Changes in Cellular Composition of the Lymph Caused by Ionizing Radiations

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THE ANEMIA and the pathogenesis of the hemorrhagic tendency occurring in man and animals after exposure to massive ionizing irradiation are not well understood. The studies now to be described indicate that these two problems are interrelated, that capillary fragility is a major factor in causation of postirradiation anemia, and that these changes may bring about a self-aggravating process causing death of the irradiated animals.

MATERIAL AND METHODS

Preparation of Exteriorized Lymph-venous Anastomosis in Dogs

The operative procedure was essentially that developed independently by Brown and Hardenbergh. A plastic (polyvinyl* or polyethylene) cannula was introduced into the thoracic duct, another into a large branch of the jugular vein, and the connecting loop was exteriorized (fig. la, b). The venous cannula extended into the superior vena cava. To prevent obstruction by blood clots, often occurring at the tip of the cannula, fenestrations were made in the cannula just above the tip. The thoracic cannula extended about 1 to 3 cm. in the thoracic duct and was fastened by ligatures at several places inside the body. When samplings or clearance studies were made the fistula was disconnected, after which the loop was reconnected, well padded, and bandaged and the dog returned to the cage and cared for as a normal animal. After operation 100 mg. of aureomycin was injected through the venous cannula on three consecutive days.

Lymph Fistula Preparation in Rats

These were made according to the technic of Bollman, Cain and Grindlay.†

Experimental Animals

The dogs were mongrels weighing 24 to 48 pounds. The rats were of the Wistar stock either of our own inbred line or obtained from the Wistar Institute, weighing from 200 to 320 Gm.

Radiation

The factors of x-radiation for rats were 250 peak kilovoltage, 30 ma., 3 ml. Al. TSD 93.7 cm., 55 r/min. For dogs, the factors were 250 kv.p., 30 ma., 3 mm. Al, TSD 117 cm.; the proximal skin dose 49 r/min. The animals were turned over at half-time exposure.

* Obtained through courtesy of the Irvington Varnish and Insulator Company, Irvington, New Jersey.
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(a) Connected loop of the exteriorized lymph-venous anastomosis.
(b) Same disconnected with syringe containing blood withdrawn from the vein and bloody lymph collected from the thoracic duct fistula.
Examination of Cells of the Lymph

Lymph and blood from 43 irradiated, cannulated rats and from 30 controls were studied. The cell counts were made a few hours after establishment of the fistula, since red blood cells appear transiently in the lymph of some rats due to operative trauma.

In a detailed study of changes occurring during the first 10 hours after irradiation, lymph fistulas were established in 3 normal rats, and the animals draining lymph were exposed to 750 r. The viability of cells was tested before and hourly after irradiation by observing motility in a phase microscope after 15 to 20 minutes of incubation at 37.5 C., and by the safranine technic of Schrek. Examinations of cells of blood and lymph in 11 irradiated and 11 control dogs were similar to those in rats.

Results

The Lymph of Irradiated Rats

The most significant changes induced by irradiation consist of the appearance of large numbers of erythrocytes in the lymph of irradiated rats and of rapid reduction of the number of lymphocytes in the lymph, with predominance of large lymphocytes and appearance of many abnormal leukocytes.

The normal lymph of rats is milky-white and contains 0 to 2 red blood cells per 100 white blood cells. The increased number of erythrocytes in the lymph

Fig. 2.—Erythrocyte counts in the lymph of rats exposed to 650-750 r of x rays. Counts on animals on which the autopsy findings indicated a biologically low dose of x rays.
Fig. 3.—Figures a–i are lymph smears from rats; figures k, l, from a dog. All smears are stained with Wright-Giemsa stains.

(a) Normal lymph smear. Note predominance of small lymphocytes and absence of erythrocytes. $\times$ 400

(b and c) Multinucleated lymphocytes at 6 hours after exposure to 750 r.

(d and e) Lymph smear of the same animal at 8 hours showing “lumping” of chromatin and a binucleated cell. $\times$ 1000

(f) Mitotic figure at 11 days. $\times$ 1000

(g) Mitotic figure at 11 days. $\times$ 400

(h) Numerous erythrocytes and predominance of large lymphocytes at 11 days. $\times$ 400

(i) Numerous erythrocytes and 3 large lymphocytes at 14 days. $\times$ 400

(j) Numerous erythrocytes and 2 abnormally lobed large lymphoid cells at 14 days. $\times$ 400
was evident within a few days after irradiation, but a marked rise did not occur until the fourth or fifth day, and the peak was reached in about 9 to 14 days, when the erythrocyte counts were in the neighborhood of 1,000,000 (fig. 2).

