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Mitotic Activity and Cytology of Human Bone Marrow after Accidental Exposure to Ionizing Radiation

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Early detection and quantitative evaluation of the degree of radiation induced damage to the hemopoietic tissue in man is important for therapeutic and prognostic considerations after accidental exposure to ionizing radiation. Recent radiation accidents have indicated that the number of circulating blood cells are not in themselves adequate to allow satisfactory prediction of the probable clinical course soon after exposure. Lymphocytes fell rapidly even after relatively low radiation doses. The number of neutrophils, red cells, and platelets remain within normal limits or even increase during the first 2 to 3 days after exposure[1].

More definite and direct criteria of radiation exposure are necessary which might serve as biologic dosimeters and aid in a better assessment of the clinical situation. The accident in Oak Ridge in 1958[1] presented an opportunity to study radiation induced bone marrow damage. The purpose of this paper is to describe some of the abnormalities seen in the interphase cells and mitotic figures, particularly at 12 and 44 hours after the accident.

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Material and Methods

On June 16, 1958, eight men were exposed to mixed neutron-gamma radiation. Five of them received doses between 236 and 365 rads, three of them received 23 to 69 rads. Bone marrow aspirations * were performed 12 hours after the accident in the "heavy dose" group of five and from then on at about twice weekly intervals for four weeks. Marrow aspirations were done in the "low dose" group approximately once a week.

Summary of the Hematologic Findings. Details of the accident and of the hematologic data were reported by BRUCER (1959) and the overall picture of the mitotic activity by FLIEDNER et al. (1959): the total white count remained at high normal values for two days, then decreased until about the 9th day. Between the 9th and 16th day there was a slight rise in the total white count. Minimal levels were reached about the 29th and 30th day, with subsequent rather rapid recovery. Giant neutrophils appeared in the peripheral blood after about 4 days. Lymphocytopenia was present within 48 hours after exposure in the "heavy dose" group. The number of platelets remained within normal limits during the first 15 days and then showed a progressive decrease reaching lowest levels between 25 and 35 days. The hematocrit remained within normal limits in 3 of the "heavy dose" group and fell below normal levels in 2 during the 5th week.

The mitotic index was determined in Feulgen stained squash preparations[2]. A first minimum was reached after about 4 days and on this day a clearcut dose dependency was noted. There was a slight increase in the mitotic index around the 8th day. A second minimum was reached in the 3rd week. Normal proliferative activity as indicated by the mitotic index was reached in the 5th week, at the time of the most severe depression of the number of blood cells. Four and one half months after the accident, the mitotic index was significantly above normal limits.

Results

Radiation Effects upon Cell Division. The number of mitotic figures per 1000 nucleated bone marrow cells during the first 4 days was counted in ordinary bone marrow smears (Fig. 1). The mitotic index in the "heavy dose" group marrow smears fluctuated considerably. In the 12 hour sample a slight depression, and in the second sample a slight increase is possible but this was not significant before the mitotic index reached low levels.

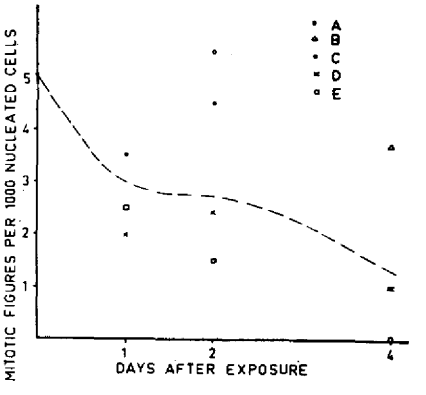

Fig. 1. Mitotic index in bone marrow smears after accidental irradiation (Normal: 5 per 1000; JAPA, 1942).

* The marrow aspirations were performed by Dr. KRETSCHMAR of the Oak Ridge Institute of Nuclear Studies, whose cooperation we gratefully acknowledge.

In Pappenheim stained bone marrow smears it is sometimes difficult to distinguish between normal and abnormal mitoses, especially those in prophase and metaphase. In the first marrow aspirates after 12 and 44 hours, however, a relative increase of anaphases and telophases was noted. In these smears 60 to 90 % of all mitotic figures showed abnormalities. Atypical mitoses were seen in both the myeloid and erythroid series.

Chromosomal stickiness was recognized in metaphases and the later stages of cell division. In some metaphases, 2 or 3 chromosomes stick together forming a

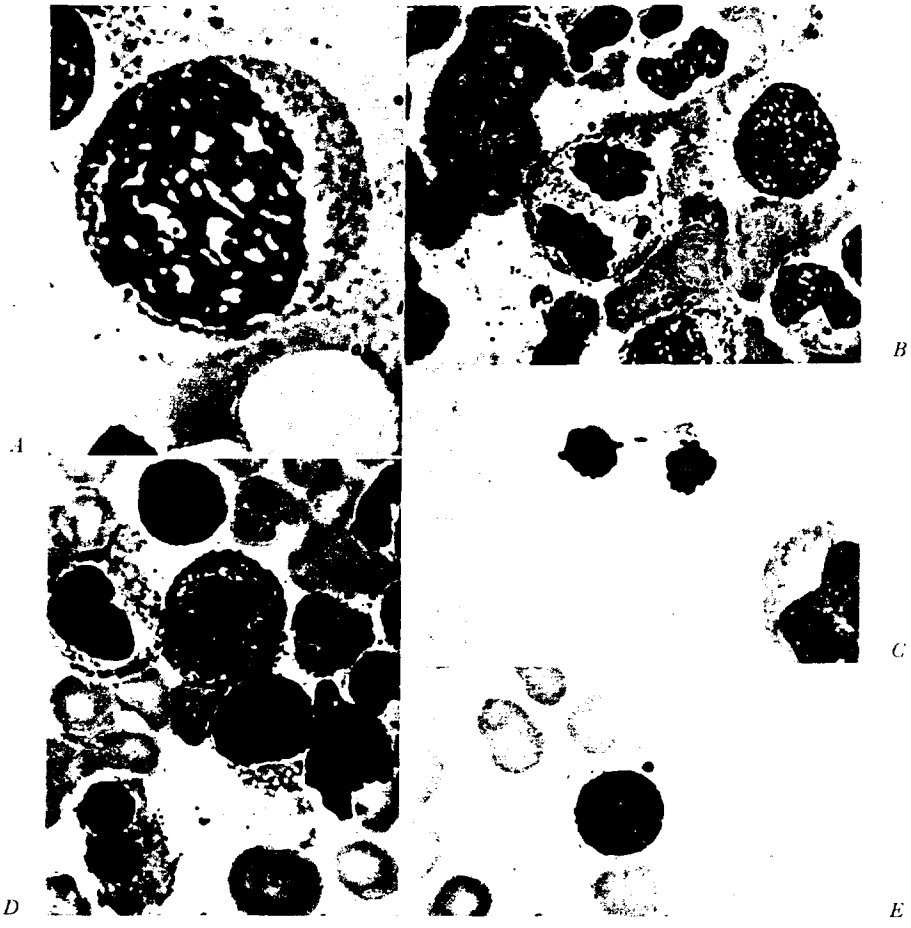

Fig. 2. A: Late metaphase with chromosomal stickiness 12 hours after the accident: B: Myelocyte division, 44 hours after accidental exposure with a chromosome fragment. C: Normoblast division, 12 hours after exposure with a chromosome fragment. D: Myelocyte division, 12 hours after exposure with two lost chromosomes. Note binucleated normoblasts and in one of them a cytoplasmic chromatin clump. E: Chromatin clump in the cytoplasm of an abnormal large oxyphilic normoblast, 12 hours after exposure.

