Some Observations on the Morphology of Erythropoietic Cells in Human Lead Poisoning

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ALTHOUGH MUCH HAS BEEN WRITTEN about the blood morphology and blood chemistry in lead poisoning, the changes in the bone marrow have received comparatively little consideration. So far as we can ascertain, the literature up to date contains 20 papers dealing in various ways with the bone marrow in human lead poisoning, but relatively few observers have had an opportunity of studying more than two or three isolated cases. Moreover in only six reports has the diagnosis of lead poisoning been verified by laboratory data (lead in blood or in excreta and/or porphyrinuria).

Most clinical observers give data showing the relative frequencies of myeloid leukocytes and erythroblasts in the marrow of intoxicated patients. While some of them also outline the presence of stippled erythrocytes, others have merely been concerned with their occurrence in the bone marrow.

A small number of papers have dealt with qualitative changes affecting erythropoietic cells in lead poisoning. Thus, nuclear changes, mainly denoted as “karyorrhexis,” have been observed by Cadwalader, De Weerdt, Leitner and Rohr, stippled erythroblasts have been reported by De Weerdt, Movitz, McFadzean and Davis. Deviations of normopoiesis has been also noted: Pellegrini found megaloblastic hemopoiesis, Pelay-Rodondo noted “some forms with megaloblastic tendency,” Gart mentioned macrocytic hemopoiesis, and Heilmeyer observed “erythroblasts similar to megaloblasts.” Kienle called attention to the atypical mitoses in lead poisoning. With the exception of two reports there are, however, no detailed descriptions and illustrations of all these changes.

In a study on bone marrow changes in lead poisoning and in abnormal lead absorption which has been carried out at this Institute during the last 8 years, we have found (1) predominance of erythrocytogenesis as evidenced by a low myeloid-erythroid ratio, and (2) certain qualitative changes, in all cases of manifest lead poisoning. The present report is a part of the study comprising only qualitative changes of erythropoietic cells. Statistical data concerning the cellular composition of bone marrow cells and the incidence of abnormal cells will be published elsewhere.

SUBJECTS AND METHODS

The group studied in this report consisted of 17 male and 2 female patients. All but two cases of lead poisoning were of occupational origin (9 workers in a storage battery plant, 7 workers in a lead smelting plant, and 1 potter), and the two others of alimentary origin (due to the ingestion of lead from lead glazed earthenware vessels). The patients were admitted to hospital with acute lead colic and three of them also with lead palsy. The...
diagnosis of lead poisoning was in every patient confirmed by the following laboratory findings: high values for the lead in blood (50–195 μg. per 100 ml.), coproporphyrinuria (132–428 μg. per 100 ml. of urine), high stippled cell count in the peripheral blood (0.3–2.9 per cent of RBC) and the various degrees of anemia (3.18–4.16 millions of RBC per cu. mm.). The lead in blood was determined by a modified dithizone method according to which values higher than 60 μg. per 100 ml. are considered to be abnormal. The ether soluble porphyrins were determined in urine by a modified method of Fischer. The upper limit for coproporphyrinuria by this method is 20 μg. per 100 ml. The bone marrow was obtained by sternal aspiration. The blood and sternal marrow films were fixed and stained within 24 hours of their preparation.

All studies of patients were made shortly after their admission before any therapeutic measures had been undertaken. Only those patients who have had no history or evidence of any other disease with a blood dyscrasia have been taken into consideration in this study.

Findings and Discussion

The myeloid-erythroid ratio, ranging from 0.6:1 to 2:1, showed a marked tendency for relative increase of erythrocytogenesis. The following qualitative changes were found in erythropoietic cells: (a) basophilic stippling, (b) polyploidy, (c) nuclear alterations, and (d) aberrant chromosomes in mitosis.

Basophilic stippling (punctate basophilia) (fig. 1). This was the most conspicuous phenomenon found in a certain number of erythroblasts in each case studied. Values as high as 371 and 337 per 1000 with a group mean of 220 per

Fig. 1.—Basophilic stippling of the erythroblasts.
1000 erythroblasts were recorded to contain stippling. Punctate basophilia was noted not only in the orthochromic but also in polychromatophilic normoblasts. It is noteworthy that stippling was always coarser in erythroblasts than in the erythrocytes in peripheral blood.

As shown by Saita and Kopasz stippled erythroblasts in the bone marrow may occur in various conditions, but they are undoubtedly most numerous in lead poisoning.

In some more immature normoblasts we have noted that the cytoplasm gave the appearance of a course vacuolation, basophilic substance exhibiting a netlike or basketlike structure (fig. 2), best visible in erythroblasts showing defective hemoglobinization. Similar changes have been noted and described by McFadzean and Davis. We are tempted to regard this structure as an intermediate stage in the development of stippling.

**Polyplody** (fig. 3). It has been well documented and is now generally accepted that plurinuclear erythroblasts may be found in normal marrows. Their greater incidence, however (in Berman’s material greater than 5.1 per 1000 erythroblasts) is to be regarded as abnormal. Instead of “plurinuclearity” we use the term “polyplody” according to the terminology used by Undritz. This term comprises not only plurinuclear but also the giant uninnucleolar or even denucleated cells (see, if referring to the red blood cells). As evidenced by the
work of several authors the mechanism of plurinuclearity and that of cellular gigantism is namely essentially the same. While the plurinuclear cells are the sequelae of a suppressed cytokinesis after the completed karyokinesis, uninuclear "gigantoblasts" may be regarded as the sequelae of the supression of both cytokinesis and karyokinesis. In these giant cells chromosomes have been divided and embedded in their single large nuclei which thus retain the tetraploid or even the octoploid number of chromosomes. After denucleation such a giant erythroblast with one or more nuclei becomes a "gigantocyte." In our material polyploid cells were mostly binuclear erythroblasts ("tetraploids," fig. 3). Their incidence varied between 22 and 137 per 1000 erythroblasts. Trinucleated examples as well as a number of uninuclear gigantoblasts (polyploid erythroblasts with apparently single nuclei, fig. 3) and gigantocytes were also frequently encountered. The polyploidy of erythroblasts was found at all levels of maturity (fig. 3).

The type of maturation of erythroblasts in our cases corresponded to those of normoblastic marrows. Megaloblasts, observed by several authors in animals and in human cases of lead poisoning were in no instance found in the present study. While it is highly probable that the earlier workers used the term "megaloblast" to denote an early stage in the normal development of the red blood cells, it is quite certain that the more recent investigators used the

Fig. 3.—Polyploidy of erythropoietic cells.
term in its present and generally accepted meaning ("anemia perniciosiforme," "algunas formas de tendencia megaloblástica," "megaloblastenähnliche Erythroblasten"). In our opinion these findings may be explained on the basis of polyploidy. Without careful inspection some polyploid uninuclear or denucleated cells may be easily confused with megaloblasts and megalocytes respectively.

Nuclear alterations. Various terms and descriptions have been used to denote nuclear changes in erythroblasts both in human and animal lead poisoning ("irregular nuclear figures," "fragmentation," "characteristic nuclear destruction," "lobulated nuclei"). As stated above, most clinical observers, however, used the term "karyorrhexis." According to Rohr karyorrhexes are not normally present in the bone marrow. They may be found in erythropoietic cells in pernicious anemia, in bone marrow invaded by tumor cells, and under the influence of mitotic poisons such as arsenic and colchicine. Erythroblasts showing these nuclear alterations in our material varied from 30 to 311 per 1000 erythroblasts. Orthochromic erythroblasts were mostly affected.

Regarding the mechanism of these nuclear changes we should like to point to some peculiar nuclear patterns found in late erythroblasts of our cases of lead poisoning. These are highly suggestive of an interruption of mitotic movement in various stages of karyokinesis (fig. 4). The resulting "karyorrhectic" figures

Fig. 4.—"Karyorrhectic" patterns in erythroblasts indicative of an interruption of mitotic movement in various stages of karyokinesis.
are evidently due to the fusion of chromosomes in any stage of karyokinesis and not to the lobulation or even “rhexitis” of the original resting nucleus. According to the stage of the arrested karyokinesis various forms and alterations of the shape of nuclei may be seen. While the fusion of chromosomes may not be clearly recognized in the prophase, their fusion in the metaphase usually gives the appearance of a rosette. It is probable that the mechanism of karyorrhexis in our cases is at least partly related to the tendency for production of plurinuclear erythroblasts. Since the divisional capacity of nuclei of late erythroblasts is not sufficiently preserved to bring division to the end, their chromosomes clump together before the completion of karyokinesis which would have otherwise produced a plurinuclear (polyploid) erythroblast. The greater the degree of maturity of erythroblast the greater this tendency for fusion of chromosomes. In other words, while the karyokinesis in a polychromatophilic erythroblast may reach the stage of telophase and thus eventually produce a plurinuclear cell, the karyokinesis of a more mature erythroblast is arrested in the stage which corresponds to the level of its maturity; the nucleus of the latter cell will then exhibit a “karyorrhectic” figure. Thus it must be assumed that the occurrence of “karyorrhexis” in lead poisoning may be closely related to polyploidy of erythroblasts. In this regard it is interesting to note that a hereditary anomaly of red cell precursors (“familial erythroid multinuclearity”) has been described in which
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the coincidence of polyploidy and karyorrhexis was the outstanding feature. The presence of basophilic stippling in the cytoplasm of erythroblasts in the reported cases makes the similarity with our findings in lead poisoning even more striking.

Aberrant chromosomes (fig. 5) were frequently seen in various stages of division of erythropoietic cells. Aberrant chromosomes (“karyomeres”) as well as the scattered chromatin particles (products of “chromosomorrhexis”) gave rise to the frequently found accessory nuclei which varied in size (fig. 1) according to the number of chromosomes or particles assembled in a group during the telophase.

As shown by repeated sternal marrow examination performed in 7 cases, all these changes in erythroblasts were of reversible nature and of individually variable duration. Nevertheless, stippled erythroblasts, though in a discrete number, could be revealed even several months after the disappearance of symptoms and signs of manifest lead poisoning.

Conclusions

Morphologic changes of erythropoietic cells which have been demonstrated in this paper indicate that the erythroblasts in lead poisoning may exhibit some abnormal though not specific phenomena.

A consistent and characteristic feature was the presence of stippled erythroblasts, mostly at the late normoblast level of maturation. By careful examination of bone marrow cells it is possible to find erythroblasts whose “persistent cytoplasmic basophilic substance in granular form”28 (punctate basophilia) has a rather netlike appearance.

So far as we can ascertain polyploidy of erythroblasts has not been previously described in connection with lead poisoning. In our material, however, this phenomenon has been regularly found in an abnormally high number of erythropoietic cells as compared with normal data given by the authorities on subject.24, 25 Megaloblasts or “erythroblasts similar to megaloblasts” described or mentioned by others have in no instance been found in our study. It is probable that the red cell precursors exhibiting a dissociation of cell-nucleus division (i.e., polyploid erythroblasts) might have been confused with megaloblasts.

The occurrence of nuclear alterations, reported by several authors as karyorrhexis, was also observed in our cases. We are inclined to believe that this phenomenon in lead poisoning is in close relationship with polyploidy; the fusion of chromosomes does not permit the nucleus of a late normoblast to complete its division; instead of a plurinuclear erythroblast a cell with “karyorrhexis” results.

Aberrant chromosomes observed in all cases were responsible for the frequent occurrence of accessory nuclei in erythroblasts.

Whether these phenomena are the temporary consequence of a rapid proliferation due to an increase of erythropoiesis in lead poisoning, or whether there is another common denominator for all quantitative and qualitative changes under the influence of lead, are questions we are at present unable to answer. A detailed discussion of the problem lies beyond the purpose of this paper.
Morphologic changes of erythropoietic cells in the bone marrow of 19 lead intoxicated patients are reported. In addition to clinical manifestations the toxic action of lead was verified by the laboratory findings: high lead blood values, increased coproporphyrinuria and characteristic findings in peripheral blood.

Qualitative changes of erythroblasts were constantly present in all smears examined. Abnormalities most frequently observed were: (a) basophilic stippling, (b) polyploidy, (c) nuclear alterations ("karyorrhexis"), and (d) aberrant chromosomes. The incidences of some of these changes have been given.

The mechanism by which these changes might be brought about is discussed; the role of interruption of cytokinesis and karyokinesis is emphasized.

SUMMARY IN INTERLINGUA

Es reportate alterationes de cellulas erythropoietic in le medulla ossee de 19 patientes con saturnismo. A parte le manifestationes clinic, le action toxic del plombo esseva verificate per le constatationes laboratorial: alte valores de plombo sanguinee, augmento de coproporphyrinuria, e tractos caracteristic in sanguine peripheric.

Alterationes qualitative de erythroblastos esseva uniformemente observate in omne frottis examinate. Anormalitates de major frequencia esseva: (a) Punctation basophilic, (b) polyploidia, (c) alterationes nucleari ("caryorrhexis"), e (d) chromosomas aberrante. Le frequentia de certes inter iste alterationes es listate.

Es discutite le mecanismo per que iste alterationes es possibilemente effecctuate. Es sublineate le rolo de interruption de cytocinesis e caryocinese.

REFERENCES

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