The normal lymph had an average leukocyte count of about 26,000, of which 98 to 100 per cent were lymphocytes (fig. 3a). There was a marked drop in total white cell count in both blood and lymph within 24 hours after irradiation, but there was no parallelism between total leukocyte levels in blood and lymph, as the disappearance of lymphocytes preceded that of granulocytes. There was, however, a close parallelism between the initial drop and subsequent rise in lymphocyte levels of blood and lymph.

It is believed that circulating leukocytes are more resistant to irradiation than their precursors in hemopoietic centers. If so, reduction in numbers of lymphocytes in the lymph is expected to precede that of the blood. Therefore, a more detailed study was made of the sequence of destruction and disappearance of lymphocytes in blood and lymph during the first 12 hours postirradiation.

A transient rise of the lymphocyte count in the lymph 1 to 2 hours after irradiation preceded the precipitous drop. At 5 to 7 hours after irradiation there was a transient rebound, followed by a severe drop, reaching about one-thirtieth of normal at 10 hours.

The morphologic changes in lymphocytes of the lymph after irradiation are illustrated in figure 5. No changes were detected during the first 2 to 3 hours after irradiation. At 3 to 4 hours small vacuoles appeared in the nuclei and cytoplasm of some cells. Bi- and multinucleated cells (fig. 3b, c) were found in lymph at 3 to 6 hours after irradiation. One to two hours later a precipitous drop in lymphocyte counts occurred with appearance of many degenerating cells in which the chromatin was “lumped” (fig. 3d, e) or nuclei were pyknotic or fragmented. The largest numbers of dead cells, as indicated by motility and safranine tests, occurred at 4 to 8 hours. At 10 hours, most injured cells and debris had disappeared.

Recovery began about two weeks after irradiation, but normal cell counts in the lymph were approximated only after about 30 days. During the regenerating phase many large lymphocytes appeared in the lymph. In normal lymph (fig. 3a) large lymphocytes numbered only about 2 to 5 per cent of all white cells; in irradiated animals (fig. 3b–j), as much as 70 per cent.

The peak of the per cent of large lymphocytes in the lymph was reached in 8 to 11 days, but an elevation was present even during the fourth week after irradiation. This, however, was only relative. The absolute number of large lymphocytes in normal lymph was about 1300 per cu. mm., while after irradiation the greatest number was 900 on the fourteenth postirradiation day. Subsequently, the large lymphocyte count decreased to about 200 at 21 days, and then increased again as the total cell counts approached normal. In normal rats, whose lymph was drained continuously, there was also a marked drop in lymphocyte counts, and an increase in large lymphocytes, the latter not greater than 15 per cent. The large lymphocytes in the lymph may be taken as an indication of regenerative efforts in lymphopoiesis.

(k) A vacuolated large lymphoid cell and a plasma cell-like leukocyte in the lymph of a dog at 6 days after exposure. X 1000

(l) Numerous erythrocytes, a granulocyte and plasma cell-like leukocyte of same dog at 8 days. X 1000
Many of the large lymphocytes in the irradiated animals were abnormal. Some had a highly basophilic and vacuolated cytoplasm. Mitotic figures were not uncommon (fig. 3f, g). Some large lymphoid cells with lobed or eccentric, round nuclei could not be identified with certainty. Since all transitional forms between large lymphocytes and large cells with multi-lobed nuclei (fig. 3h-j) were seen, the latter were assumed to be abnormal large lymphocytes. The character of a leukemia transplantable by similar cells, described years ago, had been debated. We now believe that these "atypical" cells were pathologic lymphoblasts. The presence of neutrophils and eosinophils in the lymph was limited, with rare exceptions, to 5 to 14 days after irradiation. Their presence was probably due to the capillary fragility

FIG. 4.—Total leukocyte counts of the lymph and blood and erythrocyte counts of the lymph of dog S followed during 16 days post-irradiation.

which permitted the entry of red blood cells into the lymph. Tissue mast cells were noted in the blood (fig. 5a) 5 days postirradiation; at 5 to 15 days they numbered as much as 5 per cent of the total leukocyte count. Their disappearance from the blood coincided with the decrease in erythrocyte counts in the lymph. This observation is of interest because of the relation of mast cells to heparin production.