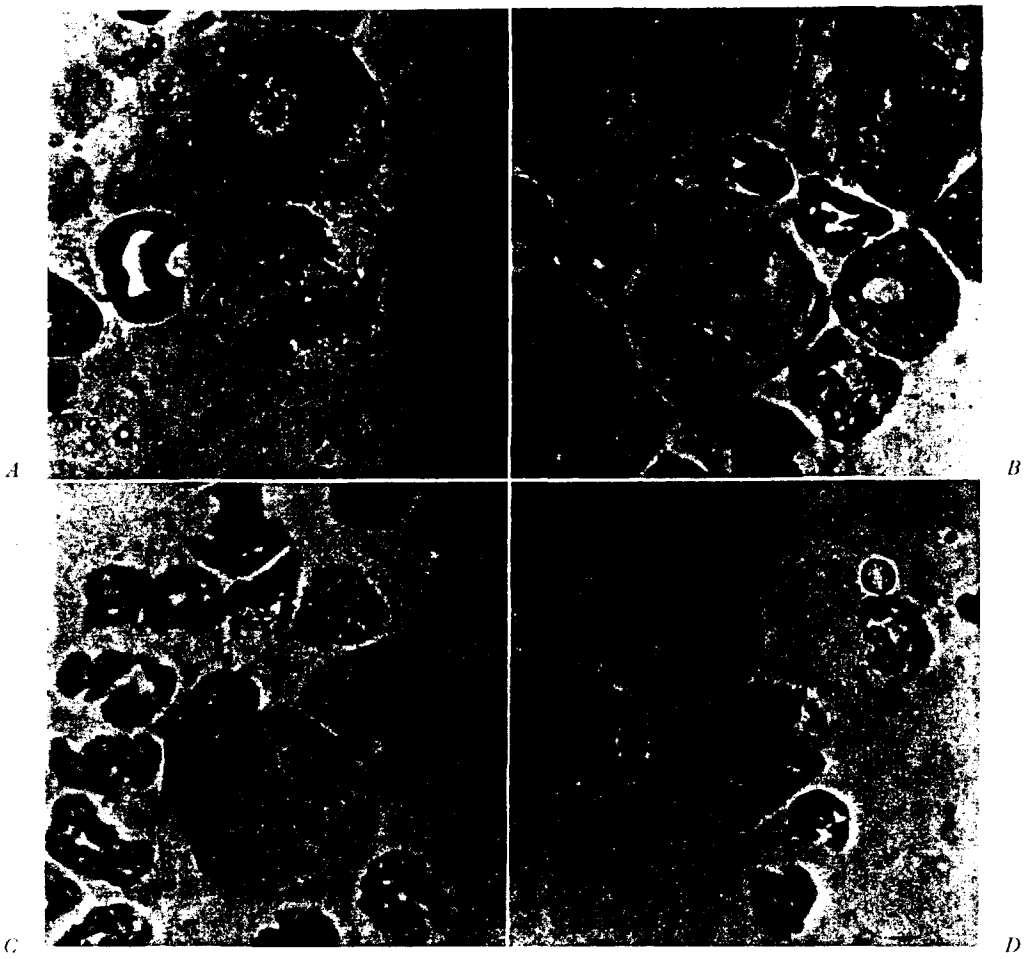

Fig. 3. Abnormalities in autoradiographs of bone marrow smears of rats after total body irradiation and injection of H^3 thymidine. A: Abnormal anaphase, 18 hours after 1500 r. Chromosomal bridges and fragments. B: Abnormal telophase, 18 hours after 550 r. with chromosomal bridges and lost chromosomes and fragmentation. C: Binucleated myelocyte with chromosomal bridge in DNA synthesis, 18 hours after 550 r. D: Fragmented nucleus in DNA synthesis, 24 hours after 1000 r.

chromatin clump, whereas the other chromosomes are more clearly outlined. In anaphases, chromosomal stickiness caused formation of chromosomal bridges which were observed frequently. These bridges consisted of 1, 2 or more chromosomes, sometimes they were almost as broad as the nucleus itself (Fig. 2), suggesting "amitotic" divisions, when recognized in the interphase cells.

Chromosomal fragmentation was easy to recognize in anaphases and telophases of erythroid and myeloid precursors (Fig. 2). One chromosomal fragment re-

mained in the equatorial layer without being moved to one of the poles. In interphase such cells which lost chromosomal fragments could be observed as chromatin clumps inside the cytoplasm. Whether or not these clumps in the red cell precursors are identical with Jolly-bodies is not known but suggestive (Fig. 2). In some metaphases and anaphases the fragmentation was incomplete and in these cases very thin chromatin strands stretched from one part of the chromosome over the fragment to the other chromosome.

In some mitotic figures entire chromosomes remained between the two poles without being moved to one side or the other (Fig. 2). This abnormality probably is due to an *injury to the centromere* and thus one or several chromosomes were lost from the dividing cell. In some cases the nuclei of interphase cells were completely fragmented forming chromatin clumps. Comparison studies in rats[3] (Fig. 3) suggested that these are altered chromosomes due to abnormal cell division, unable to move to the poles, but still able to synthesize desoxyribonucleic acid. It is uncertain whether or not such "fragmented nuclei" can survive through one or more mitoses.

In some cases the arrangement of chromosomes in anaphase and telophase was disordered. Here the abnormality was probably due to an *injury to the formation of the spindle* giving rise to unequal daughter nuclei.

In many mitotic figures the abnormalities described were combined. Therefore it was not always possible to trace the probable pathogenesis of the observed abnormalities.

