The Response of Eosinophils to Total-Body X-Radiation of the Monkey

By Earl Eldred

The number of eosinophils circulating in the blood, following exposure of an animal to ionizing radiation, varies after a complex pattern. Within the first hour after irradiation the number may rise somewhat along with the more extensive climb of neutrophils, but by the second hour the eosinophil count drops sharply and reaches a minimum after several hours.

Subsequent to the acute drop following irradiation at doses within the lethal range, the number of eosinophils may remain at control or moderately reduced levels for several days before again declining. Specific counts of eosinophils in rats, for example, return to normal by nine hours and do not decline again until the third day. In hamsters, cats, burros, swine, monkeys and, perhaps, tadpoles eosinophils are still present, though dwindling in numbers for one (mice) to four (swine, burros and monkeys) days. However, chickens are said to show an immediate and permanent drop, and in strong irradiation of portions of the human body eosinophils may not return for several days.

After these first few days eosinophils, like neutrophils, enter a period of profound depression which endures through the second and usually the third week. Eosinophils are not totally absent during this time, and there may actually be a relative eosinophilia (burros, rats).

The stage following the reappearance of eosinophils is one of particular emphasis in the present work. In the macaque this late phase is characterized by an absolute and relative eosinophilia which may last one to two months. This late eosinophilia has apparently not been described in other experimental animals, although it occurs in man. Previous accounts of its presence in monkeys were based upon data obtained from differential and total leukocyte counts. In the present work the use of specific eosinophil-counting procedures has permitted closer examination of the responses of circulating eosinophils after exposure of the monkey to a single dose of x-radiation within the lethal range.

Methods

General aspects of the response of blood elements in the rhesus macaque to x-irradiation have already been published. To the data based on differential counts in 9 animals of that work has been added information based on specific counts of eosinophils in an additional 14 monkeys. Animals were selected after a minimum of three weeks' observation in the stock animal house and were subsequently allowed two to three weeks of acclimation in permanent quarters before beginning practice captures and lancings of the ear. After
the monkeys became accustomed to the procedure, it was usually possible to effect a capture with only a brief flurry of struggling, following which the monkey, still held by hand, would lie pacifically on the animal board during the procedures and commonly appeared to be dozing. Two or three monkeys were rejected because of intractability and associated excessive lability of eosinophil levels.

From 3 to 15 early-morning total white blood cell counts, differentials and specific eosinophil counts were made on each animal before irradiation and at lengthening intervals thereafter. Two pipettes and four chambers of the Fuchs-Rosenthal counting chamber were used for specific eosinophil counts made by the phloxine-propylene-glycol technic. White blood cell counts were based upon a single pipette and 2 chambers, and cover-slip differentials upon 200 cells or, if the count was very low, upon 100 cells.

Irradiation of the unanesthetized animal was carried out in the beam of a 250 KV Maximar x-ray machine: 0.5 mm Cu, 1.0 mm Al, 15 ma., H.V.L. 1.7 Cu and 13.7 r/min. Animals received 550 r or 600 r, which is approximately the MLD/30 days. Because of intervening deaths, and excepting one animal irradiated over the body alone, only 9 monkeys of the 14 in the starting group could be followed beyond the first month. Seven of these were subjected to occasional injections of hormonal agents for the purpose of testing the response of the adrenocortical system. The general pattern of eosinophil response in these did not differ from that seen in the 11 animals (2 in the present, and 9 in the older series of reference 1) in which no tests were performed.

All animals were tuberculin negative and, with two exceptions, were subjected to gross and, in some instances, microscopic postmortem examinations. In three animals one to several cysts, probably parasitic in origin, were found in the walls of the intestine, and in one animal a small arthropod was found encysted in the lung. No further infestations were found.

RESULTS

1. Normal eosinophil levels

The 141 individual eosinophil counts taken in 14 monkeys varied from 120 to 1030. The means for individual animals ranged from 164 to 658, and the mean of these latter values was 454 eosinophils/cu. mm. Differential percentages for eosinophils fell generally between one and four per cent.