The Lymph of X-Rayed Dogs

The morphologic changes in the lymph of irradiated dogs were essentially the same as those of rats. An illustrative example is that of the dog S (fig. 4), whose lymph-venous anastomosis was maintained for 16 days. The erythrocyte count in the lymph of this dog took a sharp upward turn at the ninth postirradiation
(a) A tissue mast cell seen in the blood smear of a rat at 14 days after irradiation. × 1000
(b) Erythrocytes filling the lymph sinuses of a dog at 26 days after irradiation. × 100
(c) Erythrocytes filling the lymph sinuses of a dog at 13 days after irradiation. The normal lymphocytes have been replaced by plasma cells. × 300
(d) The sinuses of a mesenteric lymph node of a rat filled with erythrocytes at 11 days after irradiation. × 60
(e) The spleen of a rat at 7 days showing an atrophia follicle, diffuse fibrosis and moderate hemosiderosis. The black staining spots correspond to hemosiderin in macrophages.
day and on the sixteenth day, it exceeded one million per cu. mm. Changes in erythrocyte counts in the lymph of 6 additional irradiated dogs had the same pattern as dog S, although there were individual differences. The peak of erythrocyte counts was reached 13 to 17 days after irradiation (in rats, 9 to 14 days).

The morphologic abnormalities noted in lymphocytes were similar to those seen in rats (fig. 3k, l).

In one dog the lymph was studied during the first 8 hours after irradiation. The white cell count immediately before irradiation was 19,500; at 2 hours, 28,500; at 4 hours, 12,500; at 8 hours, 7,200; and at 24 hours, 2,300. At 8 hours, 85 per cent of the cells appeared still viable by the safranine test. Thus, the lymphocyte destruction was somewhat slower in this dog than in rats.

Anatomical Changes

The characteristic anatomical changes in massively irradiated dogs and rats are too well known to be detailed here, with two exceptions. The hemorrhagic appearance of lymph nodes and the fatty degeneration of the liver (fig. 1c) have been known to occur in irradiated animals of several species, but the meaning of these changes has hitherto not been appreciated.

In the present experiments hemorrhagic lymph nodes were found in every animal that died of massive irradiation (fig. 5b–d). The thoracic duct was dissected in most dogs and was found to contain bloody fluid and occasionally blood clots. These changes indicated escape of erythrocytes from the blood into the lymph system or, less likely, direct hemorrhage into lymph nodes (fig. 1c). The anatomic studies in both rats and dogs indicated that these changes are widespread and may set in abruptly or gradually, or occur intermittently during the first three postirradiation weeks. The degree of involvement of different lymph nodes varied with the animal and location. The mesenteric lymph nodes were almost invariably intensely red. The sequence of changes in lymph nodes (other than the well known atrophy of lymphoid organs) appear to be as follows: entry of erythrocytes in lymph sinuses, erythrophagocytosis, hemosiderosis and hyperplasia of large mononuclears. The hemosiderosis of the spleen (fig. 5e) has long been interpreted as evidence of erythrocyte destruction in the greater circulation. The fatty degeneration of liver (fig. 1e) was probably due to anemia since the liver cells are highly resistant to radiations but readily undergo fatty degeneration in the presence of anemia.

Discussion

There are numerous studies on changes in the lymphocytes of blood but not of those in the lymph, with one exception. Quantitative changes in lymphocytes of the blood following irradiation, noted by several investigators, include "clumping" of chromatin and fragmentation and pyknosis of nuclei, abnormal large lymphoid cells, mitotic lymphoblasts, and lymphocytes with bilobed nuclei. The sequence of changes following irradiation of suspensions of lymphocytes from blood, thymus and other organs was studied recently by Schrek who observed that the cytotoxic effect of irradiation was delayed until 3 to 4 hours after irradiation. Thus as concerns morphology and latent period of the degenerative phase, irradiation has a similar effect on lymphocytes in vivo as in vitro. The
present studies suggest that the degenerative phase is preceded by a rise in lymphocyte counts of the lymph.

Evidence for Diversion of Erythrocytes into the Lymph Compartment and Sequelae

The present studies indicate that entry of erythrocytes into the lymph system occurs even when there is no gross evidence of a hemorrhagic tendency. The massive hemosiderosis present in lymph nodes and the excessive bilirubin excretion have been interpreted to indicate destruction of erythrocytes in irradiated hosts.\(^1\) \(^2\) Observations of Clark\(^1\) indicate that if erythrocytes escape from capillaries into tissue spaces, they soon die there and are engulfed by macrophages.

The phenomenon described appears universal, varying only in degree with species and sites.\(^1\) \(^2\) Similar changes were observed by General Elbert DeCoursey\(^17\) in the lymph nodes of men who died as a consequence of atomic bomb explosions. Since this change has hitherto not been fully appreciated in either man or animals, fig. 1d and e are reproduced here through his courtesy and with the help of Dr. Muriel Raum. They are from a 45 year old soldier who was approximately 1 Km. from the hypocenter. Widespread petechiae were noted on the skin during his last five days of life when he became epilated and had bloody diarrhea. The autopsy was performed by Drs. Miyake and Hirafuku, who noted focal aplastic necrotizing pneumonia with hemorrhages, atrophy of the bone marrow, extensive necrotizing and hemorrhagic gastritis and hemorrhages in the intestine, omentum, and kidneys.* Among the Japanese casualties petechiae and ecchymoses were seen at autopsy 4 to 14 days after the explosion, but were most prominent during the third to sixth weeks. Hemorrhages in lymph nodes were not noted on gross examination until the fourth week.\(^17\) These observations suggest that the pathogenesis of the hemorrhagic syndrome in man is probably the same as that in animals.

Studies at the Naval Medical Research Institute\(^1\) \(^2\) led to the conclusion that the hemorrhagic syndrome is the result of a combination of increased vascular fragility and a blood coagulation defect. This is associated with thrombocytopenia and infrequently with a prolonged clotting time due to a circulating anticoagulant with heparin-like properties as noted by Allen\(^2\) in irradiated dogs. Evidence was presented suggesting that serum fibrinolysins may have been activated,\(^1\) \(^2\) \(^20\) and that the hemorrhagic syndrome can develop in irradiated dogs, goats, swine, rats, chickens and guinea pigs without the appearance of a prolonged clotting time and "heparinemia." Very recently Cronkite et al.\(^21\) \(^22\) presented evidence suggesting an intimate relationship between capillary fragility and platelet levels.

Although diversion of erythrocytes into lymph spaces results in merely apparent anemia, the consequences of this diversion are manifold. There is a lack of erythrocytes where needed with resultant tissue anoxia. Their presence in lymph spaces signifies the occurrence of a marked capillary damage with entry into the lymph of other elements of the blood. Massive irradiation causes a variety of independent and interdependent changes. It is not possible at present to assess

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the relative importance of factors already known in causation of death, and it is possible that the early death due to overwhelming exposure is caused by a different patho-physiologic disturbance than that discussed here.

The anemia following massive irradiation is far greater than could be accounted for by death of aged erythrocytes. The life span of the erythrocyte of dogs is estimated to be about 120 days. Therefore, aging in complete absence of erythropoiesis could account for no more than a loss of about 1 per cent of the red cell per day. The lymph volume is not known precisely, but methods are now known to approximate it. Assuming that lymph and blood plasma are equal in volume and the preirradiation red blood cell count is four million, a red blood cell count of one million in the lymph would represent an erythrocyte mass of approximately one-fourth of the original erythrocyte mass. The red cell counts in the lymph indicate the number of erythrocytes in the lymph but not the rate of their entry into the lymph. The latter, assayed by means of isotopically labeled erythrocytes, may prove to be an excellent means of timing the occurrence of capillary damage, measuring its intensity in relation to time after irradiation and in gauging attempts at therapeutic control.

The Pathogenesis of Capillary Damage

The immediate cause or causes of capillary fragility remain to be identified. Direct injury to endothelial cells is possible; if so, this would have an unusually long latency. Death of slowly multiplying cells may not be manifest for some time, but nothing is known about the life span of endothelial cells and their regenerating capacity is known to be excellent. The clinical and experimental observations that localized massive irradiation is not followed by hemorrhages at irradiation sites argue against direct injury of endothelial cells following total body irradiation at LD-50 levels.

Numerous humoral and cellular changes occur in sequence following massive irradiation. The liberation of “necrohormones” from disintegrating cells has long been suggested and histamine, either liberated from destroyed cells or discharged in excessive amount by cells stimulated by irradiation. The recent studies of Cronkite et al. present strong support in favor of the platelet theory of capillary fragility.

Whatever the cause of the capillary fragility, diversion of erythrocytes into lymph spaces causes anemia; if this process is severe, sudden shock follows with release of substances which in turn will increase the capillary damage, thus initiating a vicious cycle leading to death.

SUMMARY

Erythrocytes appear in large numbers in the lymph of rats and dogs after massive exposure to x rays. The peak of endothelial fragility, as indicated by the erythrocyte counts in the lymph, is reached on the ninth to fourteenth day in rats and the eleventh to seventeenth day in dogs. In both species the erythrocyte count in the lymph frequently exceeds one million.

Diversion of erythrocytes into the lymph compartment causes a relative anemia; excessive destruction of erythrocytes, presumably related to extravasation and not to a direct irradiation injury, is responsible in part for the absolute
anemia. It is suggested that similar changes may contribute to the anemia of leukemia and other blood diseases associated with capillary fragility.

The drop in lymphocyte counts in both lymph and blood is precipitous within 5 to 10 hours after irradiation. During the fourth to eighth hours after irradiation, injured and dead lymphocytes are present in the lymph in large numbers. During the recovery phase, the per cent of large lymphocytes in the lymph greatly increases, there are many abnormal large lymphoid cells and mitotic figures, and tissue mast cells appear in blood smears.

It is concluded that diversion of erythrocytes into the lymph caused by massive irradiation, if severe, becomes a self-aggravating process and leads to death.

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