Abnormal mitotic figures suggesting *multipolar mitoses* were observed in some cases. In these the surrounding cytoplasm was normal in its color but abnormal in its size. A probably tetrapolar polychromatic normoblast division the cytoplasm of which is about 4 times the normal size is shown in Fig. 4.

Radiation Effects Reflected in Interphase Cells. The first marrow smears were performed 12 hours after the accident. Obvious morphologic abnormalities were observed in erythroid and myeloid precursors. Some of the immature nuclei showed an increased diameter and the chromatin pattern was abnormal with loose nuclear edema or chromatin dissociation or both (Fig. 4). In some cells transitional stages to karyolysis could be observed (Fig. 4e); it was, however, rare to see definite karyorrhexis and pyknosis of nuclei. This *nuclear swelling* was similarly seen in white cell precursors, in which it often became more and more difficult to recognize the outline of the nucleus, which often looked rather homogeneous and washed out (Fig. 4d).

Nuclear swelling with chromatin dissociation became less frequent after 44 hours, and disappeared in the 3 day marrow smears. After 44 hours, and less frequently after 12 hours many large myeloid and erythroid precursors were found. Frequently the nuclear structure and staining characteristics of the cytoplasm corresponded to a more mature cell than indicated by the nuclear diameter.

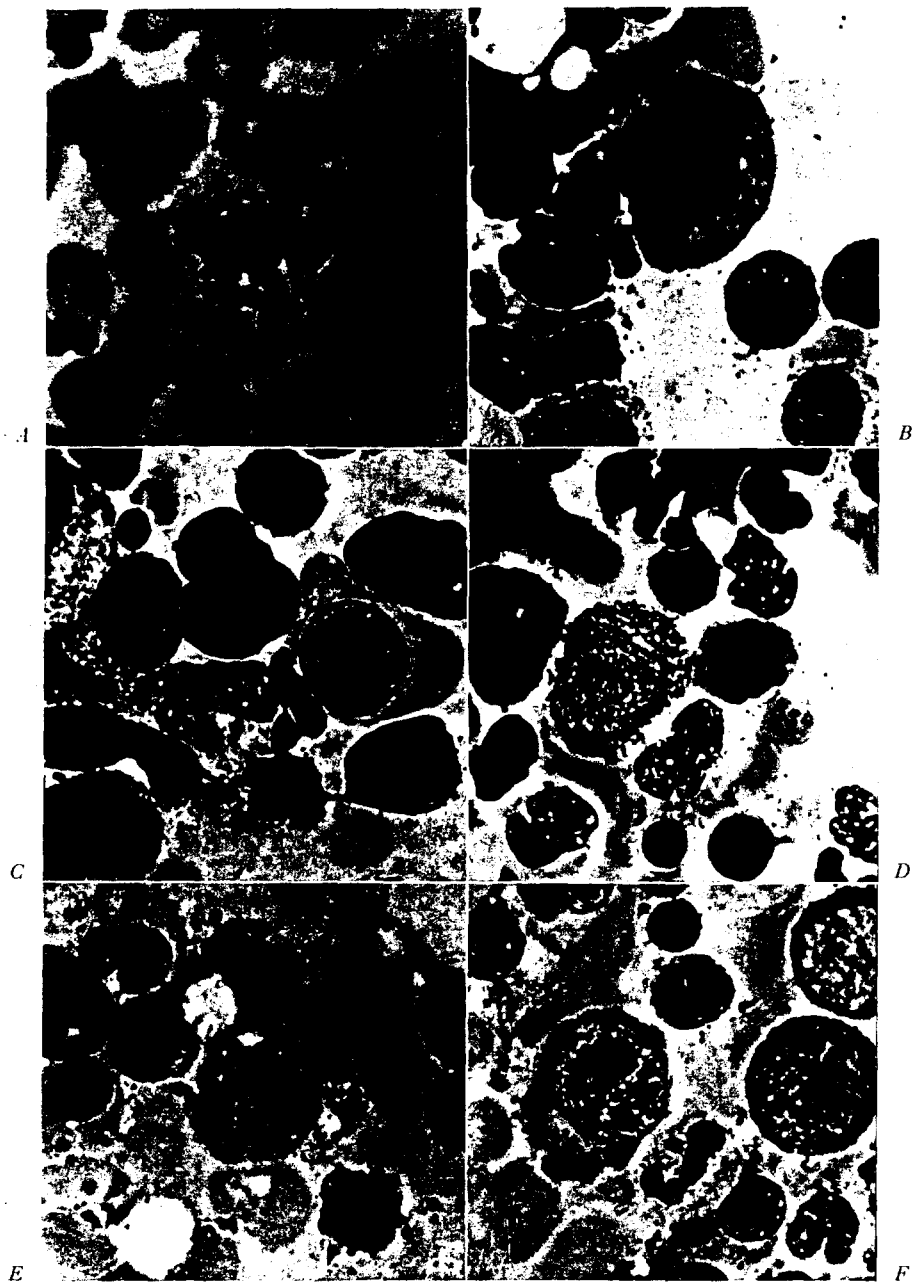

Fig. 4. A: abnormal, tetraploid mitosis in polychromatic normoblast, 44 hours after accidental exposure. B and C: Normoblasts: according to nuclear structure and cytoplasm polychromatic, according to nuclear size early basophilic normoblast, 12 hours after accidental exposure. D: "Release" of chromatin material, 44 hours after accidental exposure, washed and swollen myelocyte nucleus. E: Chromatin dissociation with nuclear swelling, polychromatic normoblast. 12 hours after exposure. F: Karyolysis in normoblast, 12 hours after exposure.

Since under normal conditions the mean nuclear volume, and thus the mean nuclear diameter decreases from one maturation stage to the next in both the myeloid and erythroid series[4, 5], the presence of large nuclei with a more mature structure and a more mature cytoplasm indicated an *inhibition of nuclear division without disturbance of nuclear and cytoplasmic maturation.* Giant neutrophils appeared in the peripheral blood after 4 to 5 days. Since giant mature myelocytes and metamyelocytes were found in the earlier marrow smears it is probable that the formation of giant blood cells is due to the inhibition of nuclear division without interference of the nuclear and cytoplasmic maturation. The same pathogenesis would cause giant red cells in the red cell series (Fig. 4, b and c).

In other cases, binucleated red and white cell precursors were seen in the early marrow smears. In these only the cytoplasmic division was altered (Fig. 2). These binucleated precursors apparently can mature and form binucleated white blood cells.