No other description of eosinophil levels based upon specific counting technics has been found, but the means of differential values for over 668 rhesus macaques, as reported by some dozen authors, lie consistently between 1 and 4 per cent, with the usual upper extremes of individual authors at less than 10 per cent.

2. Eosinophils following a single total-body irradiation

During the first to third days following irradiation, eosinophil levels, expressed for each monkey in percentages of values obtained before irradiation, were reduced an average of 49 per cent (7 animals, 12 samplings). No particular trend was noticeable during these days, but by the fourth and fifth days eosinophils were being seriously depleted and thereafter were scarce (fig. 1). No relative eosinophilia was noted during this period of depletion.

Appreciable numbers of eosinophils did not reappear until the 4th week, whereupon counts rose rapidly, so that by the 25th to 29th day control values were reached. On the 30th to 35th days there were more than 1,000 eosinophils in all animals. This wave of eosinophilia was especially prominent in the fifth to seventh weeks and was sometimes spectacular, the highest
RESPONSE OF EOSINOPHILS TO TOTAL-BODY X-RADIATION

Fic. 1.—Response of cellular elements of peripheral blood to irradiation of total body of a monkey with 550 r. Eosinophil curve is based upon counts obtained by the phloxine-propylene glycol technic. In the lower right-hand corner is indicated the percentage of nonsegmented forms seen in samples of 50 eosinophils on smears. The average percentage of nonsegmented forms before irradiation was 3 per cent.

absolute count being 16,300 (62 per cent on the differential) at 38 days. Other outstanding maxima were 13,100 at 36 days; 4,250 at 39 days; 4,070 at 48 days; 3,420 at 35 days; 3,380 at 44 days; and 2,660 at 40 days. Three months after irradiation, values still hovered between 1,000 and 2,000; in 2 animals which were followed to 237 and 302 days, eosinophilic levels still slightly exceeded pre-irradiation values. Periods of waxing and waning of eosinophil levels were sometimes present but were not consistent among all animals. Levels in unirradiated animals, incidentally, showed no important trends over periods as long as 120 days (two animals).

3. Response following repetition of irradiation

One animal which had shown a sharp, late rise in eosinophils following irradiation was subjected to an identical exposure 155 days later, at which time eosinophil counts had returned to control levels (fig. 2). A distinct wave of eosinophilia was again noted, although this wave was smaller and differed in form. A similar repetition of eosinophilia in a worker, following a second accidental exposure to radiations from an atomic pile, has been reported.29

DISCUSSION

Dunlap,21 in his review of radiation effects upon the blood, mentions that the number of eosinophils may at this time in "a few patients show definite
FOLLOWING IRRADIATION

FOLLOWING SECOND IRRADIATION

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IRADIATION

Fic. 2.—Response of eosinophil cells in peripheral blood to initial (circles) and second (triangles) exposure of monkey to 550 roentgens. Interval between exposures was 155 days.

eosinophilia amounting to as much as 10 to 20 per cent of the total white cell count. A similar eosinophilia is seen occasionally in radiologists and has been experimentally produced in laboratory animals. Re-examination of his references, however, makes it evident that this statement is more assured than justified. One of the two reports cited as support for the statement that eosinophilia may be produced experimentally concerned an eosinophilia in three dogs witnessed a few days after an irradiational exposure which failed to cause any reduction of other blood cells, and the other reference dealt with a relative eosinophilia in two of four irradiated guinea pigs, an animal which is perhaps peculiarly unfavorable for eosinophil studies. One other early report of a rise in differential percentages of eosinophils in a few guinea pigs is not applicable to the present discussion, since the eosinophilia occurred acutely and was not consistently preceded by a depression of leukocytes. Among more recent studies of blood responses following single exposures of subprimate animals to irradiation, the author has found but one instance of a late eosinophilia. The data of Brecher and his co-workers show a 250 per cent increase above control levels of eosinophils at 28 days (the last day of observation) following irradiation of mice with 400 r.

