BONE MARROW APPEARANCES IN CHILDREN SUFFERING FROM RETINOBLASTOMA*†

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RETINOBLASTOMA is a tumour which commonly spreads by direct extension. This extension may be intra-ocular or extra-ocular, extra-ocular spread occurring along the optic nerve or around extradural meningeal vessels into the bones of the skull, or across the sclera into the orbit.

Occasional instances of widespread blood-borne deposits of retinoblastoma have been recorded (Lawson, 1885; Lauber, 1907; Wintersteiner, 1907; Gardiner, 1908; Roman, 1912; Parker and Stokes, 1926; Hu, 1930; Jaffe, 1934; Ch'in, 1941). The incidence of such distant metastases is a matter of dispute. In earlier reports, Wintersteiner (1897) found direct extension to the skull bones in forty patients, but remote bony metastases in only nine (1.8 per cent.) of a series of 497 cases of retinoblastoma. Jessop (1900) surveyed 333 cases of retinoblastoma and noted that, although secondary deposits were occasionally found in the liver and other parts of the body, this was an exception to the general rule. However, Merriam (1950) found distant metastases in bones in nine of seventeen cases of retinoblastoma (52.9 per cent.) and in 25 per cent. of 24 *post mortem* examinations from the literature. Carbajal (1959) described blood-borne metastases in 50 per cent. of twelve *post mortem* cases of retinoblastoma. Taktikos (1966), in an analysis of 287 cases of retinoblastoma, reported metastases in 34 of 48 fatal cases. Unfortunately, Taktikos did not distinguish between bloodstream and lymphatic spread, or between localized and generalized metastases. It is possible that the substantially higher incidence of metastases in more recent communications is a result of improved techniques for detecting distant bony deposits.

Conflicting information also exists regarding the invasion of vascular tissue by retinoblastoma, a necessary prerequisite for blood-borne dissemination. Taktikos (1966) commented on the high incidence of invasion of vascular uveal tissue and postulated that, contrary to popular belief, spread was more frequent *via* the circulation than through the optic nerve. However, Parkhill and Benedict (1941), in 35 eyes surgically removed, found only four with extensive invasion of the choroid, whereas eighteen showed optic nerve extension.

In cases where distant bony metastases have occurred, infiltration both of subperiosteal and medullary tissues is frequently found (Willis, 1952, 1960). Replacement of bone marrow by retinoblastoma cells would therefore be expected.

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The present investigation was prompted by a report of Reese and Ellsworth (1963), who undertook a survey for metastases in cases of retinoblastoma, the most important single test being examination of bone marrow aspirated from the iliac crest. They found positive evidence of metastases in twelve (15.5 per cent.) of 77 patients from whom bone marrow was aspirated and marrow films examined.

It was decided to take samples of bone marrow from children suffering from retinoblastoma in order to detect infiltration by malignant cells.

Method

Samples of bone marrow were taken from forty children suffering from retinoblastoma. These included cases of inactive growth following treatment with cobalt plaques and systemic cyclophosphamide, active growth in treated cases, and active growth in new and untreated cases. Marrow was aspirated while the child was under general anaesthesia for fundus examination. The specimen was obtained from the iliac crest and averaged 0.5 ml. in volume. Eight fresh films were made from marrow particles removed by a wire loop from each specimen. The films were fixed in methyl alcohol and stained by the May-Grünwald-Giemsa technique. Sections were not prepared, firstly because of the difficulty in obtaining sufficient material in small children, and secondly because of the difficulty in identifying atypical non-malignant cells in sections stained with haematoxylin and eosin.

The eight films from each patient were scanned under a low-power objective and examined under high power with particular regard to the following points:

- (1) The cellularity of the marrow.
- (2) The activity and pattern of granulopoiesis. The percentage of myeloblasts in a count of 500 cells was recorded.
- (3) The activity and pattern of erythropoiesis.
- (4) The numbers and appearance of megakaryocytes.
- (5) The numbers and appearances of lymphocytes, monocytes and plasma cells.
- (6) The presence or absence of non-malignant "atypical" cells, i.e. cells commonly found in bone marrow but of abnormal or immature appearance or cells rarely found in the bone marrow.
- (7) The presence or absence of malignant cells.

Results

The results are summarized in the Table (overleaf). The forty cases studied were divided into 27 of inactive growth with previous treatment, eight of new active growth with previous treatment, and five of active growth in new untreated cases.

