Figs. 1 to 6 are from three cases of pernicious anemia.
 Figs. 1 and 2 show bone marrow. 1. Normoblast with nuclear particle. 2. Megalocyte with nuclear particle.
 Figs. 3 to 6 show blood. 3. Nuclear particle in erythrocyte. 4. Basophilic granules in erythrocyte. 5. Basophilic granules in normoblast. 6. Nuclear particle and basophilic granules in erythrocyte.
 Figs. 7 to 9 are from chronic pyridin poisoning in the rabbit (Rabbit VII pyridin); all from the blood. 7. Nuclear particle in erythrocyte. 8. Basophilic granules. 9. Nuclear particle and basophilic granules.
 All cells were stained with methyl-green-pyronin after heat fixation, and drawn with Leitz 1/12 oil immersion, ocular No. 4.

NUCLEAR PARTICLES IN THE ERYTHROCYTES *

ROGER S. MORRIS, M.D.

BALTIMORE

Evidence of regeneration of the blood is of great value not only in the diagnosis, but also in the prognosis and treatment of anemias. Among the most important regenerative signs may be mentioned the occurrence of nucleated red blood cells, of basophilic granules in the erythrocytes, and of polychromatophilia. Recently I have described another manifestation of regeneration found in the human red corpuscles in anemias and in embryonic blood, and subsequent work has served to confirm the views previously expressed, with one or two exceptions.

The small, round or oval, eccentrically placed bodies, first described by Howell and later by Schmauch, in the erythrocytes of the cat, are best seen when stains are added to the fresh blood. These have been considered identical with bodies answering to the same general description morphologically found in permanent stained preparations of blood by subsequent observers—Pol, Schmidt, Jolly and myself. There are, however, differences, though it is possible that the two are closely related.

If one adds methyl green, methyl violet or other nuclear stain to the fresh blood of a normal cat, a certain number of red cells (up to about 30 per cent.) may show round, oval or irregularly-shaped structures which are deeply stained and usually situated eccentrically in the cell, apparently always single. They are also easily seen in fresh unstained specimens of blood, and a few of them may be found free between the red corpuscles, as Schmauch pointed out. When blood films are allowed to dry in the air, the bodies are still demonstrable for a few days on the addition of stain—and, indeed, they may be seen in the unstained, dry spreads, but in preparations kept twenty-nine days I have failed to demonstrate them, although they were very numerous in films made at the same time and examined while fresh. These bodies in the red blood cells of the cat, which stain with nuclear dyes, are, then, rather unstable. Again, fixation of the films in ethyl alcohol, methyl alcohol, alcohol and ether, and alcohol and formaldehyd (Futcher-Lazear) causes them to disappear, but short fixation in 5 per cent. or 10 per cent. formaldehyd preserves them fairly well. Underfixing the films with heat (120 degrees

*From the Clinical Laboratory of the Johns Hopkins Hospital.

C. for five minutes) enables one to make permanent mounts, as Schmauch has shown, but longer fixation at this temperature or thirty seconds at the spheroidal point for water will cause these very unstable bodies to disappear. I have examined the blood of six cats both before and after venesections (as much as 60 c.c. of blood withdrawn), and the results have been quite uniform in all.

While these structures in the erythrocytes of the cat are easily destroyed by heat and other methods of fixation, I have constantly found a very few characteristic "nuclear particles" in specimens of cat's blood fixed and stained in the usual manner, i. e., hematoxylin and eosin (ethyl alcohol fixation), Giemsa's stain and Hasting's stain (methyl alcohol), carbol-thionin (Fletcher-Lazear fixation), Ehrlich's triacid (heat), Pappenheim's methyl green-pyronin (heat), etc. In no other animal have nuclear particles been found in apparently normal adults, and thus far it is only in the fresh blood of the cat that these bodies described by Howell and Schmauch have been demonstrated. This, therefore, suggests a possible relationship between Howell's bodies and the nuclear particles seen in permanent blood specimens, though I can bring no further evidence of such relationship. There are, moreover, certain well-marked differences between the two: Howell's bodies, in specimens fixed for five minutes at 120 degrees C., do not stain, or stain very imperfectly, with methyl green-pyronin; nuclear particles, in thoroughly fixed smears, take a blue color, like the nuclei of normoblasts. The nuclear particles have never been found in a large proportion of the red blood corpuscles, whereas Howell's bodies may occur in 80 per cent. of the erythrocytes (Schmauch). Howell's bodies are, as we have seen, very unstable; nuclear particles, on the other hand, are demonstrable after all the usual methods of fixation, and I have found them in the blood of a patient with pernicious anemia, fixed and stained twenty-months after the films were made. Thus the two structures differ in many respects.

