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Early and Late Cytologic Effects of Whole Body Irradiation on Human Marrow

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THE HEMATOLOGIC manifestations of whole body irradiation in man have been studied extensively.[1-12] These studies have been based on exposures in nuclear warfare,[1,2] on accidents in laboratories or fallout field,[3-10] and on large volume radiation therapy.[11,12] They have been reviewed in detail recently.[13] Serial examinations of blood cell levels (neutrophilic granulocytes, lymphocytes, platelets and reticulocytes) have been found to be an indispensable aid in the clinical assessment of the exposed individual. In most of the surviving persons, blood cell counts returned to approximately preirradiation levels within a few months, although some slight but statistically significant changes are seen to persist for years when adequate control data are available.[14] Furthermore, despite apparent recovery, chromosomal abnormalities persist in hemopoietic cells, as demonstrated by blood cell cultures or bone marrow examination,[14,15] suggesting a persisting latent injury.

In most of these studies, bone marrow examinations have not been performed or have not been reported in detail. Their value in the evaluation of the exposed person has not been clearly established and it has even been suggested that they are dangerous because of the possibility of introducing infection.

Little is known about bone marrow changes in man after whole body irradiation as a function of time and dose. However, extensive studies in experimental animals have shown characteristic cytologic and histologic consequences during the first hours and days after exposure.[17-21] Since the marrow is one of the most radiosensitive organs of the body, serial examinations after radiation exposure should provide valuable additional information both for clinical management and for a better understanding of the pathogenesis of marrow failure secondary to ionizing radiation. Samples can be obtained with ease and repeatedly, and the danger of infections is small. (They apparently have not been reported[22]).

It is the purpose of this paper to describe some of the cytologic manifestations of radiation injury in marrow smears of eight men "early" (during the first 2–3 weeks) and "late" (3.5 years) after accidental exposure to mixed neutron-gamma irradiation. From these results, some guidelines can be derived for a diagnostically more meaningful evaluation of marrow smears under such circumstances. Details about the accident, the general hematologic findings

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and preliminary results of these cytologic studies have been published previously.[23-27]

Materials and Methods

(a) Exposed persons:[*] Eight men received a mixed neutron-gamma irradiation as a result of a critical excursion during the processing of waste Uranium^{235} solution. The doses were estimated to be (whole body): Patient A: 365 rads; B: 270 rads; C: 339 rads; D: 327 rads; E: 236 rads ("high dose" group), and Patient F: 68.5 rads; G: 68.5 rads; and H: 22.8 rads ("low dose" group).

(b) Early studies: Bone marrow aspirations were performed in the high dose group 12 hours, and 2, 4 or 5, 9 or 10, 16, and 23 days after the accident, and in the low dose group 4 and 10 days after the accident. The general findings in the marrow smears and sections have been published,[7] as well as the results of mitotic index studies[28] using Feulgen-stained squash preparations. In this study marrow smears were prepared on cover slips, dried and stained with Wright's stain. They were evaluated in two ways. (1) In each smear, 500 erythroblasts (including proerythroblast, early and late basophilic normoblasts, polychromatic and oxyphilic normoblast) were counted. All "mitotically connected abnormalities" (M. C. Abn) seen during this counting in this and any other marrow cell line were recorded and are reported per 100 erythroblasts. Other cytologic abnormalities were described qualitatively. (2) In each smear made during the first few days after exposure, 2000 nucleated cells were counted to determine the initial mitotic indices.

(c) Late studies: Three and one-half years after the accident (Nov. 1, 1961) some of the studies performed early after exposure were repeated. Marrow aspirations were performed and the smears scanned for cytologic abnormalities. For these studies, at least 2000 nucleated red cell precursors were counted in each smear and the abnormalities recorded. In addition, at least 100 mitotic figures were examined in each smear for cytologic abnormalities and classified into erythrocytic, myelocytic, and "others" (not identified with certainty). The mitotic indices were determined in Feulgen-stained squash preparations with the same technic as used before,[28] by counting a total of 5000 nucleated cells (1000 on five different slides). From these total mitotic index values and from the differential count of mitoses performed on the Wright-stained smears, the specific mitotic index for erythropoiesis and myelopoiesis was computed.

(d) Normal controls: Control values for the mitotic index determinations in Feulgen-stained squash preparations, and of the distribution of mitoses in marrow smears prepared and stained by standard methods, have been reported.[28,29] These were derived from studies in seven healthy males in an age group comparable to that of the exposed persons. In the marrow smears of four of these men, a total of 10,000 nucleated red cell precursors were counted to determine the frequency of cytologic abnormalities in interphase cells and mitoses.

Results

A. *Cytologic Manifestations of Radiation Injury Early after Exposure*

As reported previously[7] the cellularity of the marrow did not appear to be markedly abnormal in the 12-hour and 2-day samples while a mild decrease was felt to be present on days 3 and 4. In these samples, the erythroid-myeloid ratio decreased during the 1st week. A more detailed cytologic examination disclosed a number of abnormalities. These are characteristic of radiation injury, although not "specific" for exposure to ionizing radiation since they can be seen in patients receiving cytotoxic chemotherapy.