In no instance was any evidence found of marrow infiltration by retinoblastoma cells, despite full scanning of all eight films from each patient.

The cellularity of the bone marrow in each case was within normal limits for young children, or increased. No instance of marrow depression due to chemotherapy was encountered. Marrows of increased cellularity were found more frequently in untreated cases of active growth (60 per cent.) and treated cases of active growth (50 per cent.) than in treated cases with inactive growth (19 per cent.). This increase in cellularity was almost always related to very active granulopoiesis, with a proportionate increase in less mature white cell precursors ("shift to the left") and, on average, prominent myeloblasts comprised over 2.5 per cent. of the total nucleated count.

TABLE
BONE MARROW APPEARANCES IN

Type of Case		Number of Cases	Cellularity	Granulopoiesis	Per cent. Myeloblasts	Erythropoiesis
Treated (35)	Inactive Growth (27)	6	Normal	Active	1·8 to 2·8	Active Normoblastic
		5	Normal	Active Mild to moderate eosinophilia	1·6 to 2·4	Active Normoblastic
		4	Normal	Active	1·8 to 2·8	Active Normoblastic
		2	Normal	Active Mild eosinophilia	1·6 to 1·8	Active Normoblastic
		2	Increased	Very active "Shift to left"	2·2 to 2·8	Active Normoblastic
		3	Increased	Very active Moderate eosinophilia	1·4 to 4·6	Very active Macronormoblastic in 1 case
		5	Normal	Active Moderate eosinophilia in 1 case	1·8 to 3·2	Active Normoblastic
	New Active Growth (8)	1	Normal	Very active "Shift to left"	3·4	Active Normoblastic
		3	Normal	Active Mild eosinophilia	1·6 to 2·8	Active Macronormoblastic in 2 cases
		4	Increased	Very active "Shift to left" Mild eosinophilia in 1 case	2·2 to 5·0	Active Macronormoblastic in 1 case
New Untreated Active Growth (5)		2	Normal	Active Mild eosinophilia in 1 case	0·8 to 1·4	Active Normoblastic
		3	Increased	Very active "Shift to left"	1·2 to 4·2	Active Normoblastic

Erythropoiesis was active in every case. In most instances it was normoblastic in pattern, but in four cases a macronormoblastic element was present. No megaloblastic erythropoiesis was seen.

Megakaryocytes were always numerous, with evidence of active platelet production and with accompanying clusters of platelets. Their numbers were definitely increased above normal in three cases. The presence of megakaryocyte precursors will be described below.

An increase in chronic inflammatory cells (lymphocytes, monocytes, and plasma cells) was noted in 40 per cent. of cases of untreated active growth, 100 per cent. of cases of treated

FORTY CASES OF RETINOBLASTOMA

Megakaryocytes		Lymphocytes, Monocytes, Plasma Cells		"Atypical" Cells	Malignant Cells
Number	Appearance	Number	Appearance		
Normal	Normal	Normal	Normal	None	None
Normal	Normal	Normal	Normal	None	None
Normal	Normal	Increased	Normal	None	None
Normal	Normal	Increased	Normal	None	None
Normal	Normal	Increased in 1 case	Immature lymphocytes in 1 case	None	None
Increased in 1 case	Immature megakaryocytes in 1 case	Increased	Normal	Osteoblasts in 1 case Primitive reticulum cells in 1 case	None
Normal	Occasional megakaryoblasts in 2 cases	Increased in 3 cases	Normal	Primitive reticulum cells in 3 cases Fibrous tissue in 1 case	None
Normal	Normal	Plasma cells 5.5 per cent.	Normal	None	None
Normal	Normal	Lymphocytes increased in all cases	Normal	Primitive reticulum cells in 1 case	None
Increased in 1 case	Immature megakaryocytes in one case	Increased in all cases	Immature plasma cells in 1 case Immature monocytes in 1 case	Fibrous tissue in 1 case Fibroblasts in 1 case	None
Normal	Normal	Increased in 1 case	Normal	None	None
Increased in 1 case	Immature megakaryocytes in all cases	Increased in 1 case	Immature monocytes in 1 case	Primitive reticulum cells in 1 case	None

active growth, and 48 per cent. of cases of treated inactive growth. An associated eosinophilia was present in eleven of the 23 instances in which chronic inflammatory cells were increased. In addition, an eosinophilia was found in five cases of treated inactive growth with no increase in chronic inflammatory cells.

