the red series; among them some cells with nucleus in pyknosis are encountered with difficulty, elements less developed predominating (basophiles and polychromatic erythroblasts).

Examination of the slide of sternum marrow: Very rare fatty cells. Megakaryocytes are common. Numerous myelocytes, metamyelocytes and eosinophiles leucocytes. Numerous neutrophiles leucocytes. Rare reticulo cells. "Plasmazellen" and myeloblasts are easily met with. In the red series, elements at all stages are seen, with predominance of elements less developed (basophiles erythroblasts).

This patient responded excellently to the treatment with reduced iron in large doses, giving birth to two healthy children.

This is, most likely, a case in which diaphysary marrow would present an aspect entirely different from that met with in the sternum marrow. Taking for granted a prompt blood recovery, the marrow cells should be in an advanced evolutorial stage and, just like as in all cases of our observation, we should have met with the normoblastic stage in the diaphysary marrow.

Interpretation of results

In 24 cases of necropsies of individuals between 3 and 68 years old, the morphological appearance of marrow from femur diaphysis presented a noticeable constancy.

The macroscopical appearance is characterized by a uniform red coloration. This fact stands out in bold relief, as this is the only organ in which this coloration is to be noticed, contrasting very much with the pale tone of the other organs, resulting from the anaemia and the subsequent fatty degeneration.

As to the macroscopical appearance here described, we met with only one exception in which marrow appeared greyish yellow (Case 17); nevertheless, even in this case the microscopical examination revealed a frankly regenerative state.

The microscopical appearance was fundamentally characterized by an intense regeneration of the noble parenchyma with a great numerical decrease in fatty cells. In the white series, we always met with an intense myelocytic reaction (eosinophiles myelocytes); the megakaryocytes presented now an increase, now a decrease. We encountered no relationship between megakaryocytic and myelocytic reaction, as might be supposed owing to the fact that blood coagulability in anchylostomiasis keeps up a certain relationship with peripheral eosinophilia. In some cases (Cases
23, 24), we remarked an intense eosinophilous reaction of marrow, the day after the verification of a normal percentage of eosinophiles leucocytes in peripheric blood. This fact, already observed by other authors, finds its explanation in the existence of a strong intestinal eosinophilous reaction. Concerning the changes of haemoistioblasts, «plasmazellen», reticulo-cells, no observation was possible in the sections on account of the difficulty in recognizing exactly these cells, owing to the technique to be followed; in the slides we found no constant changes. As to the red series, there was always to be seen a great quantity of foci where orthochromatic erythroblasts with nucleus in pyknosis or rhexis predominate. The red coloration, macroscopically seen in the marrow, is due to the great number of these cells laden with haemoglobin.

Therefore, in anchylostomiasis there is always a synthesis of this proteic complex in a noticeable quantity.

The structure of these cells, their staining characters, their size and shape are identical of those observed in normal marrow. Megaloblasts are not to be seen in any case.

An appearance differing from that described above was observed in two cases, in marrow removed from sternum. In the former (Case 21), the femur marrow presented an intense erythroblastic regeneration, and in the other one (Case 25), blood regeneration owing to the treatment applied with usual quickness and intensity. These marrows were characterized by an intense myelocytic reaction, though with a great paucity of elements of the red series.

In three other cases (Cases 14, 16, 24), sternum marrow presented the same intense erythroblastic regeneration as the marrow from femur diaphysis. In Case 16, it looked like that from the diaphysis of tibia, femur and humerus. In this case, marrow regeneration reached a maximum degree, for marrow along with the progression of anaemia regenerates itself first in vertebrae, then in sternum, ribs, femur and finally in tibia (8).

We have not studied the marrow of tibia and humerus diaphysis in the other cases. The similitude of different marrow regions in this case, makes believe that the regenerative process begins on all long bones at the same time, and it is probable that this complete regeneration is of general occurrence in necropsies of hookworm cases.

The paradoxical fact of marrow intensely regenerated and of a aregenerative blood was accoutered for after the works of Whipple, Castle, Witts and others who showed the necessity of certain substances for the development of marrow cells. After having determined the plastic appearance of marrow in anchylostomiasis, we went, for this anaemia,
in search of a process analogous to that described by these authors in other anaemia. Out of the hematopoietic factors known as yet, we observed that only one has a clear action upon blood regeneration in anchylo-
 stomiasis. Iron ion when administered in doses sufficient for absorption, quickly produces the return to the norm in the features of the red cell. As there are already anaemiae induced by deficiency of this metal, we inquired, in the papers of the authors who were engaged in their study, for information concerning the morphological appearance of marrow, and in a recent paper of Witts (6) we found iron as being one of the substances which are indispensable for the normal ripening of the normo-
 blast up to the stage of an erythrocyte. This appearance of marrow to-
 gether with other fundamental facts supporting a new interpretation of the anaemiant process in hookworm disease is outlined in our previous paper (3). As we believe, the etiological agent of this disease does not act directly upon blood causing a destruction through any process what-
 ever, nor upon marrow producing an exhaustion of its regenerative capacity owing to excess of excitation due to intestinal haemorrhages, nor to a paralysing toxic action. In our opinion, the parasite acts upon the iron metabolism causing a quantitative decrease of this element in the organism, the haematopoietic system being offended by this deficiency, with en-
 suing abnormality in the evolution of the normoblast. Taking into con-
 sideration the characters of coloration, the nucleus and plasma superfcies and mainly the rapid formation of red cells by means of a martial treat-
 ment, we think it most likely that the normoblasts, met with in a great number in marrow of anchylostomiasis, are normal cells, i. e. cells able to be transformed into normal red cells in the presence of iron. Taking for granted the production and storing up of haemoglobin in the mar-
 row of anchylostomiasis, it is possible that iron, apart from its function of forming haemoglobin, plays another rôle in the transformation of the normoblast into an adult normal red cell.

