Effects of Total Body X-Irradiation on the Peripheral Blood of the Monkey

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Although the effects of irradiation on elements of peripheral blood are well known for most laboratory animals, the authors were able to find but one reference to such studies being performed on monkeys, and this involved smaller dosage levels than those in the present study. Because of this lack of data in an animal where information would seem to have the most direct applicability to man, the following observations, obtained in connection with other research, are reported.

Method

Data were obtained from 9 healthy Macaca mulatta monkeys, weighing from 5 to 7 pounds, which were also used in a study of the effects of irradiation upon the electroencephalogram. The unanesthetized animals were rotated in the beam of a 250 K.V. Maximar x-ray machine employing 0.5 mm. Cu and 1.0 mm. Al filters, 15 Ma., and having an HVL of 1.7 Cu and a dosage rate of 13.7 r/min. One animal received 550 r, 6 received 600 r, and 2 had 700 r. Experience with irradiation of over 30 monkeys at this laboratory suggests that 600 r is an approximate LD50/30. Further details of the irradiation procedure may be found elsewhere.

Counts on free-flowing blood obtained by nicking the marginal ear vein and using Bureau of Standard pipets were made on three different days prior to irradiation, one to six hours after irradiation, and at lengthening intervals thereafter up to eight months in surviving animals. Leukocyte counts were corrected for the presence of nucleated red cells when necessary. Smears were made by the cover slip method and stained with Wright's stain. Platelets were counted by the direct method of Rees and Ecker. Since values obtained from the animals given 700 r lay within the range of those of the 600 r group, the counts of all of these animals are included in the accompanying charts. Many other scattered counts have been considered in making the interpretations. Two control animals, housed with the irradiated monkeys, showed no noteworthy changes in their blood picture over a period of eight months.

One monkey, not included in the above compilations, received 550 r and was subjected to specific eosinophil counts, based upon 4 chamber determinations from 2 pipets using the phloxine-propylene glycol method. Handling of this rather docile animal was shown on several occasions not to cause a 4 hour drop in circulating eosinophils.

Results

The patterns of response of the monkey's blood elements following irradiation agree broadly with those which have been described for other animals. The general finding consists of an initial fall in the number of circulating leukocytes followed by a period of sustained depression and a subsequent return to a normal level. Temporal and quantitative features of these changes, which are specific for the different blood cells, are presented in the following paragraphs.

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1. **White blood count**: The total white count was the determination most strikingly influenced. The counts in 5 animals made 1 to 5 hours after radiation exceeded their pre-irradiation ranges, accounting for the early rise above the total pre-irradiation average of 14,500 seen in figure 1. Over the next few days, the white blood count dropped rapidly, to remain markedly low for twelve days before increasing somewhat less rapidly during the third to fifth weeks in the 4 survivors. Lowest counts (100-750) were found in the 3 animals that died. At thirty-three days, the white blood count of each survivor had regained its pre-irradiation average and in 3 cases continued slowly to rise during the next three months. At seven months, 2 animals showed counts and differential determinations similar to those observed before irradiation.

2. **Neutrophils**: The increase in white blood cells occurring several hours after irradiation was due entirely to a rise in neutrophils, these cells being increased two- or three-fold in 6 of the animals. This elevation was associated with an increase in band forms, which rose to 10 per cent of the total cells from a pre-irradiation average of 2 per cent. Band forms were still present during the first week of dropping counts, were absent during the period of markedly depressed counts, and became moderately elevated again when the neutrophil count began to rise in the third week. At this time, occasional metamyelocytes were seen. Toxic granulation, which has been described in radiation sickness, was not seen at any phase.