The following cells were found, which were either of atypical appearance or were uncommon in normal bone marrow. Such cells could, on their appearances, be confused with malignant cells.

(1) Megakaryocyte Precursors (megakaryoblasts and promegakaryocytes)

Present in seven cases. These cells are found more frequently in the marrow of children than of adults. They were large cells, averaging 30 to 40 μ in diameter, although considerably smaller than mature megakaryocytes (Fig. 1). They resembled malignant cells in that they possessed relatively scanty basophilic cytoplasm and large nuclei of primitive appearance possessing a fine chromatin pattern. However, compared with malignant cells, they occurred singly and were very infrequent, their nuclei showed a tendency towards indentation (Fig. 2) and lobulation, nucleoli were rarely visible, and small clear areas within the nuclear chromatin network were usually apparent.

FIG. 1.—Mature megakaryocyte at top of picture; megakaryocyte precursor below it. Note the almost elliptical nucleus of the megakaryocyte precursor and the relatively scanty, basophilic cytoplasm. May-Grünwald-Giemsa. $\times 500$.

FIG. 2.—Megakaryocyte precursor. In this case, the nucleus shows considerable indentation. Two lymphocytes and a polychromatic normoblast are also present. May-Grünwald-Giemsa. $\times 1150$.

(2) Abnormal Monocytes

Present in two cases, both of active growth. These atypical forms of mononuclear cells are commonly found in the blood of patients suffering from malignant disease (*e.g.* Alexander and Spriggs, 1960; Salsbury, 1964; Griffiths and Salsbury, 1965), and can also be found in the marrow. In the present series, the cells averaged 15 to 20 μ in diameter, possessing spherical nuclei and basophilic cytoplasm (Fig. 3, opposite). The cells differed from malignant cells in possessing relatively abundant cytoplasm, a coarse nuclear chromatin network, and indistinct nucleoli. It can be seen from Fig. 3 that the cells did not resemble glandular fever cells, particularly in their relatively large nuclei and scanty cytoplasm.

(3) Primitive Reticulum Cells

Present in six cases. Normal reticulum cells can be found in any bone marrow film and are readily identifiable. However, a small proportion of primitive forms is commonly present in the marrow of children and such cells are particularly prominent if the marrow is hyperactive.

The cells seen in the six cases of children suffering from retinoblastoma bore a close resemblance to malignant cells in that they were large, averaging 40 μ in diameter, possessed relatively scanty basophilic cytoplasm, and contained large nuclei with a fine chromatin network and multiple, often prominent, nucleoli (Fig. 4, opposite). Points of distinction from malignant cells were few and fine, but assessment was made chiefly on their discrete nature and infrequency.

FIG. 3.—Abnormal monocyte. Cytoplasm is relatively abundant, deeply basophilic, and vacuolated. The nucleus is elliptical with a coarse chromatin pattern. One nucleolus is faintly visible. May-Grünwald-Giemsa. $\times 1150$.

FIG. 4.—Primitive reticulum cell. This is a large cell with a large, elliptical nucleus possessing a fine chromatin pattern. Several nucleoli are faintly visible. May-Grünwald-Giemsa. $\times 1150$.

(4) Fibrous Tissue and Fibroblasts

Present in three cases. These cells are occasionally found in marrow films from children, but only very rarely in normal adult marrow. Mature fibrous tissue presents no difficulty in identification, appearing as strands of elongated cells with very long deeply-staining and structureless nuclei, but fibroblasts may lead to confusion with malignant cells. In the one case in which fibroblasts alone were present, the cells averaged 30 to 50 μ in diameter, with relatively immature nuclei. Identification was made by the elliptical nature of the nuclei, the abundant cytoplasm containing many azurophil granules, and the arrangement of cells in syncytial strands (Fig. 5).

(5) Osteoblasts

Present in one case. Osteoblasts are also found more commonly in the marrow of children than of adults. They were large cells, averaging 40 to 50 μ in diameter, with spherical nuclei containing a fine chromatin pattern and relatively prominent nucleoli. However, cytoplasm was abundant with an indistinct margin and the nuclei were eccentrically placed, giving the osteoblasts their characteristic appearance of a "giant plasma cell" (Fig. 6).