The consequence of iron as to haematopoiesis in this disease led us to verify in the marrows observed what were the modifications the normoblasts underwent through martial medication. Ge-
 nerally, during the first three days, even with an intense iron medication, no regenerative signs appear in peripheric blood (7). Thus, among the clinical observations of our patients, looking for the remedies employed for the treatment of the disease, we could — in cases of death within the first three days after their admission to the hospital or in cases of martial treatment with insufficient doses — we could observe fatal cases in which iron had been administered.
The transformation of the normoblast into a red blood cell, or the destruction of the nucleus, normally proceeds in various manners.

Ferrata says in his text-book (8): «We may admit that the erythroblasts become erythrocytes after a pyknotic reduction of their nucleus which is expelled «in toto» from the cell (Maximow), or after its fragmentation (Weidenreich) and more rarely, after its intracellular dissolution (Israel and Pappenheim)». In pathological cases the same author quotes: «Therefore we may draw the conclusion that in pathological conditions there may happen: a) Total expulsion of the nucleus both in the megaloblast as well as in the normoblast, when the nucleus is still bulky; b) Nuclear karyorrhexis into fragments more or less voluminous; c) From the nuclear constituents in course of destruction the formation of azurophilous granulations (chromatin), of Cabot’s ring bodies (nuclear membrane) and of azurophilous polychromatophilia».

Cases 14 and 15 were subjected to iron treatment; the former died on the second day, and the treatment of the latter was made with insufficient doses; iron nevertheless succeeded in inducing a reticulocytosis on the last day of the patient’s life. In these two cases we found a manifest predominance of erythroblasts with the nucleus already segmented. In the marrow of a fatal malaria case, which disease presents intense regenerative evidence in peripheric blood, we also found predominance of erythroblasts in karyorrhexis. Yet, in our observation, out of nine cases in which no iron salt whatever had been administered, three (Cases 16, 17, 18) showed a predominance of erythroblasts with nucleus in pyknosis, and the other six ones (Cases 19, 20, 21, 22, 23, 24) showed a predominance of erythroblasts in karyorrhexis. There is, consequently, no relationship between the state of pyknosis or karyorrhexis in normoblasts observed in the marrow and the administration of iron salts.

CONCLUSIONS

1) — Marrow from femur diaphysis in twenty-four fatal pure cases of hookworm disease always presented an aspect frankly regenerative of an erythroblast-myelocytic type.

2) — Marrow removed from sternum in two cases presented an aspect resembling that found in marrow from femur diaphysis; in two other cases it presented intense myelocytic reaction with predominance of eosinophile myelocytes, yet without marked erythroblastic reaction.

3) — Concerning the elements of the white series, the cells met
with in greater proportion are eosinophile myelocytes, and concerning the red series — orthochromatic erythroblasts with nucleus in pyknosis or karyorrhexis.

There is, consequently, synthesis of haemoglobin in a valuable quantity.

4) — The observation of marrow morphologically regenerated and functionally deficient is a fact which, most likely, is due to an evolitional anomaly of orthochromatic erythroblast towards the erythrocyte.

5) — The normoblastic aspect of bone-marrow, verified and documented in this paper, is one of the facts in which we based the hypothesis that the hookworm anaemia is caused by a fault of iron in the body.

EXPLANATION OF THE PLATES LXXXII—XCVIII.

PLATE LXXXII

Fig. 1 — Case 1. Marrow from femur diaphysis. Enlargement 360 ×. The erythroblastic foci are almost exclusively constituted by cells with nucleus in pyknosis or rhesis.

Fig. 2 — Case 2. Marrow from femur diaphysis. 120 ×. Abundant parenchyma rich in cells. Predominance of red series with nucleus in pyknosis.

PLATE LXXXIII

Fig. 3 — Case 3. Marrow from femur diaphysis. 220 ×.

PLATE LXXXIV

Fig. 4 — Case 4. Marrow from femur diaphysis. 300 ×. The cells presenting a smaller nucleus and a darker stain are erythroblasts laden with haemoglobin.

Figs. 5 and 6 — Case 4. Another region of femur marrow. 132 ×.

PLATE LXXXV

Fig. 7 — Case 5. Marrow from femur diaphysis. 66 ×. There is to be seen a limit between the aplastic zone and the region where a frank regeneration is observed.

