

SOME HEMATOLOGIC EFFECTS OF IRRADIATION*

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DURING our study of the cellular changes in mammals exposed to external and internal sources of ionizing radiations, evidence was sought for correctness of the idea that small doses of radiations stimulate cellular activity and multiplication. Although this idea is held by a number of radiologists, little basis for it is found in the radiologic literature other than misunderstanding and misquotation of a few important papers. Some of our experiments offered unusual opportunities for a consideration of the question of stimulation. Thus, in those experiments in which there were progressive decreases in the amount of irradiation and in the reactions to focalized irradiation, one could expect to find evidence of stimulating effects if they actually occurred. But our findings in the organs and peripheral blood have been so consistently at variance with the idea of stimulation by small amounts of ionizing radiation that it seems desirable to summarize these observations.

Some of the confusion on this question undoubtedly originates from a loose use of the word stimulation. In the following discussion we shall discriminate between a primary stimulation which results directly in cellular hyperplasia, hypertrophy, or hyperactivity after irradiation, without a stage of obvious previous injury, and a secondary stimulation which might be considered as a reparative process resulting from the necrotizing action of radiation.

MATERIAL AND METHODS

The radiations which were employed in these studies on animals and man fall essentially into two main categories. (1) Externally originating total body irradiation from x, γ rays, fast and slow neutrons. (2) Focal irradiation by externally originating B rays and radioactive isotopes administered enterally or parenterally.

1. *Total body irradiation.* The organs of large numbers of rabbits, rats, and mice and those from a smaller number of guinea pigs were obtained for histologic study when the animals were sacrificed at intervals after varying dosages of x and γ rays, fast and slow neutrons. The general plan of these experiments was to start with the LD 50/30 days dose of each agent and to decrease it until no histologic or hematologic changes were observed. In most of the series the total amount of irradiation was given at one exposure, although there were several large and important series in which small amounts of radiation were given repeatedly.

We found it necessary to carry out these experiments for histologic purposes by killing the animals at planned intervals because cytologic examinations of animals

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which were found dead were worthless. The series was supplemented with the study of a number of moribund animals.

2. *Focal irradiation.* This occurred in the spleens and testes of a few mice given large amounts of irradiation by B rays from an external source of P^{32} . However, the great mass of our experiments with focalized irradiation effects occurred after the intravenous or intraperitoneal injection of radioactive isotopes. In a few series these materials were given intramuscularly or by inhalation. Among the elements studied were the α (Alpha) emitters Ra and Pu, the β emitters P^{32} , Sr^{89} , Y^{91} , and the β and γ (Gamma) emitters Zr^{90} , Ba^{140} , La^{140} , Na^{24} , and the degradation products of Ra. These isotopes were given in dosages starting with the LD 50/30 days and in diminishing fractions thereof.

The tissues were routinely fixed in Zenker-formol, imbedded in nitrocellulose and stained with hematoxylin-eosin-azure II, except for those portions of the tissues which were fixed in alcohol so that auto-radiographs could be prepared.

Whenever possible, studies of the peripheral blood were conducted on the same series of animals in which sacrifices were being made for cytologic study. Where this was not possible, groups of animals were studied in parallel series; in still other experiments, blood counts were made on the animal at the time of sacrifice in an attempt to correlate the peripheral hematologic picture with effects on the blood forming organs. Sampling of peripheral blood was done at intervals varying with the chronicity of the experiment. In all cases, the techniques employed were standard hematologic procedures.

From the standpoint of the hematologic studies, the experiments of most significance were: (1) those in which an acute single dose of penetrating total body radiation was given (x-rays or fast neutrons) and (2) the experiments in which rats, guinea pigs, mice, and rabbits were exposed chronically to γ rays or x-rays. In one of the chronic gamma ray exposure experiments, the radiation was given daily over an eight hour period; and in another set of experiments, the γ radiation was given twenty-four hours per day. In the chronic x-ray experiments, the daily exposure was given within a few minutes. The daily exposure of these animals, whether exposed to gamma rays or x-rays, extended over long periods up to four years.