3. **Eosinophils**: Differential counts made on monkeys irradiated at 600 and 700 r showed several features of interest: a continued presence of eosinophils
one to three days after irradiation; a virtual absence from the fourth to twenty-seventh days, followed by an absolute and relative eosinophilia of considerable proportions during the fifth and seventh weeks. Specific eosinophil counts were made in one monkey irradiated with 550 r, precautions being taken to avoid stressful stimuli (fig. 2). Twelve pre-irradiation counts ranged from 120 to 617 and averaged 254/cu.mm. On post-irradiation days 1, 2 and 3, counts were 400, 177, and 149, confirming the impression gained from the smears that the eosinophil level was still within the normal range during the first three days following radiation, rather than being markedly depressed as might be expected if the animal were in a stressed state.

**Fig. 2**—Effects of irradiation with 550 r upon the eosinophils, neutrophils and lymphocytes of Monkey #39. Note the absence of fall below the preradiation range of eosinophils during the first, second, and third days post-radiation.

From the fourth day through the third week, eosinophils were scarce or absent, but returned abruptly beginning on the twenty-seventh day and exceeded the pre-irradiation range by the fifth week. In this animal and in 3 of the others which were followed into the fifth and seventh weeks, eosinophils comprised over 25 per cent of the total white count and were about equally divided among band and segmented forms. Their cytoplasm during this period was more basophilic than normal and occasional metamyelocytic forms were observed. After three months, eosinophils were normal in appearance and occurred in more normal percentages.

4. **Lymphocytes**: Lymphocytes dropped consistently in counts made 1 to 5 hours after irradiation, one count falling from 5230 per cu.mm. before exposure to 636 1 hour after exposure. Lymphocytes, however, never became depressed to the extent noted with the neutrophils, and for one to two weeks most of the cells seen were lymphocytes. The lymphocytes did not begin to return appreciably until the fourth week, so that for a time neutrophils outnumbered lympo-
cytes, reversing the normal relationship. By the third month, lymphocytes again exceeded neutrophils and their own pre-irradiation averages.

Some lymphocytes were observed to have clumping of nuclear chromatin while in the fourth week large lymphocytes containing nucleoli were seen in small numbers and small lymphocytes showed exaggeration of nuclear indentation. At this stage, monocytic and lymphocytic differentiation was difficult. Bilobed forms were not seen.

5. Monocytes: Monocytes were not seen after the fourth post-irradiation day, but became plentiful during the third week when they averaged 22 per cent of the differential count. By the seventh week, the monocytes were more typical in appearance and were dropping to the more normal proportions found in later months.

In one monkey, the number of monocytes, after dropping to within normal ranges at seven and twelve weeks, again rose. During the third month, this animal began to lose weight and after a week of progressive anorexia, diarrhea and prostration, died 3.7 months after irradiation. Counts made the day before death showed a small elevation in total white cells from previous levels, but heterophil cells were almost completely replaced by what appeared to be atypical monocytes. These were not blast forms. Normal lymphocytes were seen in small numbers and platelets and erythrocytes were not lowered. As two other irradiated monkeys in the colony died with diarrhea at about this time, this animal may have had an acute gastrointestinal infection. It is possible, however, that this animal had monocytic leukemia.

6. Platelets: Before radiation, platelets averaged 218,000 per cm. and ranged from 103,000 to 416,000. Daily counts on several animals indicated that the number of platelets declined slowly until about the seventh day and then dropped
quickly (fig. 3). Each of the 4 animals that died during the second and third weeks had a count below 40,000. By the third week, platelets in the survivors were as plentiful as before radiation. During their rise, occasional giant platelets were seen. No megakaryocytes were observed.

7. Erythrocytes: Red blood counts declined slowly during the first week after irradiation, then dropped more rapidly to reach a minimum level at the second or third weeks. The lowest individual count was 2.4 million. At two weeks, a few polychromatophilic cells appeared, and six days later, polychromatophilia and anisocytosis were conspicuous and normoblasts were frequent. Fewer signs of red cell immaturity were present at four weeks, although the red cell count continued to rise slowly to the thirteenth week.

