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DEOXYRIBONUCLEIC ACID SYNTHESIZING CELLS IN THE BLOOD OF MAN AND DOG EXPOSED TO TOTAL BODY RADIATION

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With the use of tritiated thymidine, which is incorporated only into new deoxyribonucleic acid, and the autoradiographic technique, it has been possible to show that cells capable of deoxyribonucleic acid synthesis and thus, presumably, of division are present normally in the blood. In the present investigations the number of these cells was followed serially in five men exposed to large doses of whole body radiation in an accident involving fissionable material at the Oak Ridge National Laboratory. Similar studies were performed on irradiated dogs to confirm and extend these observations. Following exposure the number of cells falls drastically within hours (normal values approximately 6 per cubic millimeter for human being; 60 per cubic millimeter for dog). The values then rise gradually, to attain levels exceeding normal approximately ten days after exposure of the human being. A second peak exceeding normal levels is noted at approximately forty days. The cells are all of the large mononuclear type.

The possible fate and function of these cells are discussed.

IT WAS shown previously, by means of tritiated thymidine and the autoradiographic technique, that cells capable of synthesizing deoxyribonucleic acid (DNA) are present normally in the peripheral blood.[1, 2] Thymidine is incorporated solely into newly formed DNA, and thus the presence of the labeled compound indicates ability of the cell to synthesize DNA and, presumably, to divide. The cells found were mononuclear. The number present in the normal human being is approximately 6 per cubic millimeter; the number is increased in some nonneoplastic[3, 4] and some neoplastic[3-6] diseases. In preliminary publications, it was shown that the number of these cells in the peripheral blood changes in both man[7] and dog[8] following whole body irradiation. In the present communication is presented the pattern of change in the number of circulating DNA synthesizing cells in human beings accidentally exposed to large doses of radiation from a critical assembly and in dogs exposed to different doses of x-radiation.

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REPOSITORY DOE - FORRESTAL
COLLECTION MARKEY FILES
BOX No. 3 of 6
FOLDER ACCIDENT FILE #1

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MATERIALS AND METHODS

Human Exposures.—As a result of an accident involving nuclear fission which occurred in the Y-12 plant of the Oak Ridge National Laboratory in June, 1958,[7] five men were exposed to doses of total body radiation so high that hospital care was considered advisable. Details of the accident, and the clinical findings in the exposed individuals have been described previously.[8-10] The radiation consisted of a mixture of neutrons and gamma rays, and the total doses ranged from 236 to 365 rads, of which approximately one fourth was due to fast neutrons.

In connection with other hematologic studies, blood was drawn from each subject from Days 3 to 141 for the present studies.

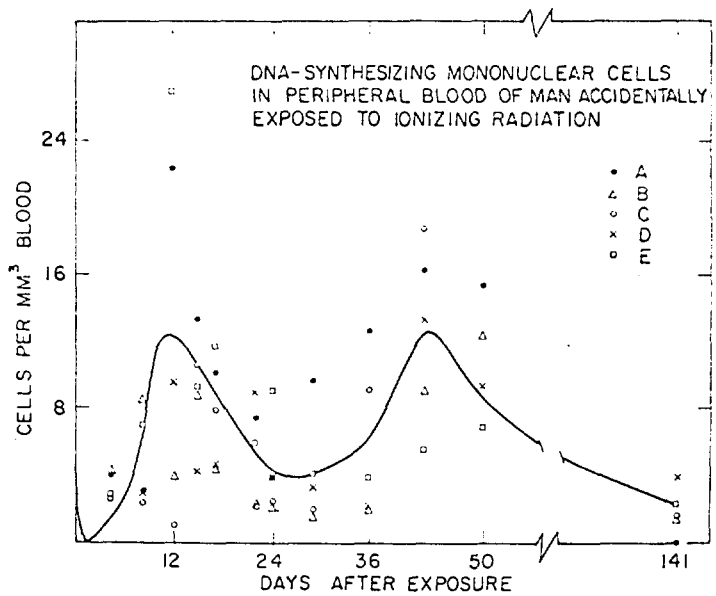

Fig. 1.—Sequence of changes in the number of labeled cells in the peripheral blood of human beings accidentally exposed to gamma and neutron radiations. All values on Day 3 were zero. The estimated total doses received, in rads, are as follows: *A*, 365; *B*, 270; *C*, 339; *D*, 327; *E*, 236.