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In agreement with previous experimental findings,[19,20] two types of cytologic abnormalities were distinguished: "Cells injured directly" and "mitotically connected abnormalities." In all five marrow smears of the high dose group, prepared 12 hours after the accident, and less frequent in the 2-day sample, cells were found, mainly erythroblasts, showing a marked degree of clumping or dissociation of the nuclear chromatin with transition into frank karyolysis. These findings were not quantitated because it was judged that their evaluation was too subjective. However, it was the impression that about 5 per cent of all erythroblasts in the 12-hour samples showed nuclear changes which were not impressive in the smears of the low dose group or of "normal" controls. From animal studies it was derived that such immediate cytologic changes presumably progressing to cell death occur before further cell division could occur and are apparently dose-dependent.

Easily recognized and quantitated without doubt were mitotically connected abnormalities. In mitoses found in marrow smears, an abnormality was considered to exist if a chromosomal bridge was found in ana- or telophase or if a chromosome and/or chromosomal fragments were apparently discarded from further karyokinesis and "left behind" between the poles of the mitosis or elsewhere in the cytoplasm. Examples of such abnormal mitoses are given in figures 1 and 2. During interphase, cells were considered to present a M.C. Abn. if one or more nuclear fragment, termed "karyomere" (E. Schwarz[30]), was found. Examples of such abnormal cells in the erythropoietic series are given in figure 1 and in myelopoietic and lymphopoietic cells in figure 2. Another type of M. C. Abn. was the occurrence of binucleated cells, seen in the early studies primarily in myelocytic elements, and in the late studies primarily in the erythropoietic cells. Examples are shown in figures 2 and 4. "Giant cells" were also classified under this type of abnormality. They were defined as cells significantly larger in overall size than normal cells of identical nuclear structure and apparent cytoplasmic maturity. They are easily recognized in the myelopoietic series and examples are given in figure 2. They occur also among erythropoietic forms, as shown in figure 1, but these were not included in the quantitative analysis of abnormalities since there is considerable disagreement about the relation of cell size and maturity in erythroblasts.

Figure 3 gives the results of M. C. Abn. during the counting of 500 erythroblasts in the smears of each of the irradiated persons. In the erythropoietic series only the cells showing one or more karyomeres are included. These structures were found in the first marrow smears 12 hours after the accident. Their frequency rose to maximum in the 2nd day samples when about 12 per cent of all erythroblasts had at least one karyomere, and decreased subsequently. Among 10,000 erythroblasts counted in the marrow smears of four normals for comparison, only 11 were found to contain one karyomere (= 0.115), the frequency varying from 0.05 to 0.2 per cent. Most of these were in oxyphilic normoblasts; none were found to contain more than one karyomere. These karyomeres may appear round like a droplet (fig. 1, D, F, G, J), or elongated (fig. 1, I). They may be single (fig. 1, L) or multiple (fig. 1, H,

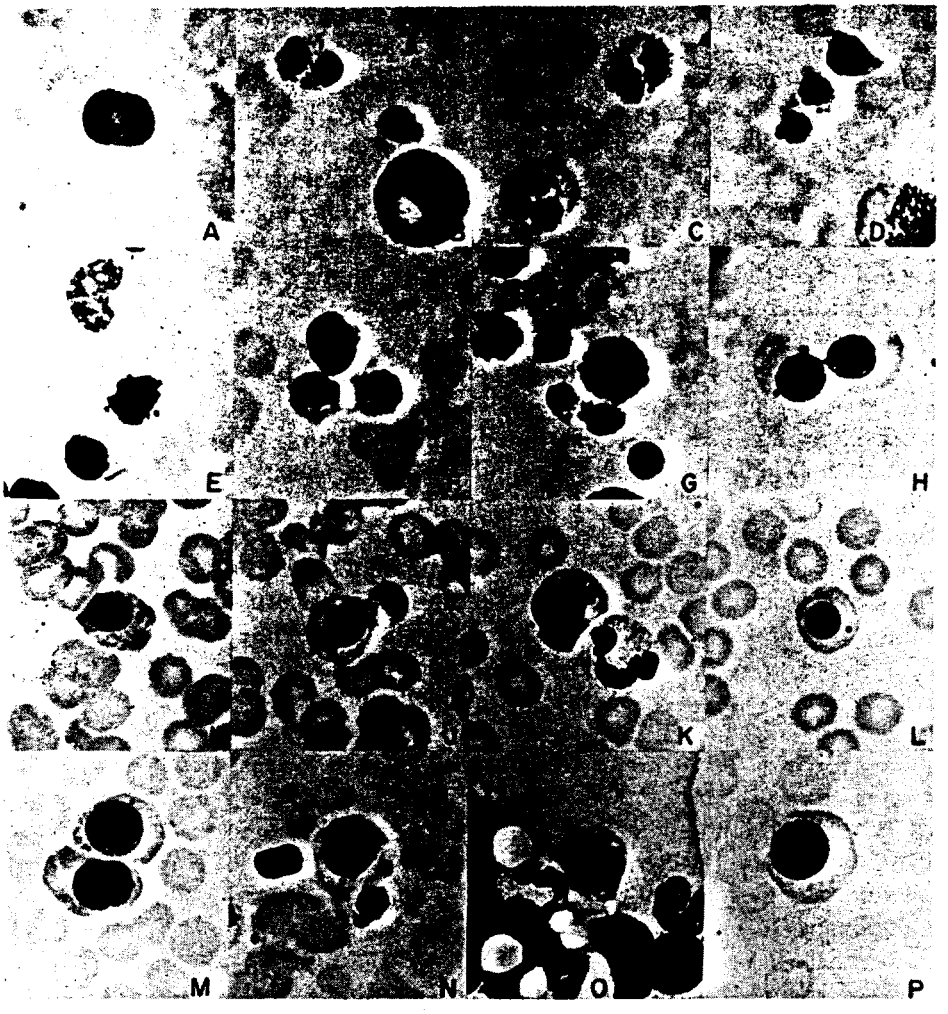

Fig. 1.—"Mitotically connected abnormalities" in erythropoietic precursors of human bone marrow during the first days after exposure. Chromosomal bridge: A, B, C; internuclear bridge: H; aberrant chromosomes in mitoses or karyomeres in interphase cells: C-N; incomplete nuclear division (?): O; "giant" oxyphilic normoblast: P.

J, M). In mitotic figures, they were found between the two poles in anaphase, or "left behind" in the cytoplasm (fig. 1, D, E, G). Not enough mitotic figures were counted in the "early" samples to give accurate values for the frequency of obvious mitotic abnormalities (bridges in ana- and telophase, chromatin fragments); but it was estimated that 20–50 per cent of mitoses were abnormal in the 12-hour and 2-day smears of the "high dose" group.

The smears of the low dose group were available only from days 4 and 10. The frequency of erythroblasts with karyomeres on day 4 was lower than in the high dose group but slightly elevated compared to normal, suggesting a dose dependency (fig. 3).

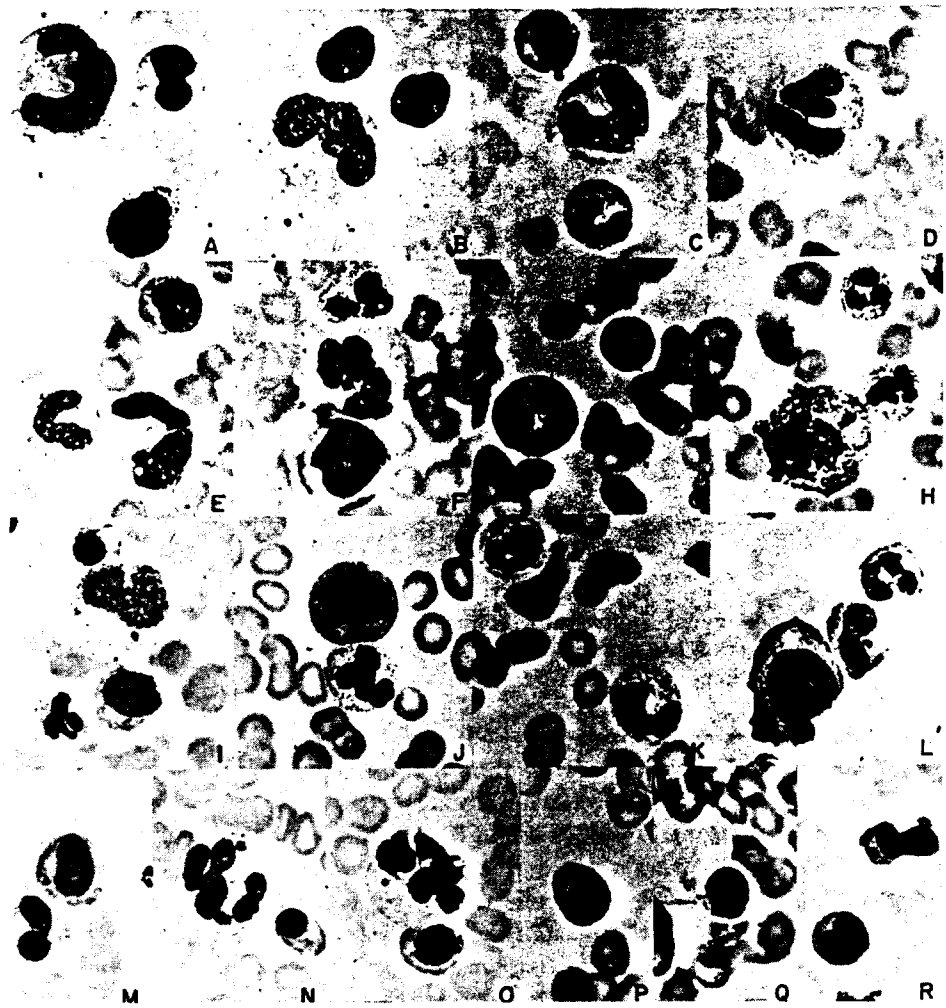

Fig. 2.—"Mitotically connected abnormalities" in myelocytic and lymphocytic "Giant cells," cells of human bone marrow during the first days after exposure. mono- or binucleated: A-G; N, O; binucleated cells with (D) or without internuclear bridges: C-G; aberrant chromosome in mitosis: H; karyomeres in interphase cells: G, I-R.