OBSERVATIONS

I. CHANGES AFTER A WIDE RANGE OF DOSES OF TOTAL BODY IRRADIATION FROM EXTERNAL SOURCES

One of the effects of total body external irradiation in doses not higher than the LD 50/30 days is to separate those organs which are sensitive to this amount of irradiation and those which are not. In the laboratory mammals which were studied with this dosage, the radiosensitive organs are the gonads, bone marrow, spleen, lymph nodes, thymus, parts of the gastrointestinal tract, skin and bone. Nerve, muscle, and most exocrine and endocrine glands are resistant.¹

It was found that all of the external ionizing radiations in equivalent doses produced similar effects.¹ If the animals had been given the LD 50/30 days dose of x-rays, or γ rays, or fast or slow neutrons, the changes which resulted could not be

distinguished from one another. That is, in examining sections of the organs of these animals, we could not tell which ionizing radiation had been used. With this amount of irradiation the very first changes observed, usually within the first hour, were degenerative changes and death of cells in the blood forming organs, bases of the intestinal crypts and foveollae of the stomach, spermatogonia, and ovarian follicular epithelium or developing ova, and early mild inflammation (mainly edema) of skin.

Mitoses were usually gone by the second hour. There was no evidence of an increase in mitosis. In rabbit bone marrow after 100r of total body x-irradiation, mitoses began to be present again after three hours. They continued through the fourteen hour interval at a level slightly above that seen in controls. At the eight hour stage some of the mitoses were abnormal. During the next hours, the debris increased in amount and was gradually phagocytized. With an LD 50 dose after a few days the blood forming organs became markedly reduced in size and the bone marrow and lymph nodes became aplastic—often completely so.

In the course of one to two weeks the blood forming organs gradually became actively hematopoietic again. Only rarely did this process reach or exceed normal in the bone marrow. Several months usually elapsed before the lymph nodes, thymus, and spleen returned completely to normal.

With progressively smaller doses the degenerative effects were less marked. They became minimal at 50r of x-rays and were just detectible in our animals at 25r of x-irradiation or the equivalent fraction of the LD 50/30 days dose of neutrons. With doses lower than 25r, no changes were found; in animals so treated there was no evidence whatever for either an injurious or stimulating effect.

In the regeneration after external irradiation the only instances of overcompensation of tissue was in bone marrow of rabbits which survived the LD 50/30 days. In them the marrow seemed hyperplastic (both erythropoietic and myelopoietic within two months after exposure.)

After the administration of single doses of either x-rays or fast neutrons to rabbits, mice, and rats, an increase in circulating heterophil-leukocytes occurred within the first twenty-four hours with doses up to and beyond LD 50/30 days. In fact, with doses of 500 and 800r in the rabbit Jacobson et al.² noted that two definite statistically significant peaks occurred at circa twelve and twenty-four hours. After doses of this magnitude, a leukopenia invariably followed the heterophil leukocytosis. With doses of 300r and below, only a modest elevation in the number of circulating heterophils occurred and this at about twelve hours. Control animals handled in a comparable manner (except for the actual exposure to irradiation) also had this latter modest peak increase after about twelve hours. Heterophils are the only circulating cells which are initially increased in number after acute total body radiation. Lymphocytes, monocytes, eosinophils, reticulocytes, and platelets are reduced.

As recovery occurs in the hematopoietic organs, however, a "compensatory increase" above normal control values was not uncommonly encountered, particularly for the heterophils and reticulocytes. No significant absolute lymphocyte increase on a compensatory basis was encountered in experiments in which doses

of 800r to 5r acute total body X radiation were administered. In fact, lymphocyte values in the peripheral blood of the rabbit are reduced over a period of ninety days after an acute exposure to 800r. This is in agreement with the slow return of the lymph nodes to normal after irradiation.

After acute doses of x-rays, or fast neutrons Jacobson et al.^{2, 3} observed an "abortive rise" in lymphocytes, heterophils, and reticulocytes in rabbits between the fourth and twelfth day after irradiation. This rise may possibly represent an abnormal stimulation in the sense that certain precursors are sufficiently altered to produce a limited succession of abnormal progeny.

