SOME EFFECTS OBSERVED IN THE PERIPHERAL BLOOD, BONE MARROW, SPLEEN, THYMUS AND LIVER OF Mc COLLEN RATS SUBJECTED TO GAMMA RADIATION AT THE IEAR-1

J. P. Silva and C. Barros
Instituto de Energia Atômica
São Paulo-Brazil

ABSTRACT

This paper describes effects of exposure, by gamma radiation, observed in the peripheral blood, bone marrow, spleen, thymus and liver of Mc Collen rats, using the technique of coloured smears by May-Grunwald-Giensa.

The rats were subjected in a IEAR-1 beam-hole, with the reactor not in operation (exposure to gamma radiation only). Most of the observations refer to rats subjected to acute full body exposure of 300 r. Besides the known quantitative alterations in the peripheral blood, qualitative modifications in some figurative elements were registered. In the smears of bone marrows, spleen and thymus, following the cell destruction phase, cell repopulation accrued as from the 5th and 6th days, in which altered chromatinic cells appeared, including giants and bi-nuclear cells. Evidence is borne concerning morphological alterations of the mitotic figures which leads to an abnormal distribution of chromosomes in the daughter-cells. It was verified that in the bone marrow the erythropoietic sector suffers more rapidly than the granulocytopoietic one, the recovery of the latter being slower besides presenting morphological alterations of a more lasting nature. Attention is drawn to the plasmocellular proliferation and to metaplasia erythrogranulocytopoietic sources in the spleen and liver.

Results on observations made of rats exposed to different conditions (300 r/30 minutes and 600 r/1 minute are presented.

BLOOD

The lesion suffered by the hemopoietic organs resulting from gamma radiation brings about more or less pronounced modifications in the cellular elements of the circulating blood. In order to accumulate data concerning said alterations following exposure of Mc Collen rats under diverse conditions, seven groups of rats were studied. The first comprised 8 non-subjected animals performing as witnesses. The remainder were subjected as follows:

Group I : Total of 8 non-irradiated witness rats.
" II : 76 rats subjected to 300r - exposure time: 1 minute
" III: 13 " " " " " " 30 "
" IV : 5 " " " " " " 60 "
" V : 8 " " " 600r " " 1 "

A description will follow of each celular type found in the blood covering the destruction, cytopenic and regeneration periods, as also the morphological alterations observed in the blood cells.

GROUP OF NON-IRRADIATED RATS

Lencocytes:

An average of 9800 lencocytes per cubic millimeter of blood was found (Graph I). The percentages of neutrophils and lymphocytes were of the order of 18.1 and 77.9% respectively. Eosinophiles represented 1.05% and no basophiles were found. Monocytes comprised 2.7% of the remainder of the blood cells (graphs 2 and 3).

Erythrocytes:

An average of 7.750.000 per cubic millimeter were found (Graph I).

Haemoglobin:

The average rate was 13.4 g/100 ml (graph 1), the values corresponding to those referred to in the literature (1, 2, 3, etc).

GROUP OF RATS SUBJECTED TO 300r - EXPOSURE TIME: 1 MINUTE

Leucocytes:

Examining the results arrived at in 59 animals, it is found that within the first two hours after irradiation, an increase existed in the number of white blood-cells, followed by a more or less regular fall-off up to 24 hours later. The fall-off started between the 4th and 6th hour being more pronounced on the 24th hour. From the second to the fifth day following exposure, the overall count of white cells remained at low levels with a trend toward normal values arrived at around the twenty first day. The observed results may be easily interpreted, recalling that the organism initially reacts to the effects of radiation, causing an afflux of white cells in the blood stream. Immediately, due to the lesion suffered by the haemathopoietic organs, a lower production of cells occurs with a resulting decrease in the number of same within the peripheral blood. It is observed that as from the second day (graph 4) a progressive increase of lencocytes occurs up to the twenty first day. On the other hand no very important leucocytosis was observed within the first hours after irradiation.

Neutrophils:

For the first six hours following irradiation, neutrophyls at low percentage levels were found (as can be seen in graphs 5 and 6), lower still than in the animals not subjected. From 24 to 28 hours after irradiation, a slight increase was found of neutrophils that undergo a slight numerical decrease followed by normalization between the 15th and 21st day.

