# STUDIES ON THE CHANGES PRODUCED BY ROENTGEN RAYS IN INFLAMED CONNECTIVE TISSUE.

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## INTRODUCTION.

Although Roentgen rays have been used for more than 20 years in clinical medicine and despite the extensive literature, very little is as yet known about the action of this form of energy upon living substance. Not only is the nature of the action not clear, but there are lacking even systematic experimental investigations on the common histological changes in the cells and tissues after treatment with x-rays. These considerations induced me to undertake the experiments described below in 1919. The material can by no means be considered complete, but as the work has been discontinued the results obtained so far will be described in the present article.

The literature on the biological action of x-rays will not be discussed at length as this has been done by previous authors.

It is generally admitted that, of all the cells of the vertebrate organism, the greatest sensitiveness towards x-rays is displayed by elements possessing a particularly high degree of reproductive capacity and, on the other hand, showing no finally settled morphology and function. Accordingly, the most striking results have been obtained with sex cells, eggs and spermatozoa. X-raying of these elements leads, in the subsequent development, to various monstrosities and eventually may cause the death of the embryo. If the sexual glands in the body are exposed, they are "sterilized;" *i.e.*, the egg and sperm cells perish, whereas the nutritive cells, the follicle and Sertoli cells, remain alive (Krause, Reifferscheid, and others).

On the other hand, among the somatic cells, the lymphocytes, being indifferent, non-granulated, lymphoid cells of the blood and the blood-forming organs, are especially sensitive towards the rays. After exposure of lymph nodes, spleen, thymus, etc., the lymphocytes are rapidly destroyed (Krause, Fromme, Soper, Grasnick, Hartmann, and others).

The Roentgen tube, radium, whose $\gamma$-rays closely correspond to x-rays, also mesothorium and thorium X seem to give in all the reported cases almost identical results. The differences are not important; for example, it is the myeloid and not the lymphoid tissue, that seems to be especially sensitive towards thorium X (da Silva Mello, Zoellner).

It is also well known that of the different types of epithelial tissue the germinal layer of the epidermis and the hair follicles are considered the most sensitive; in clinical work with x-rays this is always to be noticed and this property of the epidermis often causes ulcerations of the skin and alopecia (Rost).

References concerning other tissues will be made in the course of the following discussion.

As a rule, even in small doses the rays have a damaging and inhibiting influence upon the living substance, manifesting itself in various degenerative changes in the respective cells. Yet in recent years observations have been made which seem to make it probable that, under certain conditions, a stimulating effect might be produced by using very small doses. Development of plants may perhaps be stimulated by small doses of x-rays. Haecker and Lebedinsky, Packard, Baldwin (1919–20, 1920), and others have seen a stimulating influence of x-rays on the development of animal eggs. Murphy and his collaborators also emphasize the stimulating action of small doses of x-rays on the lymphocytes of the circulating blood and the lymphoid organs.

What parts of the living cell are chiefly affected by the rays is not precisely known, as special cytological investigations in this direction are lacking. But it has been shown since the classical studies of O. Hertwig and his school that, of all the cell organs, the nucleus and especially the chromatin content of the latter are first affected. This must be the chief cause of the abnormalities in development after exposure of germ cells to the action of rays. This specific sensitiveness of the nuclear chromatin, as indicated by fragmentation of the nuclei, etc., has since been confirmed by many authors (Hoffmann, Packard, Payne, Rost, and others).

As we do not yet possess a systematic knowledge of the cytological and histological changes produced in cells by the action of x-rays, it seems natural that even such thorough work as, for example, that of Hartmann on the influence of x-rays on the blood-forming organs in amphibian larvæ should yield mere commonplace results in the sense of a general inhibition of lymph cell formation. In the general uncertainty one meets from time to time quite unexpected and startling facts, as, for example, the remarkable sensibility of the epithelium in the crypts of the small intestine, discovered by Warren and Whipple (1922, *a*, *b*) and McQuarrie and Whipple. Profound and rapid injuries of the intestinal function and a peculiar Roentgen ray intoxication are caused, whereas the lymphoid elements of the small intestine seem hardly to be affected.

In studying the changes in the cells and tissues caused by the action of x-rays, one has to discriminate between instances in which the

rays are acting upon the whole organism, and those in which only a limited part of the latter is exposed and the action is only local. It is evident that in the first case the results must be incomparably more complicated and far more difficult to control than in the second.

My intention was to study the influence of the x-rays upon the cellular inflammatory process in the loose connective tissue. The inflammatory changes in the connective tissue have been for many years the subject of my investigations (Maximow, 1902, 1903, 1904, 1905, 1906). This problem has a certain interest in itself, as the changes produced in the connective tissue elements by the action of the rays are unknown. Furthermore, its clinical importance is not to be underestimated, as the loose connective tissue, distributed all over the body, is inevitably involved in every case of therapeutic use of the rays and its behavior must play an important part in every case of wound healing, of tumor development, etc., and must directly determine to some extent the issue of the pathological process. According to Ribbert the changes produced in the connective tissue by Roentgen rays are responsible for the origin of the Roentgen carcinoma. Fraenkel believes that the loose connective tissue is to be looked at as a single large endocrine system; he cautions against using high doses of rays in treating carcinoma, since the extremely important defense reaction of the connective tissue is apt in such a case to be imperilled or weakened.

## *Material and Methods.*

The experiments were performed on rabbits and, for inducing aseptic inflammation, the method previously described (Maximow, 1902) was used in which flat rectangular celloidin blocks, with slits cut parallel to their surface, are introduced into the subcutaneous or intermuscular loose connective tissue of the abdominal wall. Generally the appropriate place in the abdominal wall was exposed on the day after the introduction of the celloidin blocks and the exposure was repeated once a day for a certain period. The animal was stretched out, with the back downward, and covered with a tightly fitting, duly bent lead sheet of 3 mm. thickness. The lead had a round opening of about 4 cm. in diameter, corresponding to the position of the celloidin blocks, which are easily felt through the skin. Above the opening the Roentgen tube was placed with an aluminum plate of 0.5 mm. thickness as filter. The tubes used were of the Gundelach or Müller type. The distance between the anticathode and the surface of the skin was as a rule about 14 to 15 cm. The length of the daily exposure was on the average 15 minutes.

For determining the dose Kienboeck's test was used, the dose employed every time being about 12 of Kienboeck's quantimetric units, the so called x.

The first series includes eight cases of this kind; in one of them only one exposure was made, in two others there were two, in one four, in one five, in one ten, and in two fifteen exposures. After the last exposure a period of 5 to 27 days was allowed to elapse; in one instance this period was prolonged to 60 days. At the end of this time the animal was killed and the part of the body wall containing the blocks was fixed, after the skin had been carefully removed and the tissue stretched over a cork frame. The pieces of tissue were fixed in Zenker-formol or in some cases Champy's liquid, embedded in celloidin, and cut in serial sections parallel to the surface. For staining, eosin-azure or iron-hematoxylin was used, and after Champy's fixation the method of Kull. Identical celloidin blocks were always introduced simultaneously on the opposite side of the abdominal wall for control. These remained without exposure to x-rays and were fixed together with the exposed blocks.

In most cases on the exposed area of the skin alopecia of higher or lesser degree developed. In the three rabbits in which the exposure was repeated ten or fifteen times, a superficial inflammation of the skin could be observed. Ulceration never occurred and, even in the animal which had been exposed fifteen times and had lived 27 days after the last exposure, the initial inflammation again subsided.

In one instance simultaneously with the introduction of the celloidin bodies 2 cc. of lithium carmine were injected into the same place in the connective tissue; in another 2 cc. of trypan blue were used for the same purpose.

In a second series of experiments, in three rabbits, a certain area of the abdominal wall was exposed on each of 3 days before the introduction of the celloidin blocks; two exposures were made, separated by an interval of 5 minutes. In two of the rabbits celloidin blocks were introduced into the previously exposed place 5 days after the exposure, in the third rabbit 10 days after the exposure, and left there for 3, 10, and 25 days respectively. Control blocks were also introduced into other, normal places in the abdominal wall.

Lastly, the influence of x-rays on scar tissue was tested; for this purpose in two animals celloidin bodies, introduced in the abdominal wall 15 days previously, were exposed five times during 5 days to a dose of 12 x.

The process of the aseptic inflammatory reaction in the connective tissue consists of a regular series of many closely connected phenomena. An important part is played here by local sessile elements, the fibroblasts and resting wandering cells, as well as by wandering elements, special leucocytes, lymphocytes, and polyblasts, coming for the most part from the blood vessels. The exposure to x-rays of all these elements with their different sensitiveness towards the rays, together with the constant changing of the sensibility according to

their different stages of development, must evidently produce very complicated results. This complexity is still more increased by the fact that the action of the rays does not at once become manifest, and furthermore by the constant arrival of new cells from the blood vessels into the connective tissue. A preparation from a block several times exposed will thus contain, on one hand, cells which have been acted upon by the rays to a greatly varying degree and have been living and functioning for different periods of time after their last exposure, and, on the other hand, cells on which the x-rays did not act at all, but which had to exist for different lengths of time in the inflamed tissue, which had been previously affected by the rays.

Macroscopically, the tissue surrounding the foreign bodies and exposed to the rays shows only one more or less distinct difference in comparison with the control side—the inflamed tissue directly adjacent to the foreign body seems to be edematous and softer, and, especially after repeated exposures, the celloidin block appears surrounded by a more or less thick layer of a semifluid substance. For this reason careless dissection may easily permit the celloidin block to slip out of the surrounding tissue capsule. In the oldest case, killed 60 days after the last exposure, no difference was found in the macroscopic aspect of the exposed and the control tissues. In both the foreign body was closely wrapped in dense scar tissue.

In all the animals the results described below were in principle quite identical. The differences noted were only those of degree and depended on the number of exposures and on the stage of the inflammation. The effect of the x-rays was very distinct on the 5th day after a single exposure of a celloidin body introduced the day before; it was still more distinct after two exposures. The greater the number of exposures, the more distinct become the changes in comparison with the control side, and in the two rabbits exposed fifteen times they were pronounced on the 8th and 15th days after the last exposure.

### *The Granulation Tissue and the Exudate Layer Surrounding the Foreign Body.*

As an illustration of the earliest visible changes an example will be described in which the celloidin body had been exposed twice to a

dose of 12 x and had been fixed 11 days after the second exposure. The aseptic inflammation had thus had 13 days in which to develop.

In sections made parallel to the surface of the control block typical granulation tissue with beginning scar formation is found (Fig. 2). The fibroblasts (*Fbl*) have considerably increased in number and constitute a new tissue layer, enveloping the foreign body. Their flat cell bodies contain regular oval nuclei with dust-like chromatin granules and with the typical large nucleoli. Between the cell bodies are located the newly formed, thin, densely arranged, collagenous fibers (*C*). Everywhere between the fibroblasts are scattered the polyblasts (*Plb*). For a description of the latter the reader is referred to former papers (Maximow, 1902, 1903, 1904, 1905, 1906). Small, recently emigrated, lymphocyte-like polyblasts are rare. Most of these cells have already reached the resting stage and have flat, elongated or slightly branched cell bodies and typical, dark, irregularly folded nuclei. Since in this instance trypan blue was injected together with the blocks, the protoplasm of most of the polyblasts contains, on eosin-azure slides, distinct granules of a grayish blue color. Granulated special leucocytes are very scarce.

The newly formed granulation tissue is expanding outwards without sharply defined limits into the surrounding common normal loose connective tissue. Towards the surface of the celloidin is a thin layer of multinucleated syncytium-like giant cells, lymphocytes, phagocytic polyblasts of varying size, granular special leucocytes, and necrotic cell remains chiefly derived from special leucocytes. In the interior of the celloidin body the process of tissue invasion is going on—fibroblasts, singly and in groups, phagocytic polyblasts in different stages of development, innumerable, partly degenerated, special leucocytes, and outgrowths of capillary vessels are penetrating into the clefts.

The tissue enveloping the exposed blocks has a different aspect (Fig. 1). In fact, a newly formed layer of connective tissue is entirely lacking. The foreign body is surrounded by a rather thick exudate layer of peculiar structure; it has in a fixed preparation a loose net-like structure and gives at first sight the impression of clotted fibrin (Fig. 4). This can be easily confirmed by using Weigert's fibrin stain; the fine fibrils of the net are stained dark blue. Thus, the

celloidin block is embedded in a mass of clotted fibrinous exudate. It is the semifluid mass seen, as described above, on macroscopic examination and causing the ready dislodgment of the bodies exposed to x-rays. In the meshes of the net ameboid cells are scattered everywhere—special leucocytes (Fig. 4, *Lkc*), easily distinguishable by the polymorphic nuclei and the specific granules, stained bright red by eosin-azure, and polyblasts of different stages of development—every possible transition between the smallest lymphocyte-like cells (*Lmc*) and the large macrophages, crowded with trypan blue granules (*Plb*). Some of the polyblasts have branched pseudopodia or appear elongated and have a worm-like shape. Degenerating polyblasts with shrunken pycnotic nuclei are found here but rarely. Degenerating special leucocytes (*Lkc'*) are, on the contrary, numerous and show disintegrating nuclear debris and partly dissolved, partly scattered granules. They are the chief object of the phagocytic activity of the polyblasts mentioned above (*Plb'*).

On the surface of the celloidin of the x-rayed blocks are dense masses of small lymphocyte-like and large phagocytic polyblasts and great quantities of special granular leucocytes. Degenerating forms are especially common here, chiefly among the special leucocytes.

At the periphery of the layer of fibrinous exudate a wide, clear, highly edematous tissue zone is seen (Fig. 1). It is not newly formed, but old, deeply changed connective tissue. The thick, wavy collagenous fibers (*C*) appear swollen and the spaces between them are filled with clear liquid, containing in many places fibrin nets. In the direction towards the fibrinous layer these collagenous fibers are lost in the clotted mass. Towards the surrounding tissue they gradually pass over into the apparently unchanged collagenous fibers.

The fibroblasts in this edematous tissue layer lie widely separated from each other (Fig. 1, *Fbl*). Their number seems not to have increased at all and mitotic figures are not found within them. The cell body, as can be easily seen by referring to Fig. 1, *Fbl*, is no longer thin and flat, but appears considerably thickened and enlarged and shows either a polygonal or fusiform shape with long tapering ends. The protoplasm is strongly basophilic and stains dark after eosin-azure. The nucleus is also considerably enlarged; in the majority of the cells it keeps its regular oval form unchanged, but the chroma-

tin granules inside are coarser and the nucleoli appear to be much larger. In many fibroblasts the nucleus is showing unmistakable signs of fragmentation, of constriction into single parts of very different size (*Fbl''*). Near the nucleus in the protoplasm often a clear space is to be seen—a similarly hypertrophied attraction sphere.

The polyblasts in the edematous zone are much more numerous than in the capsule of the non-exposed control body, described above; they are scattered in irregular groups among the fibroblasts and look quite different. Their appearance seems to correspond to a much younger stage of development. For the most part they are still quite similar to lymphocytes; they are small, round, ameboid cells with an eccentric kidney-shaped nucleus (Fig. 1, *Lmc*). In the protoplasm the progressive accumulation of trypan blue granules is clearly seen. They are freshly emigrated lymphocytes on their way to be transformed into polyblasts (*Plb*). The minority of them are larger cells of very different, mostly fusiform shape with sharply outlined, sometimes dentated contours, with a dark, irregularly folded nucleus and with a varying quantity of trypan blue granules in the protoplasm; these are doubtless the local resting wandering cells on the point of being transformed, by a process of rounding off and mobilizing, into polyblasts (*Rwc*). In the polyblasts mitoses are not to be found; under normal conditions also, as is well known, they divide only rarely in the field of inflammation.

The edematous tissue contains also special granulated leucocytes in considerable numbers (Fig. 1, *Lkc*); they seem thus to continue their migration out of the blood vessels into this tissue even in relatively late stages.

The pathological cell forms just described become more and more rare as the normal connective tissue is approached, but one can find them occasionally as far away as between the adjacent muscle fibers.

In the clefts of the celloidin bodies no trace is to be found of an ingrowth of young connective tissue and vessels; one sees here only innumerable, mostly degenerated, special leucocytes and also some degenerated polyblasts in various stages of development.

In the cases in which the introduced celloidin bodies were exposed many times to the average dose of 12 x, a relatively high total dose, the changes are naturally much more accentuated. In two, in which,

after introduction of the bodies, fifteen exposures were made during 15 days and the tissue was fixed 8 and 27 days respectively after the last exposure, so that the total duration of the inflammation was 24 and 43 days respectively, the capsule of the control bodies is composed of common dense scar tissue with fibroblasts, containing fibroglia fibrillæ, and with a dense feltwork of newly formed collagenous fibers. Between the fibroblasts sessile polyblasts with polymorphous nuclei and often long and branched protoplasmic processes are scattered at regular intervals. This scar tissue adheres almost directly to the surface of the celloidin, and between the two are seen only few hypertrophic polyblasts and single, flat, multinucleated giant cells. In the more distant layers, especially in the second animal, nets of newly formed capillaries are seen, surrounded by groups of plasma cells and by pigmented sessile polyblasts.

In the clefts of these control preparations a fully developed connective tissue is already present. It consists of large fibroblasts with fibroglia fibrillæ, of sessile, polymorphous polyblasts with irregular nuclei, and of networks of new blood vessels with large groups of plasma cells.

In the exposed preparations on the surface of the celloidin a layer of partly degenerated small and large polyblasts and special leucocytes and for the most part degenerating giant cells are seen. The layer of the fibrinous exudate is still thicker than before. In the first rabbit it has the same foamy or net-like structure as before and contains, besides a few, mostly degenerated, leucocytes, relatively scarce, actively ameboid, chiefly small, lymphocyte-like polyblasts. In the second, 42 day case, it appears already slightly thickened and its net-like structure is no longer definitely outlined; now it seems to consist of dense, roughly fibrillated trabeculæ and irregular pieces and contains numerous, mostly small, ameboid, phagocytic polyblasts and special leucocytes. Among these cells one can find, especially now, many degenerating forms, and in some places the dense fibrinous mass is overcrowded with free pseudo-eosinophilic granules, coming from the degenerated and destroyed special leucocytes.

In the first case no real neoformation of tissue can be seen as yet. The exudate is surrounded just as before by the highly edematous tissue layer, which, however, is more extensive, spreading far between

the adjacent muscle fibers and showing still greater changes (Figs. 5 and 6).

The number of fibroblasts is little increased. As before, they lie far removed from one another. Rarely one succeeds in finding abnormal mitotic figures in them (Fig. 3, *x*); such divisions certainly can lead only slowly to real cell multiplication.

The hypertrophy of the fibroblasts has now become very marked, especially in the second case (Figs. 5 and 6, *Fbl*). With their strongly basophilic, flat, giant cell bodies and their numerous processes running in different directions and often branching, they resemble large nerve cells. The nuclei, mostly oval or kidney-shaped, and occupying an eccentric position, have attained a quite excessive size. Their interior is filled with numerous coarse, darkly stained chromatin granules partly covering the nucleoli, and contains sometimes large intranuclear vacuoles (Fig. 5, *Fbl'*). Whereas mitoses, as mentioned above, are extremely rare, amitotic constrictions of the nuclei are common (Figs. 5 and 6, *Fbl''*). The giant nucleus undergoes in such cases a constriction into two or more parts of very irregular size and shape. These parts, as a rule, occupy a peripheral position in the cell body, whereas in the middle of the latter a group of crowded centrioles is seen even after the eosin-azure stain, lying in a clear space, the sphere.

In the second rabbit there seem to appear in the fibroblasts the first signs of an altogether faint productive reaction; in some places they become arranged in flat layers of net-like texture and produce in their protoplasm distinct fibroglia fibrillæ.

Of neoformation of the collagenous fibrillar intercellular substance not much can as yet be seen. The clear edematous liquid between the hypertrophied fibroblasts still seems to contain only the old preexistent wavy collagenous fibers (Figs. 5 and 6, *C*). They appear partly swollen, partly dissociated into fibrillæ, and become lost towards the celloidin block, as they enter the fibrinous exudate.

The changes in the fibroblasts as above described must surely have been seen at least in part by earlier authors. Gassmann (1898–99, 1904) has made similar statements. As regards the hypertrophy of the cells under the influence of x-rays, Wickham may be quoted. According to him hypertrophy of various cells and, in the first instance, of their nuclei, is common after treatment with x-rays. He

finds it especially in malignant tumors, whose cells die in the condition of colossal enlargement. Edema and swelling of the collagenous fibers are occasionally mentioned in the papers of Gassmann (1898–99, 1904), Unna, Rost, and others. How far these changes can be brought into connection with the so called fibrinoid degeneration of connective tissue of Neumann is difficult to decide.

Whereas in the control preparations of the later stages the pseudo-eosinophilic special leucocytes are practically missing, they are still numerous in the exposed inflamed tissue (Figs. 5 and 6, *Lkc*). Thus, it has to be admitted that new cells of this kind are constantly arriving from the blood vessels. Many of them degenerate and are destroyed, spreading their granules throughout the tissue, or are engulfed by polyblasts (Fig. 5, *Plb'*). These granular cells proved to be especially numerous in the second of the above mentioned two cases, in which 27 days had elapsed after the fifteenth exposure.

As regards the polyblasts, they are also much more numerous than in the control preparations. Most of them belong still to the youngest lymphocyte-like variety (Figs. 5 and 6, *Lmc*). The relatively rare larger forms (*Plb*) evidently must be looked upon as the mobilized, local, resting wandering cells. Some of the latter still contain the deeply staining granular inclusions, typical for these cells in the rabbit. Their development, which in the control preparations has proceeded very far or has even been completed, appears to be in abeyance or checked, so that they all remain in a state corresponding to that of much younger stages of development. In the second case only a few of the larger polyblasts seem to tend toward quiescence, assuming a fixed, flat or elongated shape. Phagocytosis, especially in relation to the degenerated special leucocytes, is energetically continued by the polyblasts. As a result of this phagocytosis one may find in many places smaller and larger polyblasts, containing numerous acidophilic pseudo-eosinophilic granules in their protoplasm. Such elements sometimes may simulate myelocytes.

In the edematous tissue of the second of the two instances referred to above, the plasma cells are numerous (Figs. 5 and 6, *Plc*). Everywhere they, together with lymphocytes, common polyblasts, and special leucocytes, surround in large groups the enlarged capillaries. Among them many can be seen degenerating. On the other hand, once in a while, one finds mitotic figures in them.

In the clefts of the exposed blocks in the two last cases described there is no neoformation of tissue to be found. No fibroblasts are seen here, only living or degenerated leucocytes and polyblasts and their debris. In the second case the majority of the polyblasts in the clefts were transformed into large spherical macrophages, containing large numbers of peculiar needle-shaped crystalloids, deeply stained with eosin-azure, besides engulfed special leucocytes and their remains.

In the specimen of longest duration, in which the celloidin blocks were fixed 60 days after the fourth (and last) exposure, no distinct changes could be found in the tissue surrounding the foreign body in comparison with the control preparations. The celloidin block was surrounded by dense, tough scar tissue of usual structure. There could be noted only the presence of relatively numerous resting polyblasts in degenerated condition, with shrunken and disintegrating nuclei.

*Changes in the Blood Vessels.*

In all the experiments the capillaries in the edematous tissue layer were found to be enlarged to a greater or less degree; no special changes in the endothelium could be noted, except an occasional slight swelling. Of the other vessels, in the small veins only were distinct changes in the endothelium observed (Fig. 7). The cells appeared much swollen and were protruding knob-like into the lumen. The nuclei showed an exceedingly conspicuous fragmentation and underwent constriction into irregular vesicular parts (*End*).

A similar swelling of the endothelium in the blood vessels of the skin after exposure to x-rays has been described before by Gassmann (1898–99, 1904) and Linser. Such intensive changes in the other coats of the larger vessels and their obliteration, as described by Gassmann, I could not find. But that the endothelium of the blood vessels is in general very sensitive towards the Roentgen rays, apart from the presence of constant edema, can be inferred also from the observations of Bagg, who saw, after injection of radium emanation into pregnant rats, extravasations in the subcutaneous tissue of the embryos.

*Changes in the Muscles.*

A phenomenon regularly observed in all the experiments was the change caused by the x-rays in the muscular tissue of the abdominal wall, situated above or below the exposed celloidin blocks.

Corresponding data are to be found in the literature. Gassmann (1898–99, 1904) was probably the first to note in Roentgen ulcers in rabbits a peculiar degeneration of the striped muscle fibers. He has seen irregular shrinking and thinning of the fibers, with separation of the fibrils from each other, loss of the parallel position and partly of the cross-striation, resulting in transformation into a homogeneous mass; the nuclei multiplied and giant cell-like syncytia were formed. Between the muscular fibers he found connective tissue rich in nuclei and infiltrated with round cells, polymorphonuclear leucocytes, and plasma cells. Brief references are to be found also in the work of Grasnick and Fromme to dissociation of muscle fibrillæ and loss of cross-striation.

In my material distinct changes in muscular tissue are present as early as 11 days after the second of two ordinary exposures of 12 x each. In the two rabbits in which fifteen exposures were made, they naturally are much more accentuated, although still distributed very unevenly. In the first case, in which the preparations were fixed 8 days after the fifteenth exposure, the muscle fibers nearest the celloidin body (Fig. 8, *a*, *b*, *e*, *f*) are widely separated from one another by the edematous tissue with its swollen collagenous fibers (*C*). In some of them the typical picture of coagulation necrosis can be seen—highly swollen, thickened portions, where the longitudinal as well as the cross-striations are missing and the whole fiber is transformed into a homogeneous mass, partly broken up into pieces of various size (Fig. 8, *e*, *f*). The sarcolemma and the muscle nuclei may be still intact in such places (*f*); in many cases, however, the sarcolemma proves to be absent and the necrotic substance is attacked from many sides by ameboid phagocytic polyblasts and gradually resorbed (Fig. 8, *a*, *Plb*). The same fiber may show in its further course apparently normal structural conditions. In other fibers a gradual disappearance of the myofibrillæ is seen, either through the whole length of the fiber or only in some places. The fibrillæ lose their regular parallel arrangement, become separated from one another, and gradually disappear. At first their cross-striation remains unchanged (Fig. 8, *b*), but afterwards the Q-discs become indistinct and only the Z-discs remain (Fig. 8, *e*). Meanwhile the sarcoplasm accumulates at the periphery of the shrinking bundles of the myofibrillæ, under the sarcolemma, and causes here irregular bulgings on the external contour of the fiber (*e*). In such places the manner in which the Z-discs reach the sarcolemma and become attached to the latter.

so that it appears constricted and the fiber acquires a typical festooned outline, is clearly seen. During this gradual involution of the contractile substance, just as in other instances of muscle atrophy, the nuclei may also change their position and character; they often come to lie deep in clefts between irregularly separated fibrils (Fig. 8, *b*), sometimes even in the axial part of the fiber, and secondly, they undergo amitotic division (Fig. 8, *Am*), so that their number may increase considerably and they may become arranged in long rows.

In the second case, in which the foreign body was fixed 27 days after the fifteenth exposure, the muscle fibers adjoining the body present a still higher degree of change (Fig. 9). They are widely separated from each other by abundant, highly edematous connective tissue, containing many leucocytes (*Lkc*), polyblasts (*Plb*), and plasma cells (*Plc*). The swollen muscle fibers, in the advanced coagulation necrosis as described above, are no longer seen, and seem to be completely resorbed. The majority of the fibers are thin, shrunken bands of sarcoplasm (*k*), with scarce, irregularly arranged fibrillæ without any trace of cross-striation; they contain muscle nuclei, single or crowded in heaps (*N*); remains of a sarcolemma can be made out only in some few places. Often in the course of one and the same fiber, areas can be found with changes of very different degree, so that parts with a relatively well preserved general structure and especially with typical cross-striation alternate with parts that, with their crowded nuclei and their slightly granular or vacuolated protoplasm with scarce fibrils, resemble giant cells.

It is clear that the changes of the muscle fibers closely correspond to those described by Gassmann (1898–99, 1904).

### *Inflammation in Previously Exposed Connective Tissue and the Action of X-Rays upon Scar Tissue.*

In the three rabbits, in which, as stated above, the celloidin blocks on one side of the abdominal wall were introduced in areas previously exposed several times to a double dose of x-rays, comparison with the control preparations of the other side showed changes absolutely corresponding to those described above for the usual experiments.

Especially in the first two animals the same fibrinous exudate occurred around the foreign body, the same edema, the same hyper-

trophy of the fibroblasts, and the same inability of the latter to divide (Fig. 3, *Fbl*). Sometimes in the fibroblasts abnormal mitosis could be detected (*x*). Furthermore, the tissue was also overcrowded with abnormally numerous leucocytes (*Lkc*) and mostly young lymphocyte-like polyblasts of hematogenous origin (*Lmc*), whereas the local resting wandering cells seemed hardly changed (*Plb*). In the rabbit in which the inflammation lasted only 3 days and lithium carmine had been injected, together with the foreign bodies, into the connective tissue, all the polyblasts, even the youngest lymphocyte-like cells, which surely must have come from inside the blood vessels, contained distinct granules of carmine.

In the two animals mentioned above, in which scar tissue had been exposed, there were found no changes in the tissue elements resulting from the exposure.

## DISCUSSION AND CONCLUSIONS.

The action of x-rays upon inflamed tissue manifests itself in the first place by a considerable depression of the usual reaction on the part of the fibroblasts. Under normal circumstances these elements begin to divide mitotically during the first 24 hours and soon form a layer of new connective tissue, surrounding the foreign body. After treatment with x-rays they remain idle, do not multiply at all, or start very late and often the division is abnormal. They undergo a high degree of pathological hypertrophy of protoplasm and nucleus. Instead of mitosis often amitotic constrictions appear in the nucleus. The capacity for collagen formation seems also to be lost.

Simultaneously with these changes of the fibroblasts an intensive edema of the connective tissue surrounding the foreign body is to be noted and in the immediate neighborhood of the latter a thick layer of net-like clotted fibrinous exudate is formed.

No distinctive qualitative changes could be found in the leucocytes and polyblasts. Degeneration was present here only to the same extent as in common aseptic inflammation. But first the rate and the duration of the emigration of all the cells coming from the blood were increased, and secondly there was always a distinct delay in the

process of the common transformations usually undergone by the polyblasts on the field of inflammation. The transformation of the polyblasts into fixed resting forms seems above all to be delayed. Therefore, even in late stages, the tissue is overcrowded with granular special leucocytes and with mostly young, lymphocyte-like polyblasts, whereas in the early stages the local resting wandering cells only slowly undergo mobilization.

Furthermore, in the blood vessels swelling of the endothelial cells with fragmentation of the nuclei and, in the striated muscles, degeneration of the fibers can be detected. In the latter there occur partly typical coagulation necrosis, partly atrophy, accompanied by loss of striation, separation of fibrillæ from one another, relative increase of sarcoplasm, and amitotic division of nuclei.

What the ultimate result of all these changes would be, is as yet not clear. In the case of longest duration, in which 60 days had elapsed since the last exposure, no distinct difference could be found between the exposed and control preparations. Thus one might believe that the cell injuries caused by the x-rays, and above all the inability of the fibroblasts to multiply and to elaborate collagen, are again repaired in due time. However, my material is decidedly inadequate in this respect and several cases of long duration should be examined.

It is surprising that the results obtained seem not to agree with the predominating views on the action of x-rays on cells. Apart from the endothelium of the blood vessels, of all the cells present in the field of inflammation the fibroblasts undoubtedly are to be considered as the elements most highly differentiated in a specific sense. I have shown that, as a rule, they do not round up in inflammation and do not produce ameboid cells, but remain unchanged in morphology and, through mitotic division, give rise to the new connective tissue. On the other hand, there can be no doubt that the lymphocytes and the polyblasts are to be looked upon as relatively indifferent cells, endowed with great prospective potencies of development. Thus it might be expected that just the lymphocytes of the inflamed area would be affected in the first place by the rays, as they are in the blood-forming organs, and that the fibroblasts, on the contrary, would be refractory.

But the facts have proved that the most conspicuous and constant changes concern the fibroblasts. They are paralyzed for a long time and made unable to build up new tissue. The fibrinous exudate and the edema might perhaps also depend partly on a direct injury of the colloidal intercellular substance, partly on changes of the endothelium of the blood vessels, cells which are again to be considered as highly differentiated.

Noteworthy signs of degeneration could not be found in the lymphocytes and polyblasts. But here again the necessary early stages 1 to 3 days after the last exposure were not available; it is possible that the emigrated lymphocytes are destroyed by the x-rays rapidly, in an explosive manner, in 24 to 48 hours, as in the lymph nodes or the thymus, or as described by Pautrier in the chronically inflamed tissue of the skin in mycosis fungoides. Their remains might be quickly resorbed and after the last exposure new lymphocytes would have time to emigrate out of the blood vessels and to pass to the tissue. However, if we take this for granted, there remains another inexplicable fact, concerning the local resting wandering cells—their close genetic relationship with the lymphocytes is beyond doubt and yet exposure to x-rays does not seem to affect them. In this connection it may be stated that Soper found that the reticulo-endothelial apparatus, whose cells correspond to the resting wandering cells, is stimulated by small doses of x-rays and paralyzed by large doses. For deciding these problems further investigations are necessary.

The classical researches of O. Hertwig and his school have proved beyond doubt that of all the parts of the cell, the nucleus with its chromatin is affected most by the rays. It is believed that the nuclei in mitotic division are especially sensitive (Holthusen) and that the nuclei of exposed cells lose, in the first place, their capacity for normal mitosis; they either do not undergo division at all or they show pathological mitoses (Grasnick, Gaskell, and others). The observations described above fully coincide with these statements. The nuclei of the hypertrophied fibroblasts attain an enormous size, contain an abnormal amount of chromatin, are sometimes vacuolated, and appear paralyzed and incapable of mitosis or divide abnormally. Perhaps the reason that the fibroblasts in the present experiments are so

strongly affected by the x-rays is that they are the only cells which are preparing for mitotic division directly after the introduction of the foreign body, whereas the lymphocytes and polyblasts only rarely divide in the field of inflammation. The fibroblasts thus are becoming especially sensitive towards the rays, whereas for example in the scar tissue, where they remain quiescent, they are not affected.

As the inflammatory changes in the normal skin after exposure to x-rays are not known sufficiently from the histological standpoint it would be promising to study the action of x-rays on the normal loose connective tissue.

It is evident that the changes in the cells of the inflamed area, chiefly in the fibroblasts, but also in the muscle fibers, under the influence of Roentgen rays, are the result of complicated interrelations between two different agents, first, the inflammation stimulus, and, second, the radiant energy. Neither agent alone in the doses used is able to produce the changes observed. Only the combination of both gives the results described above. It seems to be immaterial, to a certain degree, which of the two stimuli is applied first—whether the foreign body be introduced in previously exposed tissue, or the latter be exposed after the introduction of the body—in both cases practically the same results have been obtained.

The strong inhibitory and deleterious influence of x-rays on inflamed connective tissue ought to be always kept in mind in the therapeutical use of this kind of energy, especially in cases of malignant tumors, in which the local connective tissue in most cases is found in a state of inflammatory irritation.

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## EXPLANATION OF PLATES.

All the figures represent sections of the tissue surrounding the celloidin blocks and cut parallel to the surface of the latter. They have been drawn from slides, stained with eosin-azure. A Zeiss achromatic immersion lens $\frac{1}{12}$ and a compensating ocular 4 have been used.

*C*, collagenous fibers; *End*, endothelium; *F*, fibrin; *Fbl*, fibroblasts; *Fbl'*, giant fibroblasts; *Fbl''*, fibroblasts showing amitotic division of the nuclei; *L*, lumen of a blood vessel; *Lkc*, special (pseudo-eosinophil) leucocytes; *Lkc'*, degenerating special leucocytes; *Lmc*, emigrated small lymphocytes (young polyblasts); *Plb* polyblasts; *Plb'*, polyblasts with remains of engulfed special leucocytes in their protoplasm; *Plc*, plasma cells; *Rwc*, resting wandering cells undergoing transformation into large polyblasts; *Smc*, smooth muscle cells; *V*, blood capillaries.

### PLATE 18.

FIG. 1. Inflammation of 13 days; two exposures of 12 x each.

### PLATE 19.

FIG. 2. Inflammation of 13 days. Same animal as in Fig. 1; control.

FIG. 3. Inflammation of 3 days in an area of connective tissue which had been previously exposed on 3 consecutive days to a dose of 24 x and after that had rested for 5 days. *x*, abnormal mitosis of a fibroblast.

### PLATE 20.

FIG. 4. Same block as in Fig. 1. Fibrinous exudate surrounding the foreign body.

FIG. 5. Inflammation of 42 days; fifteen exposures of 12 x each.

PLATE 21.

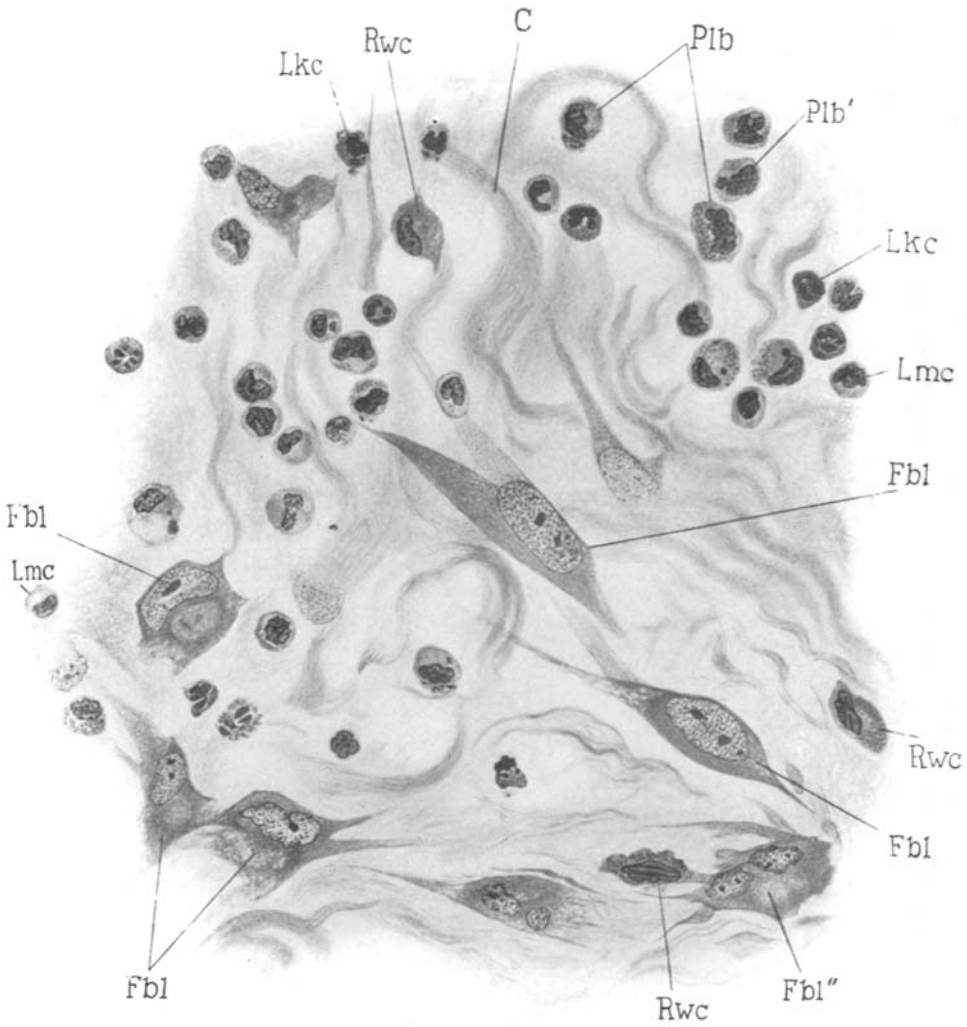
FIG. 6. Inflammation of 42 days; fifteen exposures of 12 x each.

FIG. 7. Same block as in Fig. 1. Oblique section of the wall of a small vein in the neighborhood of the exposed foreign body. Swelling and fragmentation of nuclei in endothelial cells.

PLATE 22.

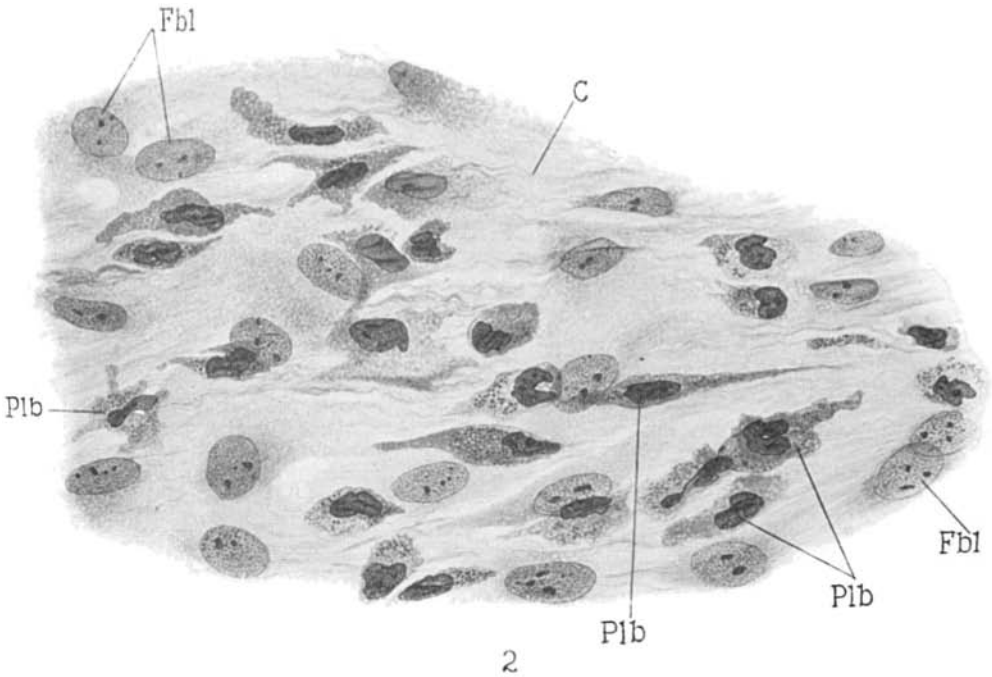
FIG. 8. Inflammation of 23 days; fifteen exposures of 12 x each. Changes of the muscular fibers adjacent to the foreign body. *a*, *f*, *b*, *e*, different stages of degeneration of the fibers; *Am*, amitosis of a muscle nucleus.

FIG. 9. Same block as in Figs. 5 and 6. Later stages (*k*) of degeneration of the muscle fibers; *N*, groups of muscle nuclei.




1

(Maximow: Roentgen ray changes in inflamed tissue.)

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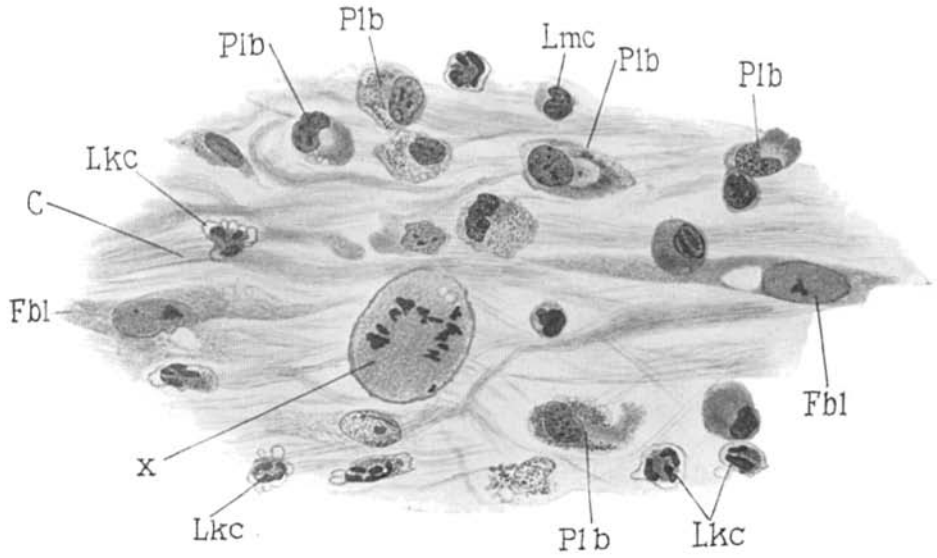
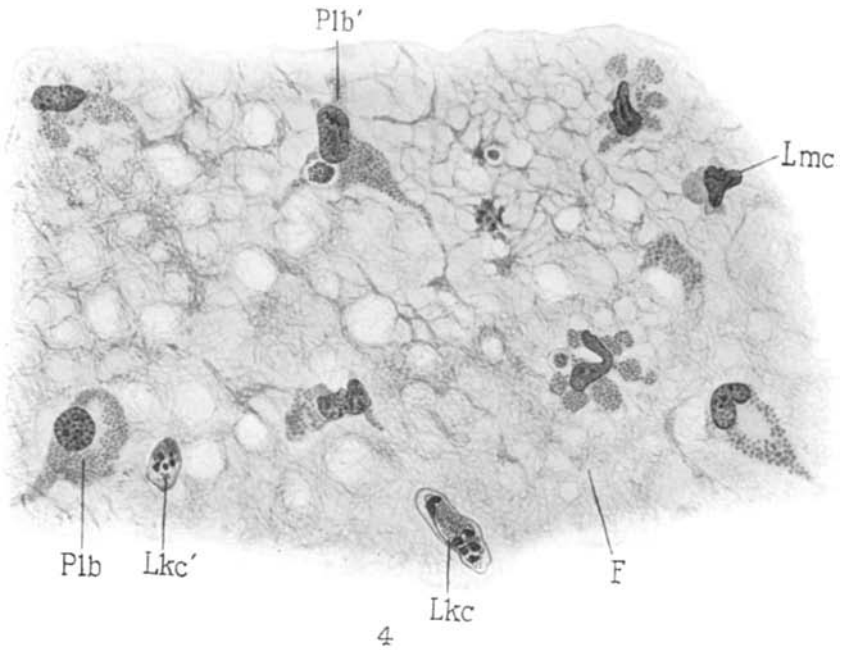



3

(Maximow: Roentgen ray changes in inflamed tissue.)

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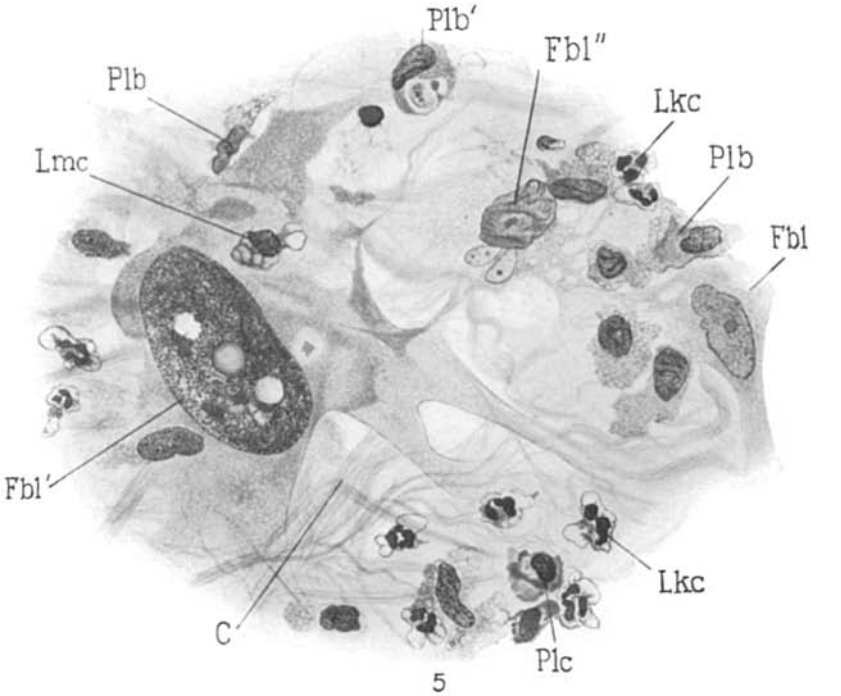
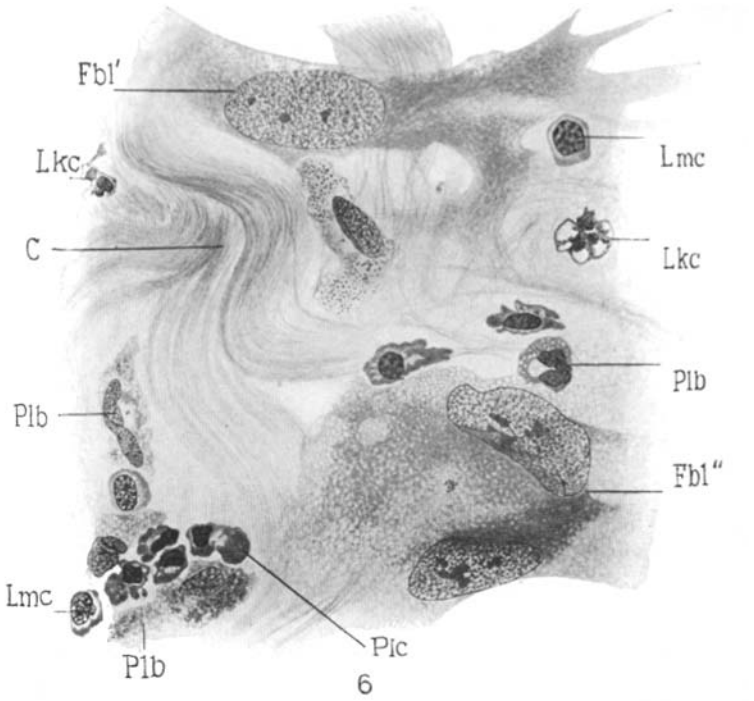
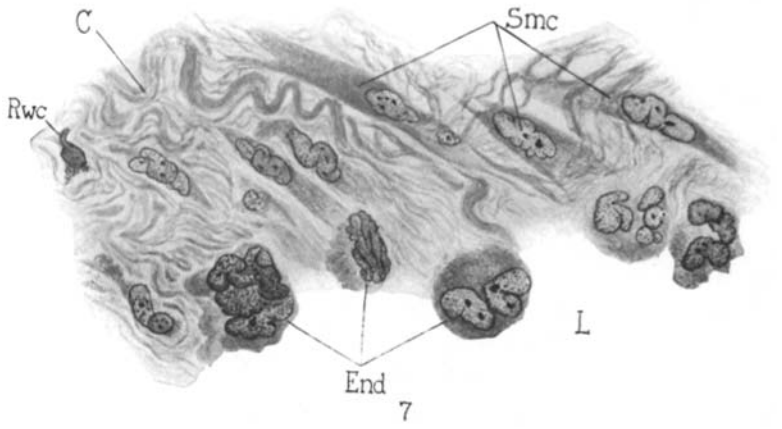



5

(Maximow: Roentgen ray changes in inflamed tissue.)

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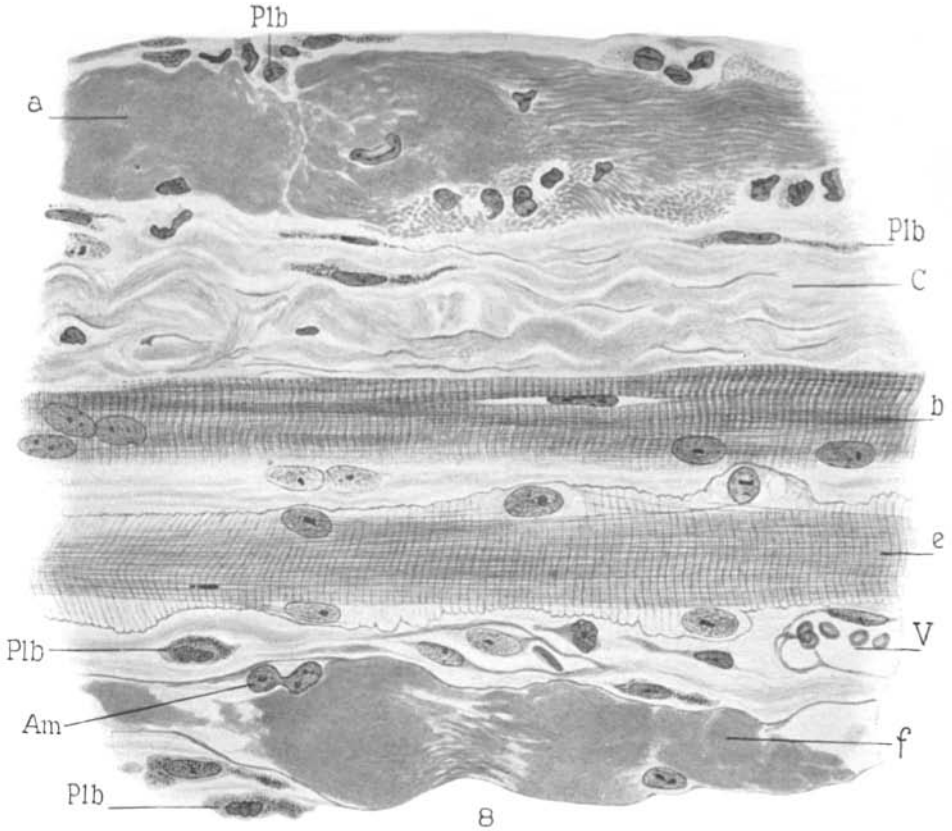
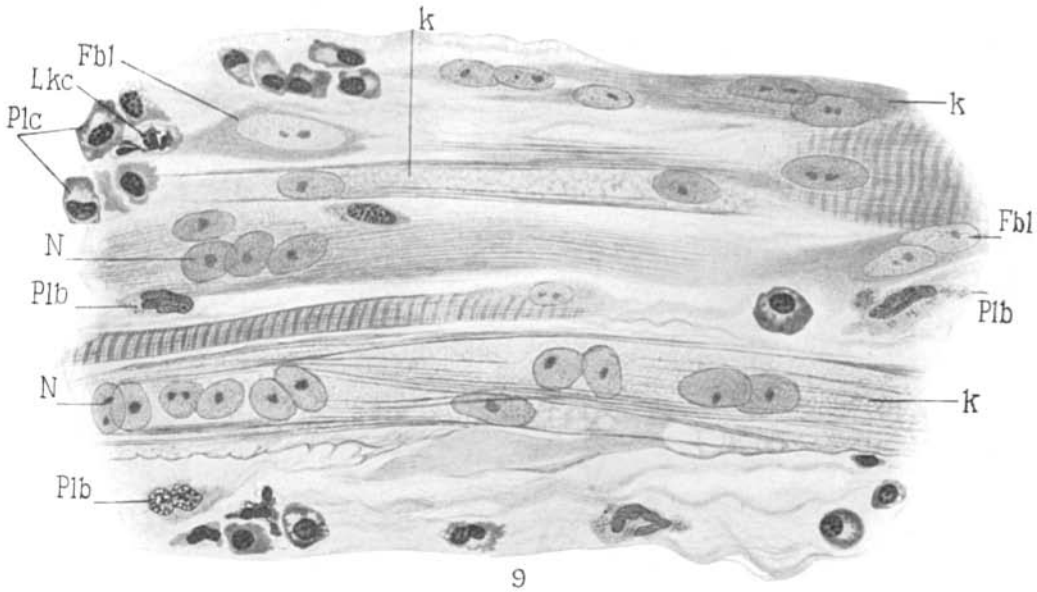



8




9

(Maximow: Roentgen ray changes in inflamed tissue.)