Dog Exposures.—Four dogs were exposed to large doses of radiation as follows: Dogs 410 and 382 received bilateral radiation, 125 r from each side, totaling 250 r. Dog 368 received 350 r, unilaterally from the dorsal aspect. Dog 359 received 350 r, from the dorsal aspect, with the right hip and leg regions covered with ⅛ inch of lead (which reduces the dose below the shield to less than 3 per cent). Radiation factors were as follows: 250 KVP; 30 Ma.; filter 0.5 mm. copper, 1 mm. aluminum; TSD 110 cm.; machine output 21.2 per minute, measured at a position corresponding to the proximal skin surface of the animals. The doses given are those derived from the output dose rates at the proximal skin surface of the animal. The 250 r bilateral exposure is in the 30 day lethal range (one animal survived beyond 30 days; the other died on Day 17). The other two exposures would be considered less than, but approaching, the lethal range.

From all dogs, blood was drawn first hourly, then every few hours, and then at daily intervals for 16 days. From one dog (Dog 410, 250 r bilateral irradiation), occasional samples were taken from Days 17 to 30.

Labeling Technique.—The technique for in vitro labeling has been described previously.[1, 3] For both human beings and dogs, 4.5 ml. of blood was added to 0.5 ml. of stock solution containing tritiated thymidine, Na_2 EDTA, and dextran for acceleration of erythrocyte sedimentation. The final concentration of tritiated thymidine was 0.5 μc per milliliter, and the

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specific activity of the material was 1.9 c per millimole. The blood was incubated for 1 hour at room temperature, at which time smears were made from cell concentrates and fixed in methyl alcohol. Films were developed after a 15 day exposure period, and were stained through the film with Giemsa stain. A total of 5,000 nucleated cells was enumerated at each of the points for both man and dog. At the time blood was withdrawn for the thymidine uptake studies, blood counts were performed in order to allow conversion of per cent labeled cells into absolute numbers of labeled cells per cubic millimeter.

RESULTS

Man.—The sequence of changes in the number of labeled cells in the peripheral blood of the human accident cases is shown in Fig. 1. In the normal human being[1-3] the labeled cells number approximately 6 per cubic millimeter. In an occasional individual, none are detected; in others, as many as 20 per cubic millimeter have been found. The cells are mononuclear, and of the types shown in Fig. 2 (cells in Fig. 2 are from the exposed patients). Some labeled cells resemble monocytes, others, immature lymphocytes, atypical lymphocytes, and plasma cells (none are typical plasma cells). On the third postexposure day, when studies were first done by this technique, the number of cells had fallen from the normal value of approximately 6 per cubic millimeter to zero. A rapid rise began on Day 4, with the number of cells in the peripheral blood being greater than normal from Days 8 to 18 (approximately). There was a second rise to above normal beginning approximately Day 28, and the number of cells in the peripheral blood was not significantly different from normal when the patients were last studied by this technique on Day 141, or 4½ months after the accident.

Fig. 2.—Cell types labeled with tritiated thymidine in the peripheral blood of human beings exposed accidentally to gamma and neutron radiations.

