THE NORMAL FATE OF ERYTHROCYTES.

II. Blood Destruction in Plethoric Animals and in Animals with a Simple Anemia.

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The findings presented in the preceding paper show clearly that phagocytosis can account for red cell destruction only in certain species, and that whatever the extracellular method of this destruction may be, it does not entail the formation of shadows, such as result from hemolysis. The evidence is against a direct hemolytic action in the spleen. The constant presence in this organ of an accumulation of poikilocytes which are subdividing, and of microcytes, and the presence of these elements in the circulating blood indicate that the red cells disappear, in part at least, by fragmentation. In a further study of methods of blood destruction, we have examined rabbits rendered plethoric by repeated direct transfusion. Such animals soon acquire the ability to dispose of large quantities of blood. This goes on in the absence of demonstrable agglutinins or hemolysins, and, according to Boycott and Douglas,[1] represents an intensification of the normal process of destruction, though what that is the authors cited could not discover. They state that the spleen is the only organ in which changes are regularly met with. It is enlarged, and phagocytes containing red cells are more numerous than usual.

Method.

Rabbits were used. From three to six compatible donors were selected for each recipient. The donors were employed in rotation, and each recipient was given nearly every day 10 cc. of whole blood from one of them. The blood was

[1] Boycott, A. E., and Douglas, C. G., *J. Path. and Bacteriol.*, 1909–10, xiv, 294.

obtained by cardiac aspiration into a syringe containing 1 cc. of 1 per cent sodium citrate in 0.9 per cent salt solution. It was injected directly into an ear vein of the recipient. Clotting was not met with. The small amount of citrate—0.01 gm. at each injection—caused no symptoms or lesions.[2] The transfusions were continued for several weeks, during which time the animals remained well and frequently gained in weight.

The rabbits were found to differ much in their ability to dispose of the excess blood. In some, it was difficult to increase the hemoglobin value even by daily injections; in others, it began at once to rise, and soon reached a high figure. Since the total blood volume from day to day was not known, it is impossible to say how much these differences are attributable to variations in the plasma. As a rule, when the animal had been transfused for some time, and the hemoglobin had increased from 80 or 90 per cent Sahli to about 150 per cent, it failed to go higher, but instead tended to drop despite the daily addition of blood. Yet very often in such animals, no demonstrable agglutinins had developed. These animals were judged suitable for our purpose. The circulating blood was examined for disintegrating red cells, the animal was etherized, and the organs were washed out one by one according to the methods outlined in our first paper. The washings were submitted to differential centrifugation, and portions of the organs were teased and examined.

The circulating blood of many of the plethoric animals showed microcytes and poikilocytes—schizocytes—in far greater numbers than did that of normal controls; but no other signs of blood destruction were seen in it. Examination of the donor rabbits showed that the increase in microcytes and poikilocytes was in general traceable to them. The removal from these animals of 10 cc. of blood every 4 or 5 days was often sufficient to cause a marked increase in the number of schizocytes in circulation, whence they were transferred to the plethoric individuals. When care was taken to use donors with few circulating schizocytes, it was found that the increased blood destruction in the plethoric rabbits was unaccompanied by an increase in these forms in the blood.

There was regularly found a notable increase in the number of schizocytes in some of the organs under circumstances which precluded an introduction of them with the donor's blood. For example, in one animal repeatedly transfused during 14 days, and always with a blood in which schizocytes were rare, the spleen was found to be packed with these forms. It was a large, brown-purple organ, from

[2] In control animals injected with sodium citrate alone, normal findings were obtained.

which several times as much blood as normal was obtained on perfusion. After washing, it was smaller than before, but still enlarged, weighing 1.1 gm. as compared with 0.6 gm. for the average normal individual of the same size. In the teased specimen phagocytosis was only slightly increased, but there were great numbers of microcytes, poikilocytes, and the peculiar dumb-bell form of fragmenting red cell encountered not infrequently in normal animals.[3] In fact, nearly all the considerable quantity of blood which failed to wash from the spleen consisted of these forms. In the washings they were far more frequent than is ever the case in normal blood. No agglutinated masses or shadows were observed.

This is a typical finding. In some spleens, small extracellular aggregates of amorphous brown pigment were frequent. Sometimes phagocytosis was increased, but often it was only normal. The one unfailing and characteristic feature was the great accumulation of schizocytes.

In the other organs, with the exception of the red bone marrow, the accumulation of schizocytes was inconstant and always negligible. The fact has already been brought out that the normal marrow contains only such schizocytes as happen to be present in the blood coursing through it. In the plethoric marrow, on the contrary, they are met in considerable numbers. Occasionally the number of phagocytes containing red cells is somewhat increased. We have utilized the peculiar affinity of the cresyl blue stain for intracellular red cells and their fragments[3] to determine whether the free schizocytes in marrow and spleen are the product of intraphagocytic digestion. This is not the case.