Lymphocytes:

The lymphocytes increase in number practically within the period of 24 to 48 hours after irradiation, keeping at high levels up to the 4th day (graph 5). Similarly, a direct numerical diminution of neutrophils was observed. From there onwards values tend toward normalization. Some authors indicate inverse alterations, mainly a reduction of lymphocytes.

Monocytes:

No important numerical alterations were observed.

Eosinophiles and Basophiles:

A comparison established between the results obtained in the animals of this group and those of the first group (Tables 3, 4), indicates no expressive numerical alterations. In two animals, on the 15th and 21st day subsequent to irradiation, 5% and 7% of eosinophiles were found in each, corresponding to absolute values of 255 and 616 per cubic millimeters of circulating blood. Among the remaining animals, the eosinophile rates varied from 0% to 3%.

Erythrocytes:

No significant variations in the erythrocyte rate was observed. In the animals sacrificed 4 hours after exposure, the counting average of erythrocytes was of 7.360.000/mm^3. Approximately on the fourth day erythrocytes values were found: average 6.350.000/mm^3. In a general sense, at the end of the 15th to the 21st day, the number of erythrocytes returned to normal. No pronounced anaemia was observed around the 15th day as indicated by some authors.

Haemoglobin:

The haemoglobin dosages showed a strong similarity with the countings of the erythrocytes (Graph 4). In a rat sacrificed following 24 hours of irradiation, a rate of 5.2 g/100ml of haemoglobin (for 3.820.000 hemacites per cubic millimeter) was found: the animal showed multiple abcesses on its lungs and liver.

GROUP OF RATS SUBJECTED TO 300r - 30 MINUTES

Lencocytes:

A strong reduction in the number of lencocytes was observed, 3 hours after irradiation, followed by a drop at the end of the 4th hour and with persistent declines throughout the 24 hours, attaining the lowest values on the 4th day (Graph 7). As from the 8th day the number of leucocytes commenced to increase. Attention was drawn to the difference in the results observed in this group and in the previous one, since the irradiation conditions were not basically dissimilar.

Neutrophils:

The initial number were somewhat higher than those observed in the control group. On the 4th day there was a fall tending toward normalization after the 8th (Graphs 8 and 9). The existing impression is that between the 3rd, 4th and 24th hour neutrophilia accrued (Graphs 8 and 9).

Lymphocytes:

Results are similar to those found within the first 24 hours in relation to neutrophiles as shown in graphs 8 and 9. For the period covered the neutrophile and lymphocyte rate was to all intents the same. On the 4th day an inversion was verified, i.e. as the neutrophiles decreased, the lymphocytes increased. On the 8th day, the initial parallel position and trend to normalization was found.

Monocytes, Eosinophiles, Basophiles and Erythrocytes:

Similar results to those in the previous animal group were observed (Graphs 8 and 9). In the case of erythrocytes, on the 8th day a certain tendency to decrease was verified, although not a significant one.

Haemoglobin:

Nothing differing from the aforementioned was observed in relation to the previous group (Graph 7).

GROUP OF RATS SUBJECTED TO 300r - 60 MINUTES

Leucocytes:

A more pronounced fall off than in the previous animal group was observed within the first 24 hours. On the 4th day an increase occurred and on the 8th there was a slight tendency for the leucocyte rate to fall off. The referred increase on the fourth day was not observed in the previous group (Graph 10).

Neutrophils and Lymphocytes:

Results are shown in diagrams 11 and 12. In so much as neutrophiles increase, lymphocytes fall off and vice versa. In 24

hours the neutrophils prevail over the lymphocytes and the prevalence observed in this group is more pronounced than in the former one. An expressive difference exists in what was observed between this group and the second one.

Monocytes, Eosinophiles and Basophiles. Erythrocytes and Haemoglobin

No significant alterations were observed in relation to the previous groups. A total disappearance of eosinophiles and Basophiles from the peripheral blood occurred. The erythrocytes and the haemoglobin did not furnish any differing indications.

GROUP OF RATS SUBJECTED TO 600r - 1 MINUTE

In this group only the constituting elements of the white series were analysed.

Neutrophils:

The inversion registered in the two preceding groups were verified. In the initial hours a fall off of amphophiles occurred up to the fourth hour, followed by an increase between the 6th and 24th hour; fall off 48 hours later and a tendency toward rate normalization after 4 days.

Lymphocites:

The values relative to the lymphocites followed an inverse curve to that of the neutrophils.