All of the labeled cells observed were mononuclear in type, and were approximately 10 to 15 microns in diameter. Most of the cells observed are of the types shown in Fig. 2. The nuclei of many showed a very dense chromatin pattern and the thin rim of cytoplasm was characteristically basophilic. In some cells, a slight indentation of the nucleus was observed; in a few cells the indentation was more pronounced, and a parinuclear "Hof" was evident in the region of the indentation. Essentially all cells seen were of the types described previously.[3]

Dog.—The sequence of changes in the number of labeled cells in the peripheral blood of the dog is shown in Fig. 3. Normally, approximately 50 labeled cells per cubic millimeter are present in the peripheral blood. These are of the types described for the normal human being; however, the predominant cells resemble immature or atypical lymphocytes. Within minutes of exposure,

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the number of labeled cells increased to about double the normal value, followed by a very rapid decrease to virtually zero by the end of 24 hours. The number increased over a period of the next several days, then decreased, and perhaps began a secondary rise on approximately Day 16. In one dog given bilateral irradiation and observed beyond Day 16, a secondary rise occurred around Day 22, with a subsequent fall (data not shown in Fig. 3). The absolute numbers in the peripheral blood from approximately the second to the twelfth day were considerably lower in the animals that received bilateral irradiation than in the animals that received either whole body irradiation unilaterally, or unilateral irradiation with one hind limb shielded. However, the general shape of the curve was the same in all four animals.

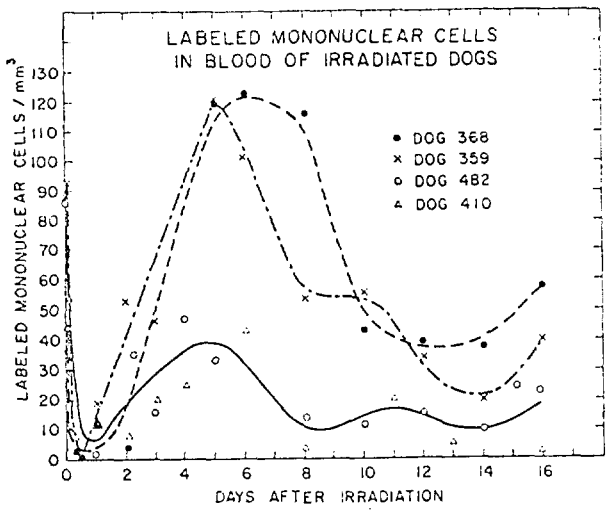

Fig. 3.—The sequence of change in the number of labeled cells in the peripheral blood of dogs exposed to 250 KVP x-radiation. Dogs 410 and 382 received 250 r bilaterally; Dog 368 received 350 r from the dorsal aspect; Dog 359 received 350 r from the dorsal aspect, with right hip and leg regions shielded.

The morphology of the labeled cells observed in the irradiated dogs was no different from that normally observed.

DISCUSSION

Although it is apparent from the data that considerable variation occurred from individual to individual, the changes in each subject were of a regular nature, and the average curves for both dog and man were similar. In both man and dog, the number of labeled cells had fallen drastically within at most a few days of exposure, and the dog data indicated that the fall occurs within the first 24 hours of exposure. In man, there were two subsequent elevations in the number of cells in the peripheral blood, which occurred on approximately the twelfth and fortieth days. A secondary rise occurred in one dog given bilateral irradiation and studied beyond 16 days. After apparently complete recovery the number of cells had returned to essentially normal values. These data indicate a definite pattern of change in the number of labeled cells following heavy exposure of both man and dog.

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The results are given in terms of the absolute number of labeled cells per cubic millimeter, independent of the precise cell type. Because of the small number of labeled cells involved, further breakdown into subcategories was not warranted. It would be valuable to know if only the number of labeled cells changed, or if the total number of morphologically similar cells, labeled or unlabeled, changed as well. Although accurate categorization is difficult in the stained filmed preparations, this was attempted in one animal (Dog 410). The numbers of morphologically similar, labeled and unlabeled cells changed in a parallel fashion, indicating that the differences following irradiation relate to all cells of that category and not only to those in DNA synthesis.

It has been shown that tritiated thymidine is incorporated in vivo and in vitro solely into DNA of cells that are in DNA synthesis and hence have the potential for further cell division.[11] It is thus assumed that the mononuclear blood cells found to be capable of incorporating tritiated thymidine have a proliferative potential, but that they do not necessarily divide within the blood stream. The origin, fate, and function of these cells and the reason for the pattern of change following irradiation are not clear.