Despite careful search, no other methods of blood destruction were observed. The findings strongly support the view that the disappearance of red cells in plethoric rabbits takes place in large part by fragmentation, if not almost wholly.

Blood Destruction in Animals Anemic from Hemorrhage.

Microcytes and poikilocytes are frequent in the blood of animals rendered anemic by hemorrhage. The conception that these forms

[3] Described in the preceding paper.

are the result of blood destruction finds here an apparent contradiction; for in simple anemia one would expect a conservation of blood rather than increased destruction.

We have examined numerous anemic rabbits by perfusion and differentiation of the washings in the centrifuge, according to the method already described.[3] To render the animals anemic they were bled on successive days by cardiac aspiration, 25 cc. being the usual amount of blood removed. After four or five bleedings, when the hemoglobin had dropped to 30 to 20 per cent, no more blood was taken. 3 or 4 days after the last bleeding, films of the blood showed that repair was in progress. There were many reticulated corpuscles, numerous large pale ones, and, in addition, marked polychromasia and numerous microcytes and poikilocytes. It was at this stage that the animal was etherized and the organs examined.

The findings may be briefly summarized. There was a striking increase in the spleen's content in microcytes and poikilocytes. Much of its residual blood consisted of these forms, and, in several instances, the organ was somewhat enlarged from their accumulation. Phagocytosis was not increased. In some of the other organs, especially in the kidney, small collections of microcytes and poikilocytes were inconstantly present. It was noteworthy that the washings from the marrow never yielded more of them than did the control specimen of circulating blood, while in the marrow tissue teased after washing, there were practically none. The point had special attention because of the widely held view that these forms originate as such from the blood-forming tissue. Our findings show that this is not the case, and that the microcytes and poikilocytes of anemia from hemorrhage are true fragmentation forms, deserving of the name of schizocytes given to them by Ehrlich.

Several facts were observed which indicate that the schizocytes of anemia are derived for the most part not from the fragmentation of cells remaining from the pre-anemic period, but from the breaking down of cells formed by the bone marrow to make up the lack in blood. Most of the schizocytes of the anemic rabbits partook of the pallor of the new-formed corpuscles. In the centrifuge, they were readily separated from the mass of cells but were themselves completely sedimented only with difficulty; and the last

to come down from suspension were practically colorless. Large numbers of the schizocytes were reticulated like the new corpuscles circulating with them. In two instances in which counts were made, 56 and 25 per cent of the schizocytes were reticulated, as compared respectively with 30 and 24 per cent of the corpuscles, a relation readily understood when it is considered that one reticulated corpuscle might give rise to several reticulated schizocytes. In the normal rabbit, as is well known, 2 per cent of the corpuscles or less show reticulation, while according to our experience, none of the schizocytes are reticulated. In the anemic rabbit, reticulated schizocytes appear almost immediately after any great increase in reticulated corpuscles. The conclusion seems warranted that the schizocytes of the anemia result from the fragmentation of new-formed, abnormal red cells. Some of the latter must begin to break in pieces almost as soon as they are put forth by the marrow. From the study of a fresh preparation of anemic blood, some idea can be had of the ease with which this may occur. As the blood drop spreads under pressure of the cover-glass, it will be observed that the well colored, normal looking red cells constitute stable objects as compared with the pale, large cells resulting from the anemia. Often one of the latter will be swept through a narrow cranny between two normal cells. The contour of these latter does not change in the least, whereas the pale cell is greatly compressed, and it flows through the aperture as if fluid. It is easy to see how such cells may be pulled to pieces in the circulation.

Altogether, the findings in anemic animals indicate that here the destruction of red cells by fragmentation goes on actively. Large numbers of the fragments accumulate in the spleen. Many, though, circulate for a greater or less time. This is not the case with the schizocytes of normal and plethoric animals, which are rapidly taken out of the blood.

DISCUSSION.

Ehrlich long ago stated[4] that the microcytes and poikilocytes of anemia result from fragmentation of the circulating cells. He

[4] Ehrlich, P., Farbenanalytische Untersuchungen zur Histologie und Klinik des Blutes, Berlin, 1891, 99.

based his view on the fragmentation *in vitro* of red cells under the action of various physical and chemical agents. His conception is still far from having gained general acceptance, many hematologists holding the view that microcytes and poikilocytes are elaborated as such in the marrow.[5] Our findings show definitely that they arise in the circulation. Ehrlich thought that their appearance during an anemia was purposeful, since it increased the blood surface, and, as a corollary to this opinion, he held that they circulated for some time. Two of our facts point against the idea of a purposeful division. The cells which break down are those least suited to combat the anemia, namely, those poor in hemoglobin; and the fragments tend to accumulate in the spleen, whence they cannot easily be flushed out. Cell fragmentation in an anemic organism would seem to be, not the result of a compensatory arrangement, but an incident in a vicious circle. Because of the anemia, the bone marrow puts forth cells that are unable to withstand the exigencies of circulation. These soon fragment and the work of the marrow must be done again. The marrow then must not only make up an initial blood lack, but while so doing must repair constant fresh losses due to the poor quality of the cells it puts forth. Or, in other words, it must take one step down for every two steps up. That there is no increase in the output of bile pigments in anemic animals may be due to a special conservation of hemoglobin, or to the relative paucity of this substance in the broken down cells, or to a diminution in the breaking down of such normal red cells as are present, or to extraneous factors such as Whipple has shown can influence the pigment output.