In myelocytic cells, M. C. Abn. were seen and recorded from the study of 500 erythroblasts. For the present study, abnormal mitotic figures and interphase cells were pooled. Examples of such cytologic abnormalities of myelocytic cells are given in figure 2. In the smears of persons used for comparison, there were no abnormal mitoses as defined in this study and no myelocytic cells with karyomeres or "giant cells." A very few binucleated myelocytic cells were seen in the course of counting 10,000 erythroblasts. The frequency of myelocytic M. C. Abn. was 0.03 and 0.02 per cent per 100 erythroblasts respectively in marrow smears of two of the normal persons used as comparisons in which 3000 and 4000 erythroblasts were counted. The changes in the fre-

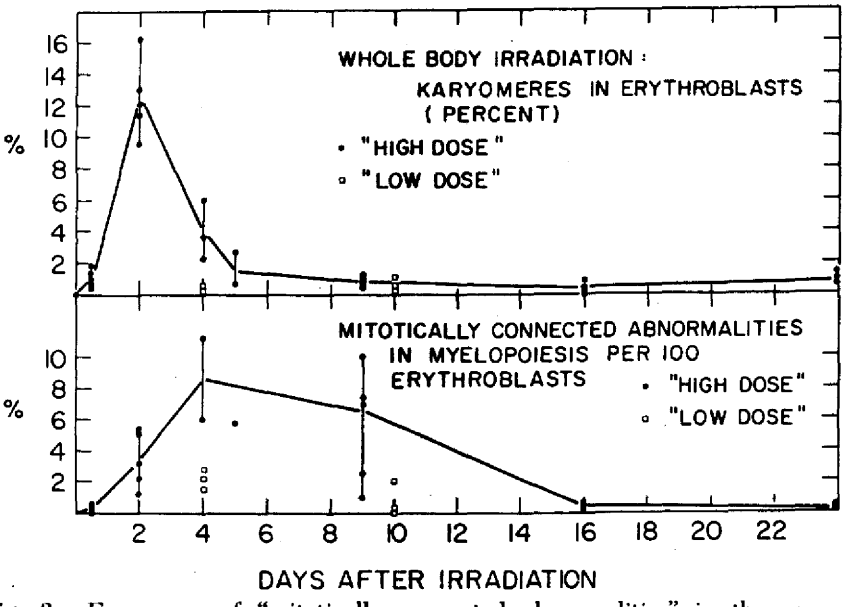

Fig. 3.—Frequency of "mitotically connected abnormalities" in the course of counting 500 nucleated red cell precursors in marrow smears of persons exposed to whole body irradiation.

quency of M. C. Abn. of myelocytic cells are shown in figure 3 together with those of erythroblasts. Myelocytic abnormalities were observed in marrow smears prepared 12 hours after whole body exposure (mainly abnormal mitoses) and were maximal on days 4 and 5. One value of each day was not included. The smears were poor and only a few erythroblasts were found. However, abnormal myelocytic cells of the types described were plentiful. If related to the few erythroblasts found, their frequency would be 100–200 per cent. Therefore, these somewhat misleading values were not included in the graph. After 4 days, myelocytic abnormalities became less frequent and had almost disappeared by the 16th day.

Of interest was the time sequence of the appearance of abnormalities at different maturation levels. Forty-eight hours after the accident, all abnormalities described were found in promyelocytes, myelocytes and metamyelocytes. In contrast, in the marrow smears of the 4th day after exposure, abnormal segmented granulocytes (giant cells, binucleated cells, and cells containing karyomeres) were plentiful. This would suggest that the time from the last myelocytic cell division (during which the abnormality presumably became manifest) to the formation of abnormal segmented granulocytes was between 2 and 4 days.

Abnormalities in myelocytic cells were found also in smears of the low dose group after 4 days (fig. 3). However, they were much less frequent than in smears of the high dose group. This may suggest a dose relationship for such M. C. Abn.

Karyomeres were found also in lymphocytes, as shown in figure 2, on day 23 after exposure, but only in smears of two men of the high dose group.

Table 1.—*Mitosis Early after the Accident as Seen in Wright's Stained Smears*

Individuals	12 Hours 6-17-58		2 Days 6-18-58		Day 4 or 5 6-20 or 6-21-58	
	No. counted	Mitoses per.1000	No. counted	Mitoses per 1000	No. counted	Mitoses per 1000
High dose group						
A	3000	5	2000	6.5	2000	1
B	2000	8.5	2000	8	2000	9
C	2000	3	2000	3.5		
D	2000	3	2000	5	2000	1
E	2000	2.5	2000	4.5	2000	2.5
Low dose group						
F					2000	5
G					2000	4
H					2000	5.5
Normal (smears: Japa[31])						5

In an attempt to establish whether or not a determination of the mitotic index is meaningful in marrow smears early after exposure, 2000 cells were counted in each marrow smear of the high dose group at 12 hours, 2 days and 4 or 5 days, and in the low dose group on day 4 after exposure. The values are given in table 1. The mitotic indices thus determined were in the range of normal.[31] As has been pointed out before,[28] mitotic index determinations performed on ordinarily prepared marrow smears are not accurate and should only be based on at least 5000 cells counted in squash preparations.

B. Manifestations of Radiation Injury "Late" after Exposure

Three and one-half years after the accidental whole-body exposure, the bone marrow was reexamined in all eight persons. In this report, results of the following studies were included: (1) Determination of the frequency of M. C. Abn. using identical criteria as for the studies early after exposure; (2) determination of the mitotic indices of the marrow; (3) determination of the relative frequency of mitoses of different cell lineages.