It is not definitely established whether alterations in the number of labeled cells in the peripheral blood after exposure parallel changes in hemopoiesis or lymphopoiesis. In general, in man, for the dose ranges considered, the blood neutrophils may rise transiently within hours of exposure, and then fall gradually. The low point is reached in the third week or later, depending on dose, and recovery of survivors begins in approximately the fifth week. The lymphocyte count falls to the lowest values attained (20 to 40 per cent of normal) within 24 hours, and remains at this low value for some weeks before recovery begins. However, the curves of serial lymphocyte counts in both the Oak Ridge and Yugoslavian accident cases[7, 10, 12] indicate questionable rises around Days 15 and 40. The data on patients exposed in two Los Alamos accidents reveal no such changes.[13] From data on animals[14] and on the Oak Ridge patients,[7, 10] it is concluded that an "abortive rise" in peripheral blood leukocytes may occur some days after exposure. It is known[15] that the mitotic index of the bone marrow also rises after an initial fall, and that many of the mitotic figures are obviously abnormal at the time of this abortive rise. Thus the changes observed in the number of labeled blood cells resemble to some degree those seen in lymphopoiesis and medullary hematopoiesis, and it is not clear whether they are related to changes in either of these hematopoietic systems. It is of course possible that they are related to neither, and that the cells belong to a separate system with a different site of formation.

The curve following exposure of a dog with one limb shielded is similar in shape to that of the animal receiving the same dose of radiation, but without shielding of the limb. The same type of curve has been noted in patients receiving partial body exposure in the course of clinical radiotherapy.[6] The absolute values in the dog which received bilateral radiation were considerably less than those in either dog which received unilateral radiation. It should be noted that bilateral radiation is more effective than is unilateral radiation, and the

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"biologically effective" dose, in terms of potential mortality, was higher with the dogs receiving bilateral radiation than with either dog receiving unilateral radiation.

It is tempting to speculate that the changes in the numbers of labeled cells following irradiation may be related to marrow degeneration and regeneration, and that the cells may have a multipotential hematopoietic function. Some support for this thesis can be derived from the well-known fact that shielding of hematopoietic tissues under various conditions in the irradiated animal results in accelerated regeneration of the bone marrow and a decrease in mortality. This could result from possible transfer of a humoral substance or of cells capable of proliferation, neither of which possibility has been established. Support for the cell transfer hypothesis is seen in the fact that radiation protection has been obtained in mice by means of blood transfusions from animals with a leukemoid reaction.[16-18] Efforts in this laboratory to demonstrate proliferation of blood cells labeled with tritiated thymidine and transfused into normal or irradiated recipient animals have not been successful to date[2]; however, the data are not considered to be definitive evidence against such proliferation under some circumstances.

Labeled cells of the types described in the peripheral blood are observed in large numbers in pleural and peritoneal effusions of some cases[6] with which a direct association with hemopoiesis would not be expected. Some of the labeled cells have been demonstrated in this laboratory to be phagocytic. Thus it is possible that at least some of the labeled cells found in the blood may be concerned with inflammation and defense mechanisms, and their apparent proliferative potential may be related to an ability to provide rapidly a mechanism for building up local defenses.

SUMMARY

1. Mononuclear cells capable of deoxyribonucleic acid synthesis, as determined by use of tritiated thymidine and autoradiography, were found in the peripheral blood of five patients accidentally exposed to ionizing radiation, and in four x-irradiated dogs.

2. The absolute number of these labeled cells undergoes apparently characteristic changes in the postirradiation period, with two peaks above normal in the human being around Days 8 to 10 and 35 to 40.

3. Some grounds for relating the changes noted to changes in the lymphopoietic or the medullary hematopoietic system can be seen; however, the fate and function of these cells is not clear at present.

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