HISTOCHEMICAL AND CYTOLOGICAL STUDIES OF THE EARLY CHANGES IN THE BONES DUE TO Sr^{90}

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Considerable importance is attached at the present time to the study of tumors arising from the action of ionizing radiation, as is shown by the extensive literature on this topic [2-10]. An interesting aspect of this problem is the investigation of the early pretumor changes in the bones, which are difficult to detect by the ordinary morphological methods. We have used histochemical and cytological methods to study the premalignant changes in bones arising from the action of Sr^{90}, a radioactive element which, if it enters the body, is fixed for a long time (the half-life period of Sr^{90} is 25 years) mainly in the bones.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out on 250 male albino rats weighing 160-180 g which received an intraperitoneal injection of Sr^{90} in a dose of 0.4 mCi/kg. The animals were sacrificed from 15 to 280 days after receiving the radioactive substance. Material for cytological and histochemical investigation was taken from the region of the metaphyses of the long bones (femur and tibia) at sites where tumors most commonly arise. The bone was punctured with a needle from a 2-ml syringe. Films were stained by Pappenheim's method. Parallel with the cytological investigations, we studied the alkaline glycerophosphatase activity by Gomori's method in histological sections taken from the bone in the region of the puncture.

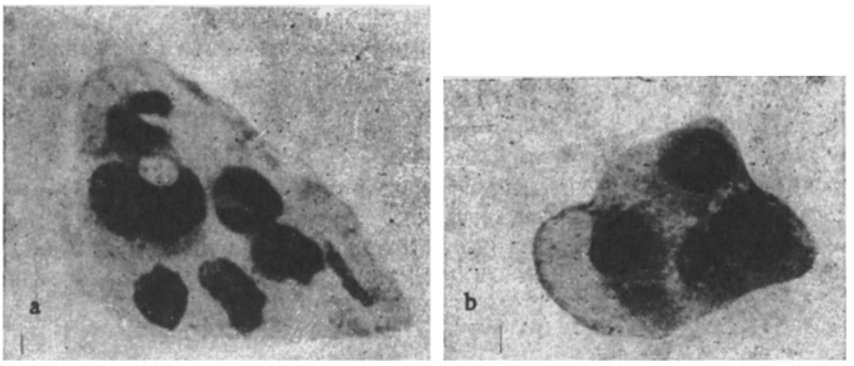
The study of the alkaline phosphatase activity is of interest if the role of phosphatases in osteogenesis is recalled. According to the literature [11, 12], a high alkaline phosphatase activity is characteristic of sarcomas of bone.

Our histochemical investigations showed that on the 15th day the alkaline glycerophosphatase activity was increased in the cells of the fibrocellular tissue filling the space between the bony trabeculae. These cells were evidently fibroblasts, reticulum cells, osteoclasts, and a few osteoblasts. An obvious increase in the activity of this enzyme was observed in the marrow cells. Cytological examination at this period showed the appearance of numerous basophilic reticulum cells of osteoblastic type. The marrow was rich in myeloid and erythroblastic cells.

Besides these changes, certain signs of disturbance of the processes of osteogenesis were also observed at this period [4,5]. The structure of the bone tissue was modified. Deformed trabeculae of bone with irregular edges and areas of newly formed primitive bone substance were seen. These structures were enveloped in fibrocellular tissue containing the cells we have described above.

On the 30th day, large basophilic cells containing nucleoli in their nucleus were observed cytologically. These cells resembled osteoblasts (see figure, a). Histochemically, at this period a lowering of the enzyme activity was seen in all the elements of the bone tissue although, just as in the puncture material, many cells with increased alkaline glycerophosphatase activity were seen. The high enzyme activity made it difficult to determine the appearance of these cells; they were evidently analogous to the cells found by cytological examination.

Later, between the 60th and 90th days, a gradual decrease in the number of marrow cells in the puncture material was observed, and endothelial and plasma cells began to predominate, together with groups of the large cells with basophilic cytoplasm mentioned above (see figure, b). Histochemical examination at this period revealed an increase in the number of cells with high alkaline glycerophosphatase activity. These were arranged singly or in groups around the deformed bony trabeculae and the newly formed bony substance, forming extensive collections together with other cells and collagen fibers.



A group of atypical cells in a puncture specimen of marrow from a rat. a) On the 30th day; b) on the 60th day after administration of Sr^{90}. Stained by Pappenheim's method. Magnification: eyepiece 10×; objective 90×, immersion.

Analysis of the changes observed on the 125th day revealed the presence of numerous atypical polymorphic cells, which were sometimes difficult to classify. For instance, some cells resembled osteoblasts in shape, while others closely resembled reticulum cells and fibroblasts. An increase in alkaline phosphatase activity was observed in all these cells. Isolated giant cells containing several nucleoli in their nucleus were seen, structurally resembling tumor cells. Occasional mitoses were also seen.

In later stages we observed proliferation of the tissue formations representing immature, neoplastic bone tissue, characterized by an even greater variety of cell forms. Cells were seen which could be classed as immature osteoblasts, others as fibroblasts or reticulum cells, and yet others as transitional forms. These atypical, immature cells were distinguished by high enzyme activity. The proliferating tissue formations consisting of the cells just described destroyed the remaining bony trabeculae and marrow.

Visible signs of a tumor were present in the animals from the 140th to the 280th days. Impressions and histological preparations of the tumor revealed collections of the atypical cells described above, of various shapes and sizes, with basophilic cytoplasm and a large nucleus, and with figures of mitotic division. As in the preceding periods, histochemical examination revealed a marked increase in the alkaline glycerophosphatase activity in these cells.

Analysis of these results suggests that the appearance of atypical cells with high alkaline phosphatase activity at a time when there are no visible signs of malignant growth is an early indication of the onset of the malignant transformation of the bone tissue. Evidence in support of this suggestion is given by the presence of analogous cells in the developing tumor, detected by means of cytological and histochemical methods.

The increase in alkaline phosphatase activity in the bone tissue cells in the early periods after administration of the radioactive material, which, according to the literature, is characteristic of tumors [1, 12], evidently may also indicate presarcomatous changes in the bones. Our cytological and histochemical findings provide information on the character of the changes arising during the malignant transformation of the bone and marrow cells caused by the action of Sr^{90}. Our investigation is only preliminary in character, and must be followed by more detailed cytological and histochemical studies of these early presarcomatous changes.

SUMMARY

Histochemical and cytological study was done of the early changes (preceding malignant growth) in the bones of rats to which strontium-90 was injected intraperitoneally in a dose of 0.4 mCi per kg of body weight.

Prior to the appearance of morphological osteosarcoma signs, as well as in the developed tumors, cells revealed atypical shape and a high activity of alkaline phosphatase. The presence of such cells in the bone tissue in the absence of any visible signs of malignant growth may serve as an early indication of the beginning tumor growth in the bones.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