In marrow smears, a total of 15,000 erythroblasts were counted in the high dose group and 7000 in the low dose group. The results were compared to 10,000 erythroblasts counted in smears of healthy persons used as comparison. In the smears of the irradiated persons, a variety of cytologic abnormalities was observed which are rare in normal smears. Most striking was the increased number of binucleated and the presence of a very few trinucleated erythroblasts. Examples are shown in figure 4. In addition, an increased number of erythroblasts with karyomeres was found. In mitoses, usually of the myelocytic series, chromosomes or fragments were found in the cytoplasm, apparently excluded from further karyokinesis (fig. 4). In two instances, tripolar mitoses were seen (fig. 4). In table 2, the frequency of binucleated red cell precursors per 1000 erythroblasts is shown. In the high dose group, the mean is 5.08 compared to 2.25 in two individuals of the low dose group receiving about 70 rad, and to 1.2 in the comparison group. The

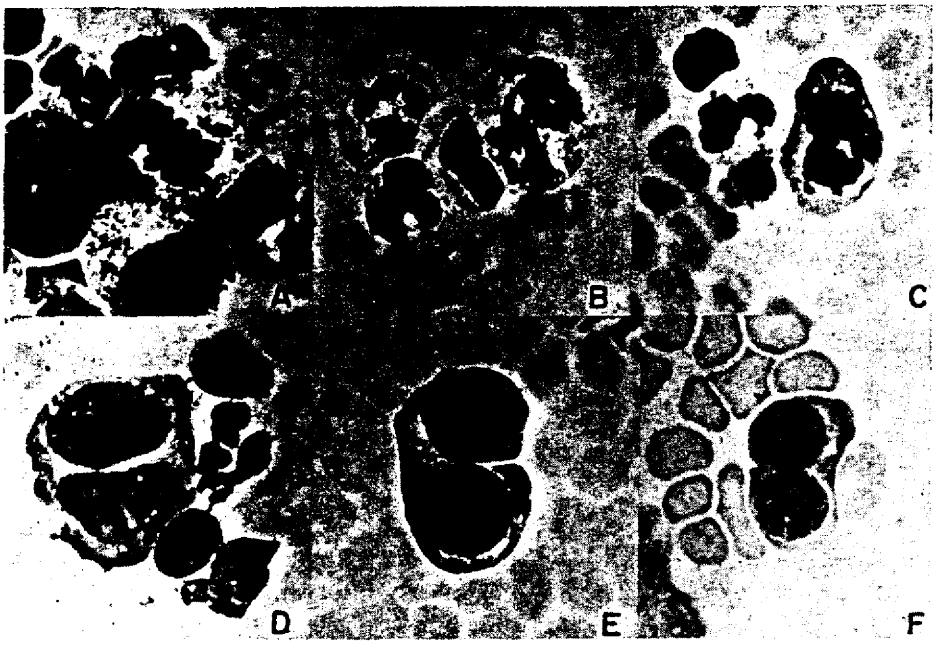

Fig. 4.—"Mitotically connected abnormalities" in bone marrow smears 3.5 years after exposure to 236–365 rads. A: Myelocyte mitosis with aberrant chromosomes; B: myelocyte mitosis with chromosomal bridge; C: tripolar mitosis in early red cell precursor; D-F: binucleated erythroblast at the proerythroblast, macroblast and basophilic normoblast maturation stage.

difference between the means of the high dose group persons and the normals is statistically significant ($0.01 > p > 0.001$), while p for the "low dose" group is 0.05.* Thus, the latter findings are not significant but suggestive. Not quantitatively evaluated was the apparent increase in red cell precursors with cytoplasmic bridges between cells with clear-cut interphase nuclei in the high dose group as compared to the normal smears. This abnormality has been described by E. Schwarz[30] who considered it to represent "residual interzonal fibers."

In a separate approach, bi- or multinucleated erythroblasts were recorded in the course of counting 100 mitoses of all cell types in marrow smears of the exposed individuals and of normal persons. It was found that the means of the frequency of such atypical erythroblasts in the high dose group were 7.4 and 3.3 per 100 mitoses in the low dose group. In smears of normal persons, a total of 161 mitoses were counted in the same way and 1.8 atypical erythroblasts were found per 100 mitoses. Binucleated cells of the myelocytic series occurred at a frequency of 2.5 per 100 mitoses of all types in the normal marrow smears, compared to 2.2 in the high dose group and 1 in the low dose

*Berman's controls[32] have a higher incidence of binucleated cells than ours. Since our controls and irradiated subjects were studied in exactly the same manner, we have chosen to make the statistical comparison with our controls rather than Berman's normal values.

Table 2.—*"Mitotically Connected" Abnormalities in Bone Marrow Cells 3.5 Years after Accidental Exposure to Radiation*

Individuals	Number of Erythroblasts Counted	Binucleated "Early" Erythroblasts per 1000	Binucleated "Late" Erythroblasts per 1000	Total Binucleated Erythroblasts per 1000
High dose group				
A	4050	1.4	5.1	6.6
B	4000	1.25	4.25	5.5
C	2000	2.5	4.0	6.5
D	2000	0.5	1.0	1.5
E	3000	1.3	4.0	5.3
				$x = 5.08 \pm 2.1$
				$0.01 > p > 0.001$ (compared to normal)
Low dose group				
F	2006	0.5	2.0	2.5
G	3050	0	2.0	2.0
H	2000	0	0.0	0.0
				$\bar{x} = 1.5 \pm 1.0$
			F and G only:	$\bar{x} = 2.25 \pm 0.63$
				$p > 0.05$ (compared to normal)
Comparisons				
SAK	3000	0	0.7	0.7
TMF	4160	0.2	0.9	1.1
EPC	1000	0	1.0	1.0
CRC	2000	0	2.0	2.0
				$\bar{x} = 1.2 \pm 0.48$

group. From these numbers it appears that there is evidence for an increase in the frequency of multinucleated red cell precursors 3.5 years after the radiation accident in the high dose group whereas the findings are not significant in the low dose group. In contrast, the differences in the frequency of binucleated myelocytic cells between the normal and irradiated persons was not significant. It should be pointed out that the occurrence of early binucleated erythroblasts must be considered abnormal in particular. Such cells are shown in figure 4. Their nuclear and cytoplasmic characteristics correspond to those of proerythroblasts, and early and late basophilic normoblasts. Such immature binucleated cells are extremely rare in completely normal marrow smears in which they are mostly polychromatic or oxyphilic.[32] Erythroblasts with three nuclei were found 3 times, and tripolar mitoses in three instances in smears of the high dose group. Among 53,167 erythroblasts of eight "normal" persons, not one pluripolar mitosis was seen by Berman.[32]