The response of eosinophils in the rhesus monkey as described by Haigh and Paterson agrees completely with the present data. They found (from differential counts) that for at least 2 days following irradiation the numbers of eosinophils were maintained near the control levels; that these cells were essentially absent from the 10th to 15th day; and that they returned in
numbers markedly exceeding control values during the 2nd, and to a lesser degree, the 3rd month. Animals receiving 600 to 650 r had a more prolonged depression and subsequent higher levels of eosinophils than did those receiving 500 to 550 r. Riopelle and his co-workers also found that eosinophil levels might rise to 2,337 cells/cu. mm. in the 6th week following a single irradiation of the macaque with 350 r. Other monkeys which received 1,000 or 2,000 r in a series of total-body exposures spread over 20 weeks manifested a very slight increase in eosinophils at 34 and 50 weeks after the beginning of the series.

Eosinophilia may occur in man following a single exposure to ionizing radiation. Moses and Platt observed a temporary period of eosinophilia in two men that developed at about six weeks following accidental exposure to radiations from a cyclotron. In another case of acute radiation injury, an eosinophilia developed which rose to levels of 3,000 cells/mm. and lasted over one year. Hiroshima survivors showed statistically significant higher levels of eosinophils than the control population of the neighboring city of Kure, but this evidence is obscured by the high average levels of eosinophils in both these populations (about 10 per cent).

Repeated subjection of the total body to small or moderate doses of radiation apparently fails to produce noticeable eosinophilia in the usual laboratory animals, judging from a number of reports on long-term radiational effects surveyed by the author. However, late eosinophilia in patients following courses of irradiation over trunk areas has been reported repeatedly. Nickson presents data on a patient who developed a persisting relative eosinophilia during the month after completion of a series of total-body exposures totalling 300 r, and on a second patient (500 r) whose marrow showed a relative increase in eosinophils. As Dunlap suggested, radiological workers may exhibit elevated levels of eosinophils. Aubertin and Pfahler, from single differential counts made upon a number of radiologists, were able to point to a few which exhibited mildly elevated percentages of eosinophils. Cornil and Rouslacriox state that the relative percentage of eosinophils was abnormal (i.e., above 4 per cent) in 43 of 182 determinations made upon 40 radiological workers. More extensive data has been brought forth by Nordensen. Although he did not test his data statistically, and unfortunately expressed his determinations in differential percentages rather than in absolute numbers, he has found what is probably a significant difference in the numbers of eosinophils in 1,566 hospital personnel who were more or less exposed to ionizing radiations, as compared with a control population of 450 individuals.

Aside from the above specific references in the literature, the phenomenon of eosinophilia following irradiation is apparently commonly seen by radiologists, and irradiation along with other major traumata like surgery and infections is considered a cause of eosinophilia. The present experiments in their demonstration of a clear and reproducible eosinophilia in the monkey after irradiation offer the possibility of experimental inquiry into the causes of this phenomenon. Results of such an investigation are to be reported in a subsequent paper.
Summary

1. Circulating levels of eosinophils in the monkey have been followed before and after total-body exposure to a near median-lethal dose of x-radiation.
2. Eosinophils are moderately decreased during the first three days and strongly depressed in the second and third weeks. During the fourth week these cells rapidly reappear, and an absolute and relative eosinophilia ensues which is maximal at the fifth to seventh week.

Sumario in Interlingua

1. Le nivellos de eosinophilos circulante in le simia esseva observe ante e post le exposition del corpore integre a un dosage de radiation X amontante quasi a DL50.
2. Le numero de eosinophilos es moderamente reducite durante le prime tres dies e fortemente reducite durante le secunde e tertie septimana. Durante le quarte septimana iste cellulas rapidemente reappare, e un eosinophilia absolute e relative se manifesta subsequentemente. Illo attinge su maximo durante le periodo inter le quinte e le septime septimana.

References

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21. Dunlap, C. E.: Effects of radiation on the blood and the hemopoietic tissues, including the spleen, the thymus and the lymph nodes. Arch. Path. 34:562, 1942.
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