Fig. 8 — Case 6. Marrow from femur diaphysis. 28 ×.

PLATE LXXXVI

Fig. 9 — Case 7. Marrow from femur diaphysis. 300 ×. There are easily to be observed orthochromatic erythroblasts in karyorrhexis.
Fig. 10 — Case 8. Marrow from femur diaphysis. 675 ×. Very numerous eosinophiles myelocytes. Some erythroblasts are to be seen with nucleus in karyorrhexis or pyknosis. In the middle, endothelial cells of a capillary are to be seen.

PLATE LXXXVII

Fig. 11 — Case 9. Marrow from femur diaphysis. Numerous erythroblasts with nucleus in pyknoses. 190 ×.

Fig. 12 — Case 10. Marrow from femur diaphysis. 600 ×: Numerous eosinophiles myelocytes and erythroblasts with haemoglobin and in pyknosis. Polychromatic erythroblasts are to be seen less frequently.

PLATE LXXXVIII

Fig. 13 — Case 10. Marrow from femur diaphysis. Erythroblastic focus in which exclusively erythroblasts with haemoglobin and nucleus in pyknosis are to be seen.

Fig. 14 — Case 10. Other focus where a great number of orthochromatic erythroblasts with nucleus in pyknosis is met with.

Fig. 22 — Case 17. Macroscopic appearance of marrow from femur diaphysis. This aspect, apparently aplastic (the figs. 23 e 24 shown the stark regeneration of this marrow), was met only in this case, in all others we observed a uniform red color.

PLATE LXXXIX

Fig. 15 — Case 11. Marrow from femur diaphysis. 675 ×. The elements of the red series are very numerous, the final evolitional stages predominating, with plasma laden with haemoglobin and nucleus in pyknosis or rhaxis. Eosinophiles myelocytes are easily observed.

Fig. 16 — Case 12. Marrow from femur diaphysis. 550 ×. Numerous erythroblasts with nucleus in pyknosis.

PLATE XC

Fig. 17 — Case 13. Marrow from femur diaphysis. 1025 ×. A focus of orthochromatic erythroblasts with nucleus in rhaxis is to be seen in the central part.

Fig. 18 — Case 14. Marrow from humerus diaphysis. 60 ×.
PLATE XCI

Fig. 19 — Case 14. Marrow from tibia diaphysis. 625 ×. Numerous eosinophiles myelocytes and orthochromatic erythroblasts in karyorrhexis.

Fig. 20 — Case 14. Marrow from femur diaphysis. 450 ×. Numerous orthochromatic erythroblasts in karyorrhexis and eosinophiles myelocytes.

PLATE XCII

Fig. 21 — Case 15. Marrow from femur diaphysis. 475 ×. Numerous erythroblasts in final evolutional stage.

Fig. 23 — Case 17. Marrow from femur diaphysis. 1000 ×: Numerous eosinophiles myelocytes and orthochromatic erythroblasts with nucleus in pyknosis.

PLATE XCIII

Fig. 24 — Case 17. The same. 130 ×.

Fig. 25 — Case 18. Marrow from femur diaphysis. 960 ×. Nest of erythroblasts where a great number of erythroblasts with nucleus in pyknosis is to be seen. On the left of the parenchymatous beam lying between the two fatty cells, a polychromatic erythroblast is clearly seen.

PLATE XCIV

Fig. 26 — Case 19. Marrow from femur diaphysis. 710 ×.

Fig. 27 — Case 20. Marrow from femur diaphysis. 450 ×.

PLATE XCV

Fig. 28 — Case 21. Marrow slide from femur diaphysis. 425 × Predominance of red series elements. Numerous orthochromatic erythroblasts with nucleus in pyknosis.

Fig. 29 — Case 21. Marrow slide from sternum. 425 ×. On the contrary of preceding Fig., the white series elements are here more numerous, with rare erythroblasts in advanced evolutional stage.

PLATE XCVI

Fig. 30 — Case 22. Marrow from femur diaphysis. 475 ×.
Fig. 31 — Case 23. Marrow from femur diaphysis. 90 ×: Numerous erythroblastic nests where cells with pyknotic nucleus predominate.

PLATE XCVII

Fig. 32 — Case 23. The same preparation with enlargement 425 ×. Strong regeneration of the red series, with predominance of orthochromatic erythroblasts with nucleus in pyknosis or rhesis. Numerous eosinophiles myelocytes.

Fig. 33 — Case 24. Slide of marrow removed from rib. 425 ×. Among the numerous elements of the red series, the presence of orthochromatic erythroblasts presenting nuclear rhesis is seen easily.

PLATE XCVIII

Fig. 34 — Case 24. Marrow from femur diaphysis. 675 ×. Numerous orthochromatic erythroblasts in karyorrhexis.

Fig. 35 — Case 25. Marrow removed from sternum. 550 ×. Very rare elements of the red series having evolved up to nuclear pyknosis. Marked predominance of white series elements among which eosinophiles myelocytes are easily to be met with.