Mitotic indices in all eight men were determined in Feulgen-stained squash preparations, using the same technics as in 1958 to study mitotic indices early after the accident.[28] The results are given in table 3. The mitotic indices in the high dose group and in the low dose group were not statistically different ($p > 0.05$) from the values found in seven healthy comparisons.[28] In the squash preparations of the high dose group, the mitoses were distributed between the phases of the mitotic cycle in the proportions: prophase 15.3 per

Table 3.—*Mitotic Indices 3.5 Years after the Exposure (Feulgen-stained Squash Preparation)*

Individuals	Slides Counted	Cells Counted per Slide	Range of Mitoses per 1000 Cells	Mean X	Standard Deviation
High dose group					
A	5	1000	6–14	9.6	4.13
B	5	1000	7–12	9.2	1.87
C	5	1000	8–11	9.4	1.09
D	5	1000	5–13	9.0	1.07
E	5	1000	12–18	13.6	2.59
			Mean	10.16 ($p > 0.05$ compared to normal)	1.94
Low dose group					
F	5	1000	6–11	8.8	1.92
G	5	1000	3–12	7.4	3.5
H	5	1000	6–14	8.6	3.12
			Mean	8.27	0.59
*Comparisons** Seven healthy comparisons	38	1000	2–18	8.97	1.0

*Data taken from Fliedner et al.: Acta. haemat. 22:65, 1959.

cent, metaphase 77.2 per cent, anaphase 5.9 per cent, and telephase 1.6 per cent. In the low dose group, the respective values were: prophase 21 per cent, metaphase 71 per cent, anaphase 6.5 per cent and telephase 2.5 per cent.

In bone marrow smears of the exposed men prepared 3.5 years after the accident, 100 mitoses were counted on each individual and classified into erythropoietic, myelopoietic, and others; the latter including all mitoses which were not clearly erythropoietic or myelocytic as well as those that occurred in megakaryocytes, plasma cells and unclassified cell types. These values are given in table 4 and compared to similar data previously obtained on normal persons.[29] The data show that the distribution of mitoses is about the same for the high dose, the low dose and the control group. It is obvious, however, that a number of the mitotic figures seen in the high dose group, and a smaller number in the low dose group were considered abnormal. Mitoses considered abnormal in these preparations showed one or more chromosomes or fragments obviously excluded from further karyokinesis (aberrant chromosomes), or clearly visible single or multiple chromosome bridges in late anaphase or telephase. In 138 mitoses counted in smears of two "normal" controls, only two such abnormal mitoses with an aberrant chromosome were seen. Since no particular search for these abnormalities was made in the other control smears counted previously, it is not certain what the exact frequency of such abnormal mitoses is in healthy persons.

Discussion

The findings presented indicate that cytologic abnormalities were present in the marrow after accidental whole body irradiation not only during the first weeks after exposure but even 3.5 years later. These abnormalities oc-

Table 4.—*Classification of Mitoses 3.5 Years after Exposure, Wright's-stained Smears*

Individuals	No. of Mitoses Counted	Per Cent Erythropoietic Mitoses	Per Cent Myelopoietic Mitoses	Per Cent Other Mitoses	Per Cent "Abnormal" Mitoses
High dose group					
A	100	68	27	5	10
B	100	76	20	5	5
C	100	72	18	10	1
D	100	82	17	2	4
E	100	73	25	2	2
Mean values		74.2	21.4	4.8	4.4
Low dose group					
F	100	52	37	11	4
G	100	60	38	2	1
H	100	71	23	6	3
Mean values		61.0	32.7	6.3	2.7
Normal comparisons					
SAK	100	72	27	1	
EPC	250	67.6	28.4	4.0	
	70	62.8	24.3	12.0	
TMF	150	75.3	20.7	4.0	
	26	77	19.2	3.8	
LEF	50	64	34	2.0	
JFB	50	64	30	6.0	
CRS	65	77	18.4	4.6	
VPB	50	68	32	0	
Mean values		69.7	26.0	4.2	

curred initially in a characteristic pattern and were clearly demonstrable during the first 1–2 days after exposure, at a time when the cells of the peripheral blood did not show marked alterations, except for a progressive lymphopenia and possible granulocytosis. If the appearance of erythroblastic karyomeres is taken as evidence for a previous abnormal mitosis, then by 2 days about 30 per cent of all nucleated red cell precursors of the persons exposed to the high dose had emerged from an abnormal mitosis. Abnormalities of mitoses were frequent during the first days (up to 50 per cent) and were rare, although present (about 4 per cent), 3.5 years after the accident. Thus it appears that the specific evaluation of marrow smears for cytologic abnormalities of patients exposed to ionizing radiation may be helpful in the assessment of the clinical situation. The evaluation of cytologic changes in fixed and stained marrow smears requires particular caution. Under normal conditions a marked variability of nuclear and cytoplasmic structure and staining properties can be found. These may vary from area to area of one preparation and among smears and they depend in part on the technics employed. Therefore, one should use only criteria that appear to be relatively independent of technical factors. It is believed that the M. C. Abn. shown in this study are examples of such criteria and can be used successfully for a cytologic evaluation of radiation effects. Less reliable are the nuclear and cytoplasmic