Monocytes, Eosinophiles and Basophiles:

The monocytes showed no alterations worth registering. Eosinophiles were present in the blood of 5 of the 8 rats while no basophiles were observed.

OTHER ALTERATIONS

Blood Platelets:

In all the irradiated rats a constant and considerable increase in the number of blood platelets were observed. In no case was thrombocytopenia verified, in opposition to what some authors have reported.

Lymphocites with bilobed nuclei:

In all the groups of irradiated animals lymphocites with bilobed nuclei were observed. It was also observed in the blood of a non-irradiated rat.

Cytological modifications:

Structural modifications were observed in the neutrophils eosinophils and lymphocytes, characterized by vacuolization pykuosis, karyorrhexis and karyolysis. Vacualization was also observed in the cytoplasm as well as the dyeing capacity decrease of lymphocytes, fragmented nuclei neutrophils and eosinophils, but cytoplasm evidencing normal granulations.

Anisocytosis with macrocytic and microcytic erythrocytes were found to be rare.

At the same time basophilia, lymphocytes and monocytes atipicals were seldom verified.

SPLEEN, THYMUS, BONE MARROW AND LIVER SMEARS

Smears were coloured by the May-Grunwald-Giensa technique. In the spleen smears of non-subjected rats histological elements of the organ were found. In the tymus smears, the same histological components were found although in differing proportions. At times, due to the abundance of the thymocytes, observation of stroma cells, the Nassal corpuscle and the connective tissue grow less frequent.

Bone marrow smears were obtained from material collected from the right femur. Dealing with young animals, collection presents no special difficulties.

In liver smears all the component cells of the histological structure were found: however, the cell borders were not easily distinguishable as if the very thin membrane were non-existent.

CELL DESTRUCTION AND ALTERATIONS

In all the animals sacrificed, even as little as one hour after irradiation, cell destruction and alterations were observed in the spleen, thymus and bone-marrow, with differing intensity however. The thymus is affected first, followed by the spleen and bone marrow. The destruction is of an intensity such that to obtain the rich and abundant material for the elaboration of smears is difficult. In the liver, 24 hours after exposure, an aspect of hydropic degeneration was observed with a distinct cell border.

The cells of the haemolympho-reticulopoietic and caracteristic organs show high sensitivity to radiation in face of their proliferating and maturing activities. The staminal cells, to ensure the uniformity of the blood mixture, perform a polymorphus activity. Mitoses are particularly performed in various stages of cell differentiation. In the liver mitosis figures are rare.

The following microphotographs illustrate the types of cell lesions found in the cytoplasm and nucleus in the spleen, thymus and bone marrow smears of the irradiated animals. Damages are frequently found of cytoplamatic vacuolization, picnoses, Karyorrhexis, karyolysis, anomalous reactions to the acid, basic and neutral dyes (both in the nucleus and cytoplasm).

Cell detritus are abundant in all spleen, thymus and bone marrow preparations. Fagocytosis figures are frequent in spleen and bone marrow smears elaborated from material collected after 48 hours of exposure; in thymus smears they are less frequent and rare in liver smears. Cell destruction in the spleen and thymus is of an intensity such that the smears reveal almost only stroma cells.

From a perfunctory examination of the preparations, an increase of the cell volume as also of cytoplasmatic basophilia is observed; giants cells appear, bi, tri and poly-nuclear. The marrow is inhibited and hypoplasiant. In the liver picnotic nuclear and frequently bi-nuclear cells were found.

Around the second day signs appear indicating the initiation of the recuperation process. In that meantime the reabsorbtion and fagocytoses of cell detritus can be observed, mainly in the spleen and bone marrow, principally due to stroma cells.

MITOTIC FIGURES

The utilization of the May-Grunwald-Giensa technique also allows the observation of mitotic figure aspects. It is known that radiation determines a temporary ceasing of mitoses in the inter-phase period. The cells that were in full mitoses and were not destroyed, continue there. In quantities inferior to normal, are found prophase, metaphase, anaphase, and telophase mitotic figures in the bone-marrow, thymus and spleen, even in the cell destruction period. That reduction is already noticeable in the preparations obtained of animals sacrificed one hour after irradiation. Six hours later they commence to increase. Bi, tri and polynuclear formations without cytoplasm division are found, even in the terminal stages of mitoses, thereby giving origin to heterophoid cells.