FIG. 5.—Fibroblasts. The cells are characteristically arranged in a syncytial strand. Note the elongation of the nuclei and abundant cytoplasm containing azurophil granules. May-Grünwald-Giemsa. $\times 500$.

FIG. 6.—Osteoblasts. These very large cells possess abundant cytoplasm with an indefinite border. The nuclei are characteristically situated in an eccentric position. May-Grünwald-Giemsa. $\times 500$.

Discussion

We have been unable to confirm the finding of Reese and Ellsworth (1963) regarding the metastasis of retinoblastoma to bone marrow. On the incidence of marrow infiltration described by Reese and Ellsworth (1963), one would have expected to find malignant cells in at least six of our forty cases. In fact, malignant cells were never seen. The absence of marrow infiltration is more in keeping with such reports of the natural history of the disease as by Wintersteiner (1897) and Jessop (1900).

The discrepancy between our figures and those of Reese and Ellsworth (1963) cannot easily be explained. However, three factors may play some part in the difference. Firstly, it is possible that Reese and Ellsworth were dealing with patients in an advanced stage of retinoblastoma, when vascular dissemination would be more likely to occur. Moreover, there is no information whether the patients had been treated or not. Our cases were largely of inactive tumours following treatment, and the cases of active tumours were all treatable with no evidence of extensive spread. Secondly, the classification of marrow infiltration adopted by Reese and Ellsworth is liable to possible misinterpretation. Although Groups 3 and 4 of their series were dependent upon the presence of definite tumour cells, Group 1 comprised over 8 per cent. "blast" cells with the presence of "atypical cells", like large lymphocytes and a lymphocytosis, while Group 2 included the presence of tumour-like cells with bizarre nuclei and nucleoli. In the absence of a more detailed description of the "blast" cells, "atypical" cells, and "tumour-like" cells, and of knowledge whether the presence of these cells constituted positive evidence of metastasis, it is impossible to attempt any correlation. Thirdly, the active marrow of children often contains unusual cells which may closely resemble malignant cells and may be misidentified as such. Such cells include megakaryocyte precursors, abnormal monocytes, primitive reticulum cells, fibroblasts, and osteoblasts. The cells have been described in some detail in this paper, largely in an attempt to emphasize that the occurrence of primitive somewhat bizarre cells in the bone marrow of children is not necessarily of pathological significance.

In a more recent paper, Reese (1967) has reported the isolation of tumour cells from the blood of two in a series of fourteen post-enucleation retinoblastoma patients. This incidence (14 per cent.) seems high for a tumour that behaves largely in a localized manner. It may be compared with an incidence of 8 per cent. of circulating malignant cells immediately post-operatively in cases of carcinoma (Griffiths and Salsbury, 1965). The work of Morley and Anderson (1961) is of interest, as it also deals with cerebral tumours. Although cells thought to be malignant were found in the blood of 14 per cent. of fifty patients suffering from intracranial glioma, similar cells were seen in 4 per cent. of a control series of fifty patients without neoplastic disease. It must be emphasized that atypical non-malignant cells, such as megakaryocyte precursors and abnormal monocytes, may be found in peripheral blood concentrates, particularly from patients with malignant disease (Salsbury, 1964).

Apart from the absence of malignant cells and the presence of various non-malignant atypical cells, the increase in chronic inflammatory cells in many of the cases, often associated with an eosinophilia, was of interest. The incidence of chronic inflammatory changes was similar in treated cases of inactive tumour and in untreated cases of active tumour, but such changes were present in every case of treated active tumour. These findings suggest that part of the change was a result of treatment (chemotherapy and radiotherapy) and that part was a reaction to active malignant disease.

Summary

Bone marrow was taken from the iliac crests of forty children suffering from retinoblastoma and examined, particularly for the presence or absence of infiltration by malignant cells. The cases of retinoblastoma included patients with treated inactive growth, treated patients with new active growth, and new untreated patients with active growth.

In no case was marrow infiltration by malignant cells found. However, many specimens contained atypical cells resembling malignant cells. Such atypical cells included megakaryocyte precursors, abnormal monocytes, primitive reticulum cells, fibroblasts, and osteoblasts. The significance of these findings and their relation to previous work on the metastasis of retinoblastoma are discussed.

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