8. Hemoglobin: The curve of hemoglobin concentration followed, in general, that of the red blood cells.

Discussion

The normal blood values found in our animals agreed with those established for the monkey by Scarborough. Although the monkey is a primate, it must be recognized that, with respect to its neutrophil-lymphocyte ratio, it resembles the rat and mouse in that the predominant cell is the lymphocyte. The carnivores, such as the cat and dog, are more similar to man in whom the neutrophil predominates. Whatever these differences in cell types may mean functionally, the progressive changes in the monkey’s blood values after irradiation followed the well known trends seen in other animals.

The response of lymphocytes in the monkey to x-ray exposure was in remarkable agreement with that of several other species, supporting De Bruyn’s conclusion that species differences are not prominently involved. With respect to neutrophils, the findings in the monkey also agreed broadly with those in other animals in showing, on the third to fifth days, a drop to a plateau of low values from which there was a gradual recovery during the third week. It was difficult to compare the alterations in eosinophil and monocyte counts following irradiation in the monkey with changes in other animals because of the scarcity of existing data.

The wave of erythrocyte depression in the monkey was less distinct than that of the white cells, but its time course from the tenth to twentieth days was in agreement with that in the rabbit, rat, and mouse. The period of platelet depression in the monkey, lasting from the seventh to eighteenth day, also agreed with the findings in the dog, rabbit, and rat. Thus, these data on at least one primate, the monkey, corresponded well with the patterns of response which have been more intensively worked out in sub-primate forms subjected to comparable radiation exposure.

The scarcity of available data makes difficult the extension of this comparison to man. Total leukocyte counts in man, taken from the atomic bomb studies, may, however, be compared with those following irradiation in the monkey and other animals. As seen in table 1, leukocyte recovery was considerably more retarded in these human bomb casualties than in any of the animals studied except the pig. LeRoi in presenting the data on the bomb casualties concluded that their responses were consistent with those of other large mammals, but the rather incomplete data from the goat do not seem in good agreement. Another
suggestiots ivould relate the rate of recovery to the life span of the species. In the case of the atomic bomb casualties, burns, wounds, infection, blood loss and malnutrition might have been a factor in the slow recovery. That malnutrition had influenced blood formation among the Japanese is suggested by the fact that 50 per cent of the hemoglobin values from a control population were below 11.5 Gm. per cent.

The findings in the eosinophil counts merit special comment. It is interesting that in the monkey exposed to 550 r, eosinophil counts remained within the pre-irradiation range until the fourth post-irradiation day, even though adrenalin had been shown capable of depressing the eosinophils by 60 to 85 per cent on four occasions prior to irradiation. This finding, suggesting the absence of stressing stimuli during the first to third post-irradiation days agrees with evidence for a maintenance of the eosinophil level during the first days of radiation therapy. Lawrence found that the urinary excretion of 17-ketosteroids did not reach a maximum until five to twelve days after irradiation of mice. Several authors showed that although adrenal cholesterol dropped immediately following irradiation, it rose again during the succeeding three to seven day period. These findings suggest the absence of stress in the initial asymptomatic period after irradiation.

Attention may be called to the fact that the pronounced fall of eosinophils on the fourth post-irradiation day is coincident with a similar depression of neutrophils and hence appears to be due to direct injury to the blood forming tissues. In the later recovery of leukocytes to normal levels, the increase of eosinophils lags behind that of the neutrophils by one week, however, suggesting differences in maturation times of these elements. Furthermore, in their recovery, the eosinophils overshoot to levels greater than normal, a phenomenon which has been noted after other types of protracted stressful stimulation.

**SUMMARY**

In monkeys subjected to a single dose of LD/50 total body x-irradiation, the periods of initial fall, continued depression and subsequent recovery were determined for total white counts, neutrophils, eosinophils, lymphocytes, monocytes, erythrocytes and platelets.

In general, the effect of irradiation upon the blood picture in the monkey.
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parallels closely that observed in lower animals. Discrepancies in the leukocyte recovery times between human atomic bomb casualties and animals, including the monkey, are noted.

Observations on eosinophils and lymphocytes which bear upon the role of irradiation as a stress stimulus are presented and discussed.

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