Chronic Radiation Experiments

Chronic exposure of groups of rabbits, mice, and guinea pigs to γ rays in daily doses of 0.11; 1.1; 2.2; 4.4; 8.8 γ for eight hours per day or twenty-four hours per day over periods extending beyond three years carried out by Lorenz et al.⁴ has not shown evidence of a stimulating effect on the blood forming tissue which was reflected in the hematologic constituents of the peripheral blood. Similar experiments were conducted with chronic daily exposure of rats to x-radiation, but different in that the daily exposures required only a few minutes.⁵ No evidence of "stimulation" was apparent in the peripheral blood of these animals. In those instances in which an effect occurred, it was invariably a reduction in the number of circulating cells.

No deliberate or well controlled human experiments have been done which are comparable in chronicity to these animal experiments. The experiments of Low-Beer and Stone,⁶ and Nickson, Cantril, and Jacobson,⁷ however, in which human subjects were exposed up to a total dose of 300r total body given in divided doses of from 5 to 20r (x-ray) produced a general reduction in the various hematologic constituents of the peripheral blood. In several cases studied by Low-Beer and Stone,⁶ however, an absolute monocytosis became apparent reaching in several instances more than 50 per cent of the circulating leukocytes. It has not occurred in the human cases we have studied nor has it been seen in the many animal experiments referred to above.

2. EFFECTS OF FOCALIZED IRRADIATION

In mice exposed to large amounts of β rays from plaques containing P^{32} there was some focalized damage in several organs adjacent to the skin. Changes were very marked in ovaries and testes. But the changes which are of interest in this communication occurred in the spleens of a few of the animals. On the dorsal surface of the spleen there was a zone of severe radiation damage. This zone gradually merged into normal spleen, the gradation consisting of progressively diminishing damage without any evidence of a zone of hyperplasia of undamaged cells. Since the β rays have only a limited range of penetration, it would be expected that near the periphery of their range the small amount of radiation would evoke a stimulating effect if such an effect does occur.

This lack of a stimulating effect at the periphery of an area of focalized radiation is also characteristic of the changes which occur after localized deposition of radioactive isotopes. When these isotopes accumulate in given areas in radiosens-

sitive organs, they produce localized areas of radiation damage—much more extensive with β emitters than with α emitters because of the longer range of the former. The penetration of alpha rays is only a fraction of a millimeter in tissue whereas the β ray penetrates several millimeters. In or near none of these areas of focalized damage is there any evidence of cellular stimulation as evidenced by hyperplasia on normal cells.

With the exception of the highly diffusible Na^{24} , the majority of the isotopes studied tended to localize in bone and most of them also accumulated in the red pulp of the spleen and in other organs. This resulted in a marked hypoplasia and aplasia of the bone marrow. As a consequence, most of the spleens of these animals showed a great increase in ectopic myelopoiesis over the normal. This obviously is in compensation for the aplastic bone marrow. In no instance was a primary stimulation produced in the blood forming tissue which reflected an increase in circulating cells. Compensatory increases were, however, noted in a few instances. The radioisotope, Sr^{89} , is fairly generally distributed to blood forming tissue within the first few days after its parenteral administration. It produces a reduction almost immediately in the nucleated cells of the peripheral blood. It translocates, however, to bone and, therefore, exerts its major effect on the bone and bone marrow. The lymphocytes, however, remain depressed for long periods even though normal lymphopoiesis is resumed in the lymph nodes and spleen. This phenomenon occurs with other isotopes which are not localized more than temporarily in lymphatic tissue.

In some of the rats to which Sr^{89} was administered osteogenic sarcomas developed eight to ten months later. This might be considered as a "stimulation."

DISCUSSION

It is clear from the histologic examination of our animals that there is no evidence of a primary stimulation of hematopoiesis by small amounts of irradiation from either generalized external, or focalized internal sources. The changes in the cells of the circulating blood after irradiation point to the same conclusion, although the analysis is complicated by factors of mobilization and localization in the various parts of the circulatory system. The heterophil increase which follows rather large acute exposures to penetrating radiations is, according to Isaacs,⁸ a hastened maturation of heterophil precursors and thus a "stimulation."