Less frequently chromatin drops were found, especially in the cytoplasm of normoblasts. Since in some cases these drops seem to be attached to the nucleus, almost like "released" chromatin, they may be the result of damage to the nuclear membrane, with *release of nuclear material* (Fig. 4 d). Whether or not these drops consist of "released" nuclear material or of chromosomal material due to a lost chromosome fragment during abnormal cell division, was uncertain.

Discussion

The findings of atypical mitoses and abnormal cells in interphase in the 12 and 44 hour bone marrow aspirates of five men accidentally exposed to mixed neutron-gamma irradiation indicate the value of early marrow examinations for the diagnosis of doubtful radiation exposure.

The clinical course demonstrated that the exposure resulted in the acute radiation syndrome in its hematological form, with leucopenia and thrombocytopenia. Although none of the persons exposed died, their health was cause for some concern at approximately 30 days and the exposures were probably in the high sublethal or low lethal region[6].

It was found in studies on the marrow of rats[3] exposed to 550, 1000, and 1500 r, that only lethal and supralethal doses caused immediate damage of many nucleated marrow cells, with signs of karyorrhexis and karyolysis. In the bone marrow smears of men examined the cellularity remained within normal limits for the first few days and no obvious abnormalities were observed at first glance.

A closer examination, however, demonstrated significant signs of injury. No detailed studies have been reported so far about the initial changes in bone marrow cytology of man after lethal and supralethal doses, but abnormalities described here might occur with greater frequency at higher dose levels. Thus a careful examination of bone marrow smears within hours after radiation exposure of doubtful severity might help with a quantitative study of mitotic and cellular abnormalities for a better assessment of the clinical situation.

The evaluation of the mitotic index of bone marrow is not a precise method for calculations. It was pointed out by JAPA[7] and UNDRITZ[8] that the mitotic index in the normal marrow smears is lower when there is an immediate block of mitotic activity[9]. Therefore only general conclusions about the mitotic activity are possible. Under normal conditions, when the generation time and the mitotic time of the multiplying cells is constant, the mitotic index reflects the degree of proliferative activity. Whether or not this is true for human bone marrow injured by radiation is not known. In mammalian and plant radiation studies it was found that radiation can cause a prolongation of the mitotic time[10, 11]. From experimental studies in tissue culture it was concluded that radiation causes a delay in different periods of the cell cycle[12], finally resulting in a prolongation of the generation time. In the bone marrow smears 12 and 44 hours after the radiation accident, a relative increase in anaphases and telophases was observed, which would be consistent with a prolonged mitotic time. Since many of the mitotic figures were abnormal, the mitotic index in itself does not necessarily reflect at the time intervals studied the proliferative capacity of the marrow. A prolonged mitotic time due to a delay in anaphase and telophase would give a misleadingly high mitotic index and thus the actual "active" mitotic index of undisturbed cell divisions is probably very low.