characteristics, although an increased incidence of cell degeneration (karyolysis and karyorrhexis) was found shortly after exposure. It may be expected that such cell changes indicating direct injury occur more frequently after lethal or supralethal exposures, as seen in animal experiments.[19,20] For the evaluation of radiation effects on the bone marrow early after a single whole body exposure (during the first 10 days), it was found satisfactory to count 500 erythroblasts per smear and to record the cytologic abnormalities encountered in all blood cell series. In *dividing cells*, the following changes were considered abnormal: (1) tri- or multipolar mitoses of erythrocytic or myelocytic precursors (fig. 4c); (2) mitoses with single or multiple chromosomal fragments "left behind" in the cytoplasm and apparently not further participating in karyokinesis (fig. 1, D-G; fig. 2, H.; fig. 4 A,C); (3) mitoses with chromosomal bridges in late ana- or telophase (fig. 1, A-C; fig. 4, B). Other changes such as marked clumping or bizarre shapes and distribution of chromosomes were not believed to be reliable enough to allow interpretation. In *interphase cells*, the following cytologic abnormalities were considered abnormal: (1) cells with one or more karyomeres in the cytoplasm (fig. 1, H-M; fig. 2, G, I-R); (2) bi- or multinucleated erythrocytic or myelocytic precursors, with or without an internuclear bridge, with or without karyomeres (fig. 1 H, fig. 2, D, E, G; fig. 4, D-F); (3) giant cells, particularly of the myelocytic series, which may be larger than any normal stage of development, i.e., huge promyelocytes or myelocytes. These cells may have the size of a more immature precursor but the nuclear and cytoplasmic characteristics of mature cells such as metamyelocytes, band forms, or segmented neutrophils (fig. 2, A-C, N-O).

All of the cytologic abnormalities described here for the human bone marrow have been found in systematic studies in animals after various doses of whole body irradiation.[19,21] In these experimental investigations, a dose relationship had been suggested for M. C. Abn. However, at supralethal dose ranges, the immediate evidence of karyorrhexis and karyolysis was more prominent than abnormalities seen in cells which attempted or completed further proliferation.

The value of a cytologic evaluation of marrow smears after possible or established radiation exposure is limited by the fact that all abnormalities described are by no means radiation-specific although characteristic for radiation effects. Such changes have been described in a variety of clinical conditions and particularly in patients who have been given cytotoxic drugs for the treatment of malignancies. Some of these cytologic alterations are also seen, although infrequently, in smears of apparently healthy persons.[33] The most extensive earlier studies have been performed by E. Schwarz[30] and L. Berman[32] who also discussed their possible pathogeneses. Thus, the possibility that cytologic abnormalities in bone marrow smears are due to etiologic factors other than radiation exposure has to be considered and ruled out. However, if they occur subsequent to known radiation exposure in persons presumably previously healthy, and appear and disappear at a characteristic time

sequence as shown in this paper, they can be used as an indicator of radiation exposure.

The pathogenesis of the various abnormalities described cannot be deduced from the marrow smears alone. However, time lapse photography of bone marrow cells[34] and of tissue culture cells[35] after exposure to ionizing radiation has elucidated some of the possible mechanisms involved.

The appearance of free chromosomes or chromosomal fragments in abnormal mitoses are the result of partial or complete inhibition of polar movements of chromosomes or chromosomal fragments.[37] When the cell enters interphase after completion of mitosis, these fragments apparently are transformed into small nuclear masses or droplets, the size of which may depend in part on the number of chromosomes in the aberrant group (karyomeres). Such aberrant nuclear fragments or karyomeres were found to be labeled with tritiated thymidine in rats when it was injected 30 minutes prior to killing at different times during the first 24 hours after whole body irradiation.[19] This would indicate that karyomeres are still capable of synthesizing DNA synchronously with the principal nucleus. Functionally, they must be considered as satelite nuclei and not signs of karyorrhexis. Politzer, in 1934,[37] suggested that such nuclear fragments display signs of kinesis simultaneously with the principal nucleus.

The appearance of chromosomal bridges in abnormal mitoses is a well-known result of radiation injury of cells and has been present in the marrow smears examined in this study. In these smears they were felt to be recognizable with confidence only in late anaphase or telophase. It is conceivable that the internuclear chromatin bridges found occasionally in interphase cells originated from such mitotic chromosomal bridges. In autoradiographs of marrow of the exposed persons incubated in vitro with tritiated thymidine, it was found in one instance that such an internuclear bridge was labeled as well as the two nuclei connected by it.[38] This indicates that such internuclear bridges are functionally still a part of the nuclei and enter DNA synthesis synchronously.