The mechanism of the spectacular monocytosis described by Low-Beer and Stone⁷ in certain of the humans exposed to divided doses of total body x-irradiation is not clear. Since it occurred only in individuals with degenerative arthritis, it may be a response associated with the underlying disease process.

Irradiation of the hematopoietic organs as reflected in changes in the peripheral blood is often stated in the literature to produce stimulating effects. The articles most frequently referred to in this connection are those of Murphy and his co-workers.⁹ Actually these authors stated, "We have further noted that by one small dose of x-ray we could obtain in a certain proportion of animals a stimulation of the lymphoid elements, preceded by a comparatively short period in which the lymphocytes were below normal."

Other statements are found in the literature which have affected the conception that x-rays produce a stimulating effect on blood forming tissue. These effects, however, have been described after large doses of penetrating x-irradiation. An increase in the erythrocytes per cu. mm. and hemoglobin in Gm. per 100 ml.,¹⁰ an increase in the platelets,^{11, 12} reticulocytes,^{13, 14} and polymorphonuclear neutrophils¹⁵ have been described by a number of authors. These and other papers on this subject, however, generally dealt with inadequate numbers of control and experimental animals and, therefore, deny statistical validation. Reports are also found in the literature describing the findings in the peripheral blood of man after chronic exposure to ionizing radiation. These reports stress the significance of a lymphocytosis,¹⁶ monocytosis,¹⁷ eosinophilia,^{18, 19} leukopenia,^{19, 20, 21} anemia either normocytic or macrocytic.^{22, 23} These reports have tended to instill a sense of security in those working with radiation because of the implication that peripheral blood findings could be used as an indication of effect on a given individual even with radiations in the "tolerance range" (0.1r total body exposure per day). There is no work to substantiate this. As significant as many of these studies have been, the re-evaluation of these concepts is indicated at this time because of studies conducted on the Plutonium Project in the past few years.

Our findings and interpretations are in accord with the general conclusions of Czepa²⁴ and Packard.²⁵ As the latter points out: "The evidence now at hand points to the conclusion that radiations do not directly stimulate normal activities of the cells; their primary effect is always an injury from which the cell may recover perfectly. But the degeneration products may temporarily quicken the tempo of some normal processes, such as protoplasmic streaming and mitosis, an acceleration which is followed by a retardation and often by very obvious injury. Such reaction is secondary, and is not true stimulation in the sense in which the term is used in radiological literature."

CONCLUSIONS

Extensive studies with acute and chronic application of externally originating ionizing radiations and internally deposited radioisotopes have failed to reveal evidence in the blood forming tissues and peripheral blood of a primary stimulation of hematopoiesis. However, secondary or "compensatory increases" in certain of the cellular constituents of the peripheral blood were seen and were invariably preceded by a reduction. The initial leukocytosis (heterophil increase) which occurs in the first twenty-four hours after acute exposure to externally originating irradiations is probably a reaction to injury mediated through a mobilization rather than a new formation of blood cells. The abortive rise in heterophils, lymphocytes, and reticulocytes is likewise probably a result of frank injury.

REFERENCES

- ¹Histopathology of Irradiation from External and Internal Sources. Edited by William Bloom. NNES (mpts) vol. 22 I.
- ²JACOBSON, L. O., MARKS, E. K., SIMMONS, E., HAGEN, C., AND ZIRKLE, R. E.: Effects of X rays on Rabbits. NNES (mpts) vol. 22B.
- ³—, AND —: Hematological Effects Produced in Rabbits by Cyclotron Neutrons. *Ibid.*