From these preliminary data and from studies in rats[3] we believe that the evaluation of the percentage of normal and abnormal mitotic figures, classified into groups according to the severity of damage, might serve as another biologic dosimeter. It would thus enhance the mitotic index, which fluctuates considerably before minimal levels are reached at the time of severe and obvious marrow damage. The observed radiation-induced abnormalities in the mitotic figures are similar to those observed in other investigations of the effect of radiation on mitoses[11]. The usual staining method of marrow smears does not permit a detailed investigation of mitotic abnormalities, since such methods are not specific for desoxyribonucleic acid. Definite abnormalities were most frequently observed in late metaphase, anaphase and telophase. In some mitotic figures chromosomes or chromosomal fragments were lost. It is not known how long these hypoploid cells can live and function. It is possible that chromosomal loss of bone marrow cells occurs in the course of duplication and maturation[13]. Observations of nuclear swelling, chromatin dissociation, and release of nuclear material caused by ionizing radiation were reported in cinematographic studies of irradiated fibroblasts in tissue culture[14]. Such abnormalities became rare after 44 hours, and were absent beyond the 4th day. It is therefore probable that "nuclear oedema" of some marrow cells is a primary effect of marrow cell damage at dose levels which are not high enough to cause immediate karyolysis or karyorrhexis.

The formation of large myeloid and erythroid precursors is a complex phenomenon. In several cases their formation was due to nuclear division without division of the cytoplasm. Thus binucleated giant myelocytes, normoblasts and mature blood cells are formed. In several cases, however, giant cells were observed

containing only one nucleus which was considerably larger than nuclei of normal cells of similar maturation stages. Since under normal conditions, the nuclear volume in red and white cell precursors decreases with the steps of nuclear division and cytoplasmic maturation[4, 5], the size of the nucleus with a normal chromatin pattern indicates the compartment. An inhibition of nuclear division but undisturbed maturation gives rise to giant cells.

Summary

The findings presented in five men exposed to mixed neutron-gamma radiation, resulting in severe depression of blood cell formation, indicate that there are significant abnormalities in the mitotic figures and in cells during interphase. The percentage of abnormal interphase cells is not high and such abnormalities must be looked for carefully. The presence in the bone marrow within a few hours after radiation exposure of nuclear swelling, chromatin dissociation, nuclear and cytoplasmic maturation without nuclear division, atypical mitoses with chromosomal stickiness, fragmentation, and loss of chromosomes indicate severe damage to the marrow. The dose in our cases was not severe enough to cause significant karyorrhexis and karyolysis resulting in immediate cell death. It is believed that serial bone marrow examinations within the first 3 days after uncertain radiation exposure may aid in a better assessment of the clinical situation and prognosis after radiation accidents.

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Discussion

D. Pecorari (Pavia, Italy): It is known that variations of the mitotic index may depend on (1) the mitotic rate and (2) the mitotic duration. It is possible that in the cases described mitosis is prolonged as shown by the high number of morphologic abnormalities observed. I ask therefore if evaluations of the mitotic duration have been made?

Secondly, we have observed by phase contrast cinematography in new erythroblasts that giant nuclei with or without morphological abnormalities often derive from metaphasic or anaphasic nuclear reconstruction leading to polyploid nuclei. I ask if Dr. Fliedner has also seen these abnormal nuclear reconstructions?

T. M. Fliedner (Reply): It was impossible to study the time of mitosis in these exposed men directly. But it can well be that there is a delay in the mitotic time resulting in an elevation of the mitotic index.

Studies on irradiated rats indicated an accumulation of cells in the second rest-period, before onset of mitosis. After about 18 hours (750 r) the mitotic block is overcome and this results in a mitotic index within normal levels. Abnormalities in blood cell precursors show a wide variation from impaired nuclear division to long chromosome bridges. Many of these cells die before they can mature to become circulating blood cells. But a few do and it seems to depend on the stage of the precursor in which the damage occurred what size and ploidy the mature cell has. Some of the erythropoietic precursors shown might be due to the process which Dr. Pecorari indicated.