With the exception of the cat and the normal pregnant rat (Jolly), nuclear particles have been found in full-grown animals only during active regeneration of the blood, i. e., after severe or repeated hemorrhages, or phenylhydrazin or pyrocin poisoning, etc. They have been found in the blood of embryonic and new-born cats, mice and rats, and I have found them in the blood of the human embryo and in the most varied conditions in human adults, in whose blood other evidences of regeneration were present. As yet I have not found them in normal blood, but have observed them in nine cases of pernicious anemia (Figs. 1 to 6), in anemia in infants (two cases), in secondary anemia (two cases), in

chronic myeloid anemia (three cases), and in one patient with anemia after splenectomy had been done (splenomegaly). Furthermore, they were present in smears of the bone marrow (femur) in two cases of pernicious anemia (Figs. 1 and 2), and in one case in large numbers. It seems quite probable that they might be found in the marrow in all cases in which they are encountered in the blood. Cabot pictures multiple nuclear particles in the bone marrow, but he apparently did not find them in the blood. Within the last year Naegeli¹ has also "seen these (nuclear) particles very often in the blood of pernicious anemia, of embryos of animals, and in different forms of anemia."

Since they are found in the bone marrow and are almost always associated with other evidences of regeneration of the blood, nuclear particles may safely be considered a sign of regeneration. Further observation in human blood has proved the greater frequency of these particles in nucleated red cells as compared with the blood of animals. In some bloods, however, single nuclear particles in the erythrocytes may be the main evidence of regeneration; this fact was well illustrated in the case of the patient after splenectomy, in whose blood, at one time, thirty nuclear particles were found in about an hour and a half (examination with mechanical stage), while only one normoblast, no cells with basophilic granules, and a few with polychromatophilia were seen. Similarly, in the blood of a patient with pernicious anemia, kindly sent to me by Dr. C. K. Winne, Jr., of Albany, nuclear particles were numerous, but no nucleated reds were present, as he had noted. Thus, in certain instances, the chief reparative sign in the blood may be the presence of nuclear particles.

It has been urged by some² that nuclear particles are identical with the basophilic granules sometimes present in the red cells and that their classification separately is superfluous. Against this view there are striking and, I believe, conclusive facts. In the first place, it has just been shown that nuclear particles may be found in the absence of basophilic granules. They are, however, often found in the same blood and even in the same cell, as I have shown elsewhere. With modified Romanowski stains (Hasting's, Giemsa's and Wilson's) nuclear particles take the same color as the nucleus, while basophilic granules take a dark blue color. Nuclear particles are practically always round, sharply circumscribed, and eccentrically placed, and are usually single, so that it is often possible to recognize both in the same cell. But the most striking

1. Personal communication, Nov. 6, 1908. See also article by Naegeli in bibliographic references at end of this paper.

2. Meyer (E.): Oral communication.

tinctorial differentiation is seen in preparations fixed by heat and stained with Pappenheim's³ methyl green-pyronin mixture. With this stain the basophilic granules are stained a brilliant red (Figs. 4, 5, 6, 8 and 9), while the nuclear particles take on a beautiful blue color (Figs. 1, 2, 3, 6, 7, 9) like that of the nucleus (Figs. 1 to 6). Since its chromatin is densely packed, the color of the nuclear particle may be even darker than the nucleus of some of the erythroblasts. When both nuclear particle and basophilic granules are present in the same cell, the picture is very striking (Figs. 6 and 9); their separate identity is at once demonstrated. It is no more justifiable to consider them to be the same than it is to look on the nucleus of the erythroblasts and the basophilic granules as identical structures.

Many writers at present, among whom may be mentioned Meyer and Speroni, consider basophilic granules to be products derived from the nucleus of the red blood cells. That the nuclear particles is of nuclear origin there can be scarcely any doubt, for with all nuclear dyes it is stained exactly like the nucleus. In animals all transitions in size may be seen between the normoblastic nucleus and the nuclear particle, and in man the latter can be seen to be derived from the nucleus, as many preparations of blood, as well as those of the bone marrow in pernicious anemia, show. Here the nuclei can be seen broken up into two or more perfectly round, sharply circumscribed masses; the extrusion or lysis of all but one of the masses gives one the single nuclear particle seen in the bone marrow and blood. Apparently it is not necessary for the nucleus to show signs of dissolution before a nuclear particle appears in the cell, for a megaloblast with active nucleus may contain one in its protoplasm.

It is possible that the differences in staining of basophilic granules and nuclear particles is due to the presence or absence of nuclear membrane. The nuclear particle is perfectly round, as a rule, and its outline very sharply defined, resembling in every way, both morphologically and tinctorially, a miniature of pycnotic nucleus; basophilic granules are less regular and sharply defined and one gets the impression that they lie free in the cell protoplasm. If this be true, the difference in staining properties is readily explained; the reaction of the basophilic granules to stains approaches that of markedly basophilic cell protoplasm. Thus, with Romanowski stains they take a blue color instead of reddish purple, with methyl green-pyronin they are red rather than blue. Their color

3. Boellke (Virchow's Arch. f. path. Anat., 1904, clxxvi, 47) first used this stain to differentiate nucleus and basophilic granules.

resembles that of the protoplasm of many of the lymphocytes with both of these stains. In other words, supposing basophilic granules, to be remnants of the nucleus, one might explain their altered staining, provided the chromatin were no longer surrounded by nuclear membrane and its reaction were more nearly that of the surrounding protoplasm. Similarly, the characteristic staining reactions of nuclear particles might be made clear if the presence of nuclear membrane about them could be demonstrated.

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