THE EFFECTS OF X-RAY ON THE RESISTANCE OF THE SKIN TO STAPHYLOCOCCUS INFECTION

by

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I

INTRODUCTION

It has been observed in clinical practice that furuncles of the skin respond favorably to treatment with Roentgen rays. Since the micro-organism usually responsible for the boils, namely staphylococci, are not themselves affected by the dosage of X-ray used therapeutically, the reasons for the favorable response must be connected with the tissue reaction. The present study was undertaken to determine the nature and extent of these tissue reactions in experