THE EFFECTS OF ROENTGEN RAYS ON THE INFLAMMATORY CELLS OF
THE MOUSE AND RABBIT

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ROENTGEN RAYS have been used regularly by radiologists1–13 for the treatment of a large variety of inflammatory conditions, but experimental morphologic studies, as shown below, have revealed no reliable evidence to explain why roentgen rays should be of value. We undertook this problem to determine whether morphologic alterations of the inflammatory cycle could be induced by a variety of roentgen ray doses applied at times before and after inciting an inflammatory reaction in mice and rabbits. Our quantities and times of irradiation include and extend beyond those used by most earlier workers.

Sturges and Levin14 were the first to study the effects of roentgen rays on leukocytes they defined as inflammatory. They found that either intravenous yeast emulsion injections or roentgen rays, when applied separately, caused a depression of the lymphocytes in the circulating blood of frogs, whereas roentgen rays superimposed on the effect of yeast emulsion had no additional action. They concluded that if the leukocytes remaining after administration of yeast emulsion were inflammatory cells, then inflammatory cells were radioresistant.

The radioresistance of inflammatory cells was further supported by Maximow15 who heavily irradiated inflammatory sites locally in the subcutaneous tissue of rabbits. The earliest examination was made eleven days following irradiation when the inflammatory site contained only leukocytes and polyblasts in a gelatinous fibrin substance. The fibrocytes had been destroyed as well as collagenous fibers; so it was concluded that either leukocytes and macrophages were extremely radioresistant or that the initial infiltration had been destroyed and replaced by a secondary infiltration.

Soto, Brunschwig, and Shultz16 produced subcutaneous abscesses in rabbits with a variety of bacteria and with croton oil. The animals were given whole body irradiation at intervals of 24 hours before injection and immediately, 5 hours, 24 hours, and 7 days following injection of the irritant. The dose applied was 600 r (200 KV, 25 ma., 50 cm. focal distance, 1 mm. Cu plus 1 mm. Al filter). The abscesses were mainly observed grossly but some were observed histologically. In neither case was any specific evidence found which indicated roentgen rays were of value clinically or the cause of histologic changes.

Hayer17 locally irradiated (600 r) an area on a dog’s thigh immediately following the subcutaneous injection of turpentine and observed that the abscess developed as in the normal animal. A similar observation was made following local irradiation of the spleen, but whole body irradiation markedly inhibited abscess development until a later period. The reason for these results was thought to be the leukopenia following whole body irradiation.

A number of authors have observed that roentgen rays decreased the number of

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inflammatory cells. Mittermair irradiated acute aseptic inflammation induced by catgut sutures in the skin and subcutaneous tissue of guinea pigs and reported a decreased number of inflammatory cells within six and twenty-four hours following irradiation. This decrease was also found by Fukase within twenty-four hours following irradiation of aseptic surgical incisions in the abdominal skin of rabbits. Fukase's result was confirmed by Tannenberg and Bayer who employed a similar technic. Mischtschenko and his co-workers examined inflammatory exudate smears two, three, and four days following irradiation of staphlococcal inflammatory sites in rabbits. They concluded that an increased leukocytic destruction as well as an increased phagocytosis was caused by irradiation.

An increased leukocytic infiltration was found by Buhtz within a few hours following irradiation of rabbits prepared as Fukase had done previously. Buhtz had his conclusion confirmed by Tannenberg and Bayer. Poumeau-Delille found an increased neutrophilic infiltration into rabbits' subcutaneous tissues within two days following irradiation of multiple aseptic abscesses with smaller doses of roentgen rays than used by the preceding authors. Doses over 150 r were said by the latter author to result in decreased infiltration. Burnet de Rochebrune reported that roentgen rays imposed upon formic acid on a rabbit's skin resulted in an exudation whereas neither roentgen rays nor formic acid had this effect when applied separately.

As a standard preparation we have used subcutaneous experimental inflammation in the mouse produced by an injected inflammatory agent. A clear description of the time sequences and cytogenic development of the acutely inflamed areolar tissue of the rabbit has been contributed by Kolouch. For a review of the earlier literature on the inflammatory article, reference is made to the elaborate discussion in the aforementioned paper, and to the textbook of Maximow and Bloom.

Kolouch studied the inflammatory cycle of rabbits and introduced the Romanowsky-stained loose connective tissue spread which was employed by the authors of this paper. This technic, which will be described below, allowed the direct comparison of cells in the connective tissue with those of the blood smears and splenic imprints.

The nomenclature of the various inflammatory cells has varied in the past with different authors. We shall attempt to follow Maximow's nomenclature as closely as possible, but convenience suggests certain modifications. The terms ameboid and resting wandering cell have been used for the resident tissue cells which are known variously as clasmatocytes, histiocytes, tissue macrophages, etc. To indicate the cytomorphic line from the lymphocyte to macrophage we have introduced, at the suggestion of Bloom, the new term intermediate polyblast.

**Materials and Methods**

An aseptic irritant composed of egg albumin into which a small amount of india ink, as a marker, had been mixed was used to incite the inflammatory cycle in the mice and rabbits. This irritant was injected in 0.07 to 0.09 cc. quantities into the loose subcutaneous connective tissue on the lateral part of the abdomen. A single injection was made into each of the mice, which were then killed at appropriate intervals with ether. About 200 mice were used in experiments to standardize the inflammatory cycle preparation (see below). The rabbits were similarly treated, except that severe inflammatory sites were made on each animal.
According to the technic introduced by Kolouch, the loose subcutaneous connective tissue of the inflammatory site was removed, placed on a clean glass slide and spread, rapidly air dried, and stained with Wright-Giemsa.

The mice were given whole body irradiation in groups of three or more in a small cardboard box. The animals were irradiated with a 7 ma. superficial therapy machine set at 100KV emitting 100 skin roentgens in 1.3 minutes with no filter and a 30 cm. focal distance. The doses varied from 25 r to 1600 r to the whole body which was applied at various times preceding and following the injection of the irritant. A total of 150 mice were irradiated in these experiments.

The rabbits to be locally irradiated were injected with a similar irritant in two sites, one on either side of the abdomen. The tissues were removed and prepared by the method of Kolouch after the rabbits had been killed at appropriate times by injected air. Twenty-four hours following injection of the irritant, the rabbits were irradiated on one side only under intravenous Nembutal anesthesia. Localization was accomplished by means of a cone three centimeters in diameter having a 15.5 cm. focal distance. The roentgen rays were produced by a 7 ma. superficial therapy machine set at 100 KV which emitted 170 air roentgens per minute with 1.2 mm. of aluminum filter.

Five rabbits were given whole body irradiation with the same machine similarly filtered but emitting roentgen rays at the rate of 64.8 r per minute with the focal distance raised to 33 cm. Four sites were injected on each rabbit's abdomen for the purpose of studying morphologic variations between the various inflammatory sites.

Eight rabbits were given whole body irradiation with a 15 ma. deep therapy machine set at 220 KV which emitted 106 r per minute with .2 mm. of copper plus .2 mm. of tin filter at a 33 cm. focal distance. The half value layer was 1.35 mm. of copper. Four sites were also injected in each animal of this series.

The Inflammatory Cycle of the Mouse

As previous literature has not described the normal cycle of inflammation in the mouse as revealed by this method, we present the following observations as a necessary basis for the irradiation experiments. The fibrocytes (fig. 1) of this species differ from those described by Maximow in the human connective tissue in having a larger number of distinct chromatin clumps in an otherwise fine reticular chromatin pattern. The fibrocyte nuclei are the largest and palest staining in the connective tissue and are round or oval in outline. The indistinctly delimited cytoplasm is slightly basophilic and more homogeneous than that of the wandering cells. The fibrocyte cytoplasm occasionally contains india ink but no other particles.

The wandering cells (fig. 1) differ from the fibrocytes in having smaller, more deeply staining nuclei with a heavier, denser chromatin network, the cytoplasm being more basophilic, granular, and vacuolated. The cytoplasm has a distinct boundary in the ameboid wandering cells and is indistinct in the fixed wandering cells. Some of the wandering cells begin phagocytosis in the inflammatory site within one hour. These resident phagocytes give rise to large histogenous macrophages by gradual transitional forms which show increasing cytoplasmic and nuclear volume. As the nuclear volume increases, the chromatin blends into the parachromatin until frequently the nucleus appears homogeneous. This cell is relatively more frequent in the earlier hours and is considerably less frequent after the first day of the inflammatory cycle. Kolouch called this cell the activated clasmocyte.

The lymphocytes of the mouse are morphologically similar to those in the human. The nuclei are either round or indented, with chromatin clumps which blend into the parachromatin. The scanty cytoplasm is deeply basophilic and occasionally
contains small vacuoles and azurophilic granules. Lymphocytes and intermediate polyblasts are very occasionally found in the normal connective tissue.

The lymphocytes (fig. 3) infiltrate into the inflammatory site about the capillaries within four hours after injection of the irritant, and large numbers are present within twelve hours.

A continuous, variable infiltration, secondary to the heavy initial immigration, was seen throughout the 108 hour period of observation. Within two hours after the initial infiltration began, some lymphocytes were acquiring an increased nuclear and cytoplasmic volume which characterized early intermediate polyblast development (fig. 4). In the inflammatory site the evolution of the nuclei of the lymphocyte to that of the intermediate polyblast and finally to the macrophage could be followed in detail. The clumped chromatin of the lymphocyte forms strands and gradually differentiates into a heavy reticular pattern containing several distinct chromatin blocks (figs. 5b, c). Associated with the formation of the polyblast chromatin pattern was a tendency for many intermediate polyblasts to acquire a kidney shaped nucleus (figs. 4 and 5). As the chromatin became more like that of the reticulum cell, the nucleus filled out to the round nucleus of the macrophage (fig. 5e). Both cytoplasm and nucleus increased in volume during this process with the former becoming less basophilic and more granular and spongioform. This development of the macrophage was completed in some cells within twenty-four hours and was assumed to be from those lymphocytes first infiltrating. Due to the continual infiltration of lymphocytes (fig. 6) a complete transitional series could be seen up to 108 hours. The intermediate polyblasts even in the early stages of development were seen to be capable of phagocytosis.

Although the great majority of lymphocytes formed intermediate polyblasts, an occasional lymphocyte underwent nuclear and cellular pyknosis and fragmentation. The degeneration was identical with the degeneration of lymphocytes in the spleen as discussed elsewhere. Rarely a pyknotic or fragmented nucleus could be found in cells resembling the intermediate polyblasts or macrophages. More rarely these degenerate cells contained phagocytosed material.

The fibrocytes were seen in mitosis in both the control and inflamed tissues; and especially in the later stages of inflammation. Rarely macrophages or intermediate polyblasts were found in mitosis.

The neutrophils of the mouse differed from those found in the human in having one or several lobes which formed a closed ring. A study of neutrophil development in the mouse clearly indicated that the single lobed, thick ringed nuclei were younger than the multilobed nuclei. The single lobed cell was especially prominent in the primary infiltration of neutrophils which had begun within one hour following the injection of the irritant. Large numbers of neutrophils underwent cellular fragmentation (fig. 2) and were ingested by histogenous macrophages within a few hours following infiltration. As in the case of the lymphocytes, secondary infiltration occurred throughout the period of observation. The neutrophils were found in the normal tissue in about the same frequency as the lymphocytes.

The connective tissue of the mouse also contains mast (fig. 1c) cells which are filled with metachromatic granules which obscure the nucleus. The entire cell is
Fig. 1.—Connective tissue spread from the mouse: normal tissue showing fibrocytes (a), and wandering cells (b), and a mast cell (c). Wright-Giemsa. X 600.

Fig. 2.—Connective tissue spread from the mouse: inflamed tissue showing neutrophilic infiltration and degeneration 4 hours after injection of the irritant. (a) neutrophil with a thick ring nucleus, (b) polymorphonuclear cell, (c) pyknotic neutrophil. Wright-Giemsa. X 600.

Fig. 3.—Connective tissue spread from the mouse: inflamed tissue showing lymphocytic infiltration (a) and early intermediate polynuclear development (b) six hours following injection of the irritant. Wright-Giemsa. X 600.

Fig. 4.—Connective tissue spread from the mouse: inflamed tissue showing typical intermediate polynuclear development 14 hours after injection of the irritant. Wright-Giemsa. X 600.

Fig. 5.—Connective tissue spread from the mouse: inflamed tissue showing intermediate polynuclear development and secondary neutrophilic infiltration four hours after injection of the irritant. (a) lymphocyte, (b) early intermediate polynuclear, (c) late intermediate polynuclear, (d) inactive macrophage, (e) active macrophage, (f) degenerate lymphocyte. Wright-Giemsa. X 600.

Fig. 6.—Connective tissue spread from the mouse: inflamed tissue showing a secondary infiltration of lymphocytes and neutrophils 84 hours following injection of the irritant. The small dark granules are from a ruptured mast cell. Wright-Giemsa. X 600.
somewhat larger than the nucleus of a fibrocyte. These cells underwent no specific morphologic change during the inflammatory cycle although several were ruptured in the inflammatory site with some consequent scattering of the granules.

Effects of Irradiation on Inflammatory Cycle of Mouse

I. Whole body irradiation on the twelve hour inflammatory site of the mouse. As the control series showed that the time of maximal lymphocytic infiltration in the inflamed areolar tissue occurred at twelve hours, this preparation was studied most thoroughly. The inflammatory cell population at this stage, in addition to the large number of lymphocytes, consists of invasive neutrophils, cf lymphocytes which have differentiated into intermediate polyblasts and of histogenous macrophages. Thus the effects upon each of these cell varieties may be determined.

Irradiation of the twelve hour inflammatory site produced vacuolation in the intermediate polyblasts with dosages of 250 r to 500 r. Throughout the cytoplasm of the affected cells appeared large numbers of small clear vacuoles. A similar type of vacuole is very occasionally seen in the normal cell but never in any large numbers. Figure 7 shows the appearance of the intermediate polyblast twenty-four hours after a dose of 400 r. The vacuoles at these dosages were observed to appear at twelve hours, reach their greatest frequency at twenty-four hours, and decrease in the later stages. It would seem then that they represent a reversible alteration and hence they have been differentiated from the frank degenerative changes—fragmentation, karyorhexis, chromatolysis, and irregularity of nuclear outline—that appear, in addition to vacuolation, at doses of 700 r or more. The preparations receiving 1200 r and 1600 r showed extensive degeneration of the intermediate polyblasts at twenty-four hours (fig. 8). This resulted in a decrease in the number of inflammatory cells relative to the fibroblasts in the tissue spreads.

The macrophages showed a greater resistance to the irradiation than did the intermediate polyblasts. Vacuolation similar to that described above, appeared at 900 r with degeneration showing at 1200 r and more (fig. 9), though differentiation in this stage cannot be made between the hematogenous and the histogenous macrophages. Consequently the relative radiosensitivities of these two cell lines cannot be ascertained. Evidence presented below indicated that up to 1200 r, the wandering cells of the tissue are unaffected.

A decrease in the number of lymphocytes and neutrophils became apparent in those preparations which had received 900 r and larger doses.

Loss of ability of the inflammatory cells to localize the irritant was evident in the preparations exposed to doses higher than 700 r. The india ink, used as a marker for the egg white, was spread out over a larger area following these heavy exposures and contrary to the normal picture, tended to be phagocytosed by the fibroblasts. Whether these usually inactive cells were activated by the large doses or were responding to lack of competition of the normal scavenger cells is problematic.

2. Irradiation of the four hour inflammatory site. In this series, only dosages of 700 r or less were tested. The effects were of the same nature as those described in the previous section though of lesser severity.

3. Irradiation of the inflammatory site at the time of injection or at or shortly preceding
the time of injection. Low dosages in this series produced no alteration of the inflammatory site. At 1,200 r doses, no degeneration of macrophages of tissue origin or of intermediate polyblasts was seen (fig. 10). A normal infiltration of both lymphocytes and neutrophils was found up to twenty-four hours with a decrease noticeable in the later stages. As a severe leukopenia was noted in the circulating blood in less than twenty-four hours, normal infiltration at this stage seemed the more remarkable. It was further noticed that the cells derived from the invading lymphocytes did not undergo degeneration as in the other experiments where the inflammatory site was irradiated after they had invaded the tissues. A plausible explanation for the greater resistance, under these circumstances, of the infiltrating lymphocytes and their derivatives, the intermediate polyblasts and hematogenous macrophages,
is that the more radiosensitive lymphocytes had already been destroyed in the blood and only the more resistant ones had infiltrated.

No degeneration of the histogenous macrophages was seen with these massive doses, indicating the high resistance of this cell type.

4. Irradiation 24 hours, 48 hours, and 72 hours prior to injection. Three mice only were used in this part of the experiment, one for each of the periods. They were killed twenty-four hours after the irritant was injected. All showed a greatly decreased lymphocytic infiltration and scarcity of intermediate polyblasts. The histogenous macrophages were present in normal numbers and consequently outnumbered the hematogenous inflammatory cells, a numerical relationship which is the reverse of that seen in the controls. A slight infiltration of lymphocytes was present.

### Table 1.—Numerical Summary of Mouse Experiments

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### Effects of Irradiation on Inflammatory Cycle of the Rabbit

1. Local irradiation on the inflammatory cells in rabbits. Twenty-one rabbits were irradiated locally on the twenty-fourth hour following the injection with dosages ranging from 80 r to 1000 r. Examination was made at intervals from 2 to 96 hours following the irradiation and no deviation from the controls were found with regard either to the morphology or the numerical frequency of the inflammatory cells. In addition one rabbit was exposed to 1000 r twenty-four hours before the injection and examined twenty-four hours later. No deviation from the control was noted.

2. The effects of general body irradiation on the rabbit. Twelve rabbits were given whole body irradiation at 700 r or 1000 r and examined at intervals ranging from 5 hours to 96 hours. Of 5 animals given 1000 r (at 220 KV), 4 showed a markedly reduced infiltration of leukocytes. In the fifth only a moderate decrease in the leukocytic infiltration was seen. In all of the experiments, no abnormalities of the inflammatory, or tissue cells were observed.

### Discussion

Our experiments did not reveal any acceleration of the inflammatory cycle at any dosage, though they were specifically designed for detecting such a phenomenon if
it should exist. No effects of any kind were observed at dosages below 250 r in the mouse or 1000 r in the rabbit. General body irradiation prior to the setting up of the inflammatory cycle decreased the infiltration of neutrophils and lymphocytes in dosages of 700 r or more in mice, and 1000 r in rabbits. Our evidence would indicate that the decreased infiltration is for the most part a reflection of the profound leukopenia induced. Local irradiation in the rabbits produced no such effect.

A striking resistance of all the inflammatory cells was noticed. The lymphocytes in the tissues showed no degenerative changes, indicating a great radioresistance in contrast to those observed in the hematopoietic organs but in agreement with experiments on blood. The intermediate polyblast, which proved to be the most easily affected of the inflammatory cell population, showed no structural abnormalities, under the conditions of the experiment, in response to doses of less than 250 r and exhibited frankly degenerative phenomena at irradiations of 700 r or more. It was apparent that the macrophages of tissue origin (as well as their precursors, the wandering cells) are more radioresistant than at least some of the macrophages developing from lymphocytes. Large doses (1200 r) given at a time preceding the inflammatory stimulus resulted in no degenerative phenomena in these cells following the onset of the inflammatory cycle. Degeneration did occur in macrophages when irradiation at this dosage was performed twenty-four hours following the injection of the inflammatory agent. With 900 r, vacuolation could be observed in macrophages in those sites irradiated after the formation of the lymphocyte derivatives. No degenerative phenomena were observed in the infiltrating lymphocytes at the greatest dosage (1200 r). Neutrophils which had invaded the inflammatory site likewise proved resistant to all doses studied.

In our results, there is nothing to correlate with the conclusions of many clinicians that roentgen therapy is of great value in a number of inflammatory conditions. It is possible that a study similar to this on septic inflammation, or even the inclusion of the late stages would have made our results more confluent with previous literature. We wonder, though, whether the phenomenon of decreased infiltration which we observed in response to massive whole body exposure has been misinterpreted by some who were looking for an acceleration of the inflammatory cycle by x-rays.

Our study of the inflammatory cycle in the mouse substantiates the study by Kolouch in the rabbit. The neutrophils began invasion of the inflammatory site within one hour following injection of the irritant and reached a maximum within four to six hours. They quickly underwent degeneration and were ingested by the macrophages. Lymphocytic infiltration occurred within four hours after the injection of the irritant and immediately or very shortly afterwards began a transformation into intermediate polyblasts. The maximum number of lymphocytes was found in the tissues within twelve hours following injection of the irritant. Kolouch’s paper dealt with the sequence of cell types and not quantitatively with the populations. Our findings on the variability of the secondary infiltrations of both granulocytes and lymphocytes indicate that a large number of preparations are necessary in order to deal adequately with the latter question.
Conclusions

1. No acceleration of cell differentiation of the inflammatory cycle is induced by treatment with roentgen rays.

2. The decreased infiltration of the inflammatory site caused by massive whole-body exposures before the inflammation was set up or early in the cycle is due to the leukopenia induced in the circulating blood.

3. Of the inflammatory cells of the mouse, the intermediate polyblast (a lymphocytic derivative) is relatively the most radiosensitive with structural abnormalities noticeable following 250 r. The macrophages derived from the intermediate polyblasts show alterations following doses of 700 r or more. Wandering cells, histogenous macrophages, infiltrating lymphocytes and neutrophils show no morphologic abnormalities following doses as high as 1200 r.

4. The ability of the tissue to prevent the spread of the inflammatory agent is decreased following dosages of 700 r or more.

5. The inflammatory cells of the rabbit are markedly more resistant than those of the mouse.

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References


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