The view that red cell destruction normally takes place for the most part by fragmentation finds striking support in the findings in both anemic and plethoric animals. In the former, the red cells are less hardy than usual. Fragmentation is the result. In the latter, a greater blood destruction than usual must be accomplished. Again fragmentation is increased. Furthermore, in our plethoric animals, which would seem especially suited to the investi-

[5] For the latest statement of this view see Barker, L. F., The Clinical Diagnosis of Internal Diseases, New York, 1916, iii, 25.

gation, no other method of destruction than fragmentation, saving only the phagocytosis already known to exist, has been encountered. Phagocytosis is often increased in these animals, but not always. In some cases, fragmentation is practically the only process of destruction found.

The accumulation of schizocytes in the spleen is striking, both in anemic and plethoric animals. That this organ has some important function in connection with such elements cannot be doubted; and the findings in plethoric animals suggest that the bone marrow may share the function if blood destruction is great. When human beings are severely burnt, many red cells break into hemoglobin-containing fragments. According to good authority,[6] these collect in the spleen so rapidly that within a few minutes practically all are removed from the blood. Not improbably the blood of normal and plethoric animals is kept free of fragments in much the same way. They are not taken so completely from the blood of anemic animals. Perhaps a change in the protoplasm, such as may be thought to accompany normal aging of the cell, is necessary for this. But even in anemia, microcytes and poikilocytes accumulate in the spleen to a noteworthy extent.

The schizocytes are undoubtedly reduced at last to a fine hemoglobin-containing dust. How this is disposed of, whether in a special organ, like the spleen, or by dissolution in the blood is not evident. But it must be disposed of rapidly, or the blood of plethoric animals would be rich in it. The hemoglobin, wherever and however given off, will of course be quickly utilized by the liver.

Taken together, the facts afford a simple and rational explanation of the normal method of blood destruction, and indicate why the problem has presented difficulties. A constant rapid fragmentation of the effete red cells, one by one, while still circulating, and a prompt utilization of the products of destruction will readily account for the high general standard of cell resistance that has puzzled observers. Whether, indeed, as the red cells fragment, their resistance lessens, as determined by the ordinary tests, remains to be determined. The

[6] Krehl, L., and Marchand, F., Handbuch der allgemeinen Pathologie, Leipsic, 1908, i.

fragmentation does not involve a loss of hemoglobin, and *a priori* there is no reason why it should be accompanied by a decreased resistance to hemolysins or hypotonic fluids, the ordinary test-agents.

Meltzer[7] has suggested that the red cells are normally destroyed through mechanical wear and tear, and has studied in this connection their fate when shaken. But cells shaken *in vitro* are not destroyed according to the method we have encountered in the body. Only a few of the shaken cells break into microcytes and poikilocytes. The vast majority are reduced to shadows. Shaking is obviously a different process from the perpetual sieving and squeezing of the cells that go on in the finer capillaries. Yet it is possible that the resistance of the corpuscles to shaking may after all give some indication of their tendency to fragment. Experiments to determine the point are now under way.

GENERAL CONCLUSIONS.

1. The increased destruction of red cells in animals rendered plethoric by transfusion takes place predominantly by a fragmentation of the corpuscles without loss of hemoglobin.

2. The microcytes and poikilocytes observed in animals with a severe anemia due to hemorrhage are not put forth as such by the bone marrow, but are portions of cells fragmented while circulating.

3. The cells thus fragmented are for the most part those newformed to meet the exigencies of the situation. Such cells are in large part unable to withstand the wear and tear of function. There results a vicious circle. The anemia renders the bone marrow unable to put forth proper cells, and those it does produce are soon destroyed, thus prolonging the condition. A similar state of affairs probably exists in many human anemias.

4. The occurrence of large accumulations of microcytes and poikilocytes in the spleen of anemic and plethoric animals indicates that the organ exercises some important function in connection with these forms. The same is true of normal animals, for the findings in them are similar, though less striking.

5. The normal fate of the red corpuscles, in those species in which

[7] Meltzer, S. J., *Rep. Johns Hopkins Hosp.* (*Welch Festschrift*), 1900, ix, 135.

phagocytosis is negligible, is to be fragmented one by one, while still circulating, to a fine, hemoglobin-containing dust. The cell fragments are rapidly removed from the blood, but their ultimate fate remains to be determined. The facts indicate that they are removed from the blood by the spleen, and under exceptional conditions, by the bone marrow.