- ⁴LORENZ, E., HESTON, W., JACOBSON, L. O., ESCHENBRENNER, A., SHIMPKIN, M. AND DERINGER, M.: Biological Effects of Long Continued Whole Body Irradiation by Gamma Rays in Mice, Guinea Pigs, and Rabbits. *Ibid.*
- ⁵STEARNER, P., SIMMONS, E., AND JACOBSON, L. O.: The Effects of Total Body X irradiation on the Peripheral Blood and Blood Forming Tissues of the Rat. Unpublished data.
- ⁶LOW-BEER, B. V. A., AND STONE, R. S.: Studies on Total Irradiation. NNES (mpts) vol. 20.
- ⁷NICKSON, J. J., CANTRILL, S. T., AND JACOBSON, L. O.: Blood changes in humans following total body irradiation. Unpublished data.
- ⁸ISAACS, S.: The relation of cell types in leukemia to sensitivity to radiation. *Fol. Hemat.* 52: 414, 1934.
- ⁹MURPHY, J. B.: The Lymphocyte in Resistance to Tissue Grafting. Monograph 21, Rockefeller Institute for Medical Research, 1926.
- NAKAHARA, W., AND MURPHY, J. B.: Changes in the lymphoid organs after small doses of X rays. *J. Exper. Med.* 29: 83, 1919.
- TAYLOR, H. D., WITHERBEE, W. D., AND MURPHY, J. B.: Studies on X ray effects. II. Stimulative action on the lymphocytes. *J. Exper. Med.* 29: 75, 1919.
- THOMAS, M. M., TAYLOR, H. D., AND WITHERBEE, W. D.: Studies on X ray effects. II. Stimulative: action of the lymphocytes. *J. Exper. Med.* 29: 75, 1919.
- ¹⁰SEGEL, P. W.: Die Veraenderungen des Blutbildes nach gynaekologischen Roentgen- Radium- und Mesothoriumbestrahlung und Ihre Klinische Bedeutung. *Strahlentherapie* 11: 1, 1920.
- ¹¹DUKE, W. W.: Variations in the platelet count. *J.A.M.A.* 65: 1600, 1915.
- ¹²BUCKY, T., AND GUGGENHEIMER, H.: Steigerung der Knochenmarksfunktion durch Roentgenreizdosen. *Berl. klin. Wchschr.* 1: 11, 1922.
- ¹³MARDERSTEIG, K.: Der Einfluss der therapeutischen Roentgenbestrahlungen auf die Reticulozyten. *Strahlentherapie* 59: 609, 1937.
- ¹⁴MOLDAWSKY, J. W.: Ueber den Einfluss der Berufsarbeit der Radiologe auf die erythropoetische Funktion des Knochenmarkes. *Fol. Hemat.* 36: 145, 1928.
- ¹⁵AUBERTIN, C., AND BEAUJARD, E.: Action des rayons sur le sang et les organes hematopoietiques. *Compt. rend. Soc. de biol.* 58: 217, 1905.
- ¹⁶RUSS, S.: Some contrasts in the effects of X rays and radium upon the blood cells. *Brit. M. J.* 2: 268, 1921.
- ¹⁷WETTERER, J.: Handbuch der Roentgen und Radium Therapie. Leipzig-Muench. Vol. 1, 1922.
- ¹⁸GOODFELLOW, D. R.: Leukocytic variations in radium workers. *Brit. J. Radiol.* 8: 669, 1935.
- ¹⁹HEIM, K.: Blutveraenderungen bei der Grossfelderbestrahlung. *Arch. f. Gynaek.* 116: 291, 1922-23.
- ²⁰MOTTRAM, J. C., AND CLARKE, J. R.: The leucocytic blood count of those handling radium for therapeutic purposes. *Am. J. Roentgenol.* 7: 335, 1920.
- ²¹NORDENSON, N. G.: Blood changes in radiologic work. *Acta Radiol.* 27: 416, 1946.
- ²²MARTLAND, H. S.: Microscopic changes of certain anemias due to radiosensitivity. *Arch. Path. & Lab. Med.* 2: 465, 1926.
- ²³LARKINS, F. E.: Acute aplastic anemia. *Lancet* 1: 801, 1921.
- ²⁴CZEPA, A.: Wachstumsfoerdernde und functionssteigernde Roentgen- Radium Wirkung. *Strahlentherapie* 16: 913, 1923-24.
- ²⁵PACKARD, C.: Biological effects of short radiations. *Quart. Rev. Biol.* 6: 253, 1931.