Alterations occur in the chromosomic structures, giving rise to an unequal distribution of chromatin to the daughter cells with abnormal arrangement of the chromosomes and originating a generation of abnormal cells that continues to survive and multiply. This fact was observed in the thymus, spleen and bone marrow smears from the start of the recuperation phase. Such alterations were being reproduced in the daughter cells 30 days following irradiation (time-limit of our observations). The microphotographs show mitotic figures at different stages with the most differing morphological aspects, at times creating chromosomic bridges obstructing cell division, as also with abnormal distribution in the aster and multipolar mitoses. The abnormal aspects of chromatin colourability are more evident in cells with abnormal mitotic figures.

RECUPERATION

As from the second day, signs of recuperation are already apparent. From the fourth day onward the restoring process of the spleen, thymus and bone marrow is pronounced and is not achieved

solely at the cost of the more primitive cells that were undamaged but by the stroma cells and principally by them. On the fourth day, the white spleen pulp is already quite restored. The fibrous connective tissue cells that in normal animals show up as authentic fibrocytes, now appear in groups taking on the aspect of histiocytes. The cytoplasmatic basophilia is discreetly increased with the fortuitous appearance of small azurophile granulations. The connective tissue appears with more looseness, richer in fundamental substance and the reconversion in histiocytes allows for its participation in the stroma restructure. Within 15 days the connective tissue cells reacquire their normal aspect. The stroma cells that appeared in the form of macrophagus return to primitive conditions. Between the fourth and tenth day, they frequently exhibit aspects of haemopoietic differentiation, i.e., in a lymphoblastic, myeloblastic and, more rarely, a megakaryoblastic sense. In the liver, the Kupffer cells at times appear as macrophagus or show myelocytic differentiation or the Ferrata paradoxal phenomenon. At times, real haemopoietic metaplasia sources are found.

The major position of mesenchymatose cells existing in the bone marrow and spleen, in relation to that occuring in the thymus, should contribute to a speedier recuperation of those organs. Nevertheless, the recuperation of these organs is fulfilled at the same rythm.

In the spleen, as from the 4th day, the haemocytoblasts deriving from rare remnants and from the limphoblastic differentiation of cells of mesenchymatose potentiality intensify the mitotic rythm giving origin to numerous lymphoblasts, prolymphocytes and lymphocytes that, little by little, repopulate the organ. At this stage, binuclear and some giant cells are found, the altered chromatinic ones being numerous. The cytological picture of the spleen is one that would be equivalent to the liberation of the fundamental capacities of Leukon that is revealed in the peripheral blood resulting from the progressive return to normality.

In the thymus smears of irradiated animals in the recuperation phase, stroma epithelial and Hassall corpuscle cells are found, and rare thymocytes and thymoblasts. The connective tissue

aspect is similar to that of the spleen, although neither polimorphus haemopoietic differentiation, nor metaplasia was observed. After 15 days, rare neutrophile myelocytes and basophile granulocytes were found. Intermediary figures between the stroma cells and the thymoblast were also observed. The organ that initially showed macroscopic atrophy, progressively recovered returning to normal size.

In relation to the bone marrow, it is interesting to note that the involutive process attains its climax 24 to 48 hours following irradiation. Within that period, the yellow marrow was undergoing a gelatinous degeneration process, thus rendering it easier to obtain the smears, the latter less rich in cells however. In the meantime, six hours after irradiation, it was verified that the basophile erythroblasts were very scarce. The intermediary forms disappeared, only scant erythroblasts and some orthochromatic cells remaining. Anomalous figures are rare notwithstanding binuclear cells being common. From the 6th day onward, the marrow again appears rich in crythroblasts, becoming exhuberant after the 15th day. The mean life of the rat enythiocytes being of the order of 40 days justifies the relatively tardy appearance of small scale anaemia, despite the intensity of the marrow lesions. Fast recovery of the Erythron functionality also contributes towards this fact.

The granulocytopoietic sector is less sensitive than the erythroblastic one, yet its lesions are more severe as a result of much slower recovery. The preparations even up to the 30th day reveal great cell polymorphism. Within this sector changes in the chromatin contents and tingibility alterations are more frequent. Modifications can be observed in the microphotographs of cell sizes, cytoplasmatic basophilia, of the contents and aspect of the specific and unspecific granulations and even of the neuclo-cytoplasmatic maturity disproportion and of the profound alterations in cell maturing giving origin to polymorphus nucleus and bizarre segmentation.

The marrow bone that up to the end of the destructive phase shows only a few myeloblasts and granulocytes gets progressively enriched in component evoluted cells of each of the granulous leucocytarious types. The eosinophiles at times increase, at others decrease. The basophiles always increase in an unusual manner in relation to the rest. In opposition to what some authors sustain, no

remarkable alterations in megakaryoblasts and megakaryocytes were verified. The staminal cells, essentially represented by haemohistioblasts and some haemocytoblasts (the latter infrequent in the normal marrow of rats), increase in volume and at the same time in cytoplasmatic basophilia. In the recuperation phase, the finding of the Ferrata paradoxal phenomena and myelocytic differentiation was frequent by means of haemohistioblasts: it is to be noted that prior to the myelocytic differentiation appearing, they showed up rich in azurophile granulations. Macrophagus are frequent but rarely haemosiderim pigment.

GRAPHS

1 - Peripheral blood cells, showing morphological and tingibility alterations caused by gamma radiation. Note in third row, pictures 2 and 3 - neutrophils alterations in bone marrow forerunners of those found in the peripheral blood. In last row, binuclear lymphocyte and lymphocyte in karyorrhexis.

2 - Note morphologic and quantitative alterations produced in cells in mitosis of rat bone marrow in complete recuperation phase after full body radiation. Observe chromosome fragmentation, bridge formation and disarrangement of same in several moments of the mitotic process.

3 & 4 - As in Graph 2, note mitotic multipolarity and giant cell formation - Magakaryocytes.

5 - Note additive cell nest of fibrous trunk and different aspects of liver cells following full body gamma radiation. Verify binuclear and trinuclear cells, vacuolized aspect of cytoplasm and ergastoplasm distribution.

6 - Observe in microphotograph, top and left, numerous cell remains following radiation; in the other photographs, intense phagocytotic activity of bone marrow and spleen reticular cells.

7 - Microphotographs above normal bone marrow of rat and in the others, different aspects of additive tissue during recuperation phase and cell in telophasis in spleen.

8, 9 & 10 - Four Kupffer cells of irradiated animals, thymus and bone marrow aspects during destruction phase and repopulation initiation of thymus and spleen, showing the increase of plasmazellen, stroma cells and cells in mitosis.

REFERENCES

1 - Wills, J. and Nehta, N.H. - Determination of normal blood standards, for the meditional laboratory's shocks albino rat. Indian Journ. Med. Res. 18, 307, 1930-31.

2 - Scarborough, H.A. - The blood ficture of normal laboratory animals. Yale Journ. Biol. and Med. 3: 63, 168, 282, 359, 431, 547, 1931.

3 - Wintrobe, M.M.; Shumacker, H.B. Jr. & Schmidt, W.J. - Valves for number, size and hemoglobina cont. of erythrocytes in normal doze, rabbits and rats. Amer. Jour. Physiol. - 114: 502, 1936.

4 - Cameron, D.G. & Watson, G.N. - The blood counts of tehe adult albino rat. Blood 4: 816, 1949.

5 - Errera, M. & Forsiberg - 1961 - Mechanics in Radiobiology Sc. Press - N.Y. & London.

6 - Miale, J.B. - 1962 - Laboratory Medicine - Hematology. C.V. Mosby Company.

7 - Report of the United Nations Scientific Committee on the effects of Atomic Radiation - Sup.Nº16 (A/5216) United Nations.

8 - Sodeman, W. - 1961 - Pathologic Physiology - W.B. Saunders Comp.

9 - Blair, H.A. - 1954 - Biological effects of external radiation McGraw - Hill Brook Comp. - N.Y.

10 - Hollaender, A. - 1954 - Radiation Biology. McGraw - Hill Brook Comp. - N.Y.

11 - Zirkle, R. - 1954 - Biological effects of external x and gamma radiation. McGraw Hill Brook Comp. - N.Y.

12 - Bloom, W. - 1958 - Histopathology of irradiation from external and internal sources - McGraw - N.Y.

13 - Wintrobe M. - 1962 - Clinical hematology - Lea & Febiger - Phil.

14 - Bloom, W. and Fawcett D. - 1962 - A textbook of Histology. W.B. Sanders Comp. Phil.