The pathogenesis of giant cells with one or more nuclei is obscure. It is conceivable to regard bi- or multinucleated erythrocytic or myelocytic cells as the result of an undisturbed karyokinesis with an inhibited cytokinesis which may be partial or complete. On this basis, one extreme would be to find two cells connected only by a long fiber-like cytoplasmic bridge. An increased frequency of such cytoplasmic bridges between two cells was suggestive particularly in the marrow smears taken 3.5 years after the accident. All transitions between a binucleated cell with a cytoplasm of normal outline and two cells connected by a long cytoplasmic bridge could be found. The pathogenesis of cells with three nuclei or of tripolar mitoses is not well understood although theories as to their formation have been proposed.[30,36] If bi- or multinucleated cells are able to complete their maturation, they can be found in the circulating blood. This was particularly true for the granulocytic series in this study which showed abnormally large hypersegmented blood

cells during the first 2–4 days after exposure. They are known to occur under experimental conditions[17] and may be as frequent as 50–60 per cent of all granulocytes after very high radiation doses.[39] In the erythropoietic series, the final enucleation of multinucleated cells may result in the appearance of some macrocytes.

An alternative possibility for the formation of multinucleated cells after radiation exposure is the fusion of two mononucleated cells. This has been demonstrated by time lapse photography of marrow cells[34] and of tissue culture cells.[35] It is unknown what proportion of these abnormal cells originates from inhibited cytoplasmic division with complete nuclear division and what proportion from cell fusion.

Although not proven, mononucleated giant cells may be the result of a disturbed cyto- and karyokinesis with continued DNA synthesis and uninterrupted cellular maturation. The occurrence of a skipping of cell division in erythropoiesis has been postulated by Stohlman[40] in various states of erythropoietic activity and is suggestive in this study also for the white cell series early after irradiation. Such skipping of cell division with undisturbed cell maturation results in the net production of only $\frac{1}{2n}$ (if n is the number of skipped divisions) of the cells produced normally. Thus, the slope of granulocyte diminution early after whole body exposure is not only the result of interrupted new production at the stem cell level[41] but is modified by the degree of mitotic inhibition of precursor cells.

The presence of cytologic abnormalities 3.5 years after the accident, as indicated by some mitotic abnormalities, and an increase in the frequency of binucleated early erythroblasts is poorly understood. Chromosomal abnormalities were demonstrated in blood leukocyte cultures of these men by Bender.[15] The abnormalities seen in the bone marrow can be found in all those clinical conditions associated with an increased regeneratory activity of hemopoiesis, such as various forms of anemia, but they are also seen in leukemias.[32,33] The cell counts of the peripheral blood in these men,[42] as well as the mitotic indices in the marrow, were well within normal limits. Thus, the abnormalities shown may indicate a slight increase in an "ineffective" hemopoiesis although the pathogenesis and significance are not established and must await long-term follow-up studies.

Summary

1. Serial marrow studies were performed during the first few days in eight men accidentally exposed to a mixed neutron gamma irradiation. They showed the occurrence of a wave of cytologic abnormalities. These were identical with those seen in animal experiments 1–3 days after whole body irradiation. They were considered to be "mitotically connected" (M. C. Abn.) and included the occurrence of chromosomal bridges and chromosomal fragments in mitoses. In interphase cells, the main abnormalities were nuclear fragments ("karyomeres") in the cytoplasm of erythroblasts, myelocytic cells and lymphocytes; bi- and multinucleated cells; and giant cells. The peak of ab-

normalities in the erythropoietic forms was reached after 2 days; that in the myelopoietic cells 4 days after exposure. On the 4th day, there was a distinct dose-dependent difference in these abnormalities between the high dose group (236–365 rads) and the low dose group (22–68 rads).

2. Some cytologic abnormalities, as seen in increased regeneratory activity of the marrow, were found in marrow smears 3.5 years after the accident, although the peripheral blood counts and mitotic indices of the marrow were within normal range. Their significance is obscure.

3. A careful cytologic evaluation of serially aspirated marrow samples during the first hours and days after whole body exposure proves to be an additional important aid in the assessment of the exposed individual and may well prove to be useful in determining the degree of injury and thus the prognosis.

SUMMARIO IN INTERLINGUA

1. Serial studios del medulla esseva effectuate durante le prime dies in octo homines accidentalmente exponite a un mixte irradiation neutronic gamma. Esseva notate le occurrentia de un ver unda de anormalitates cytologic. Istos esseva identic con le anormalitates incontrate in animales experimental inter 1 e 3 dies post le irradiation del corpore total. Illos esseva reguardate como "mitoticamente connectite" e includeva le occurrentia de pontes chromosomal e de fragmentos chromosomal in mitoses. In cellulas de interphase, le major anormalitates esseva fragmentos nucleari ("karyomeros") in le cytoplasma de erythroblastos, de cellulas myelocytic, e de lymphocytos e le presentia de bi- e multinucleate cellulas e de cellulas gigante. Le culmine del anormalitates in le formas erythropoietic esseva attingite post 2 dies e in le cellulas myelopoietic post 4 dies a partir del exposition. Le quarte die, un distincte differentia in iste anormalitates esseva notate secundo le dose inter le gruppo a alte dosage (236 a 365 rad) e le gruppo a basse dosage (22 a 68 rad).

2. Certe anormalitates cytologic, manifeste in un augmentate activitate regeneratori del medulla, esseva trovate in frottis medullari 3,5 annos post le accidente. Tamen, a ille tempore le numeration in le sanguine peripheric e le indices mitotic in le medulla esseva intra le limites del norma. Le signification de iste anormalitates remane obscur.

3. Un precise evalutation cytologic de serialmente aspirate specimens de medulla durante le prime horas e dies post le exposition del corpore total se revela como un importante adjuta additional in le examine de un exponite subjecto. Il pare ben probabile que un tal evalutation va provar se utile in determinar le grado del injuria e assi le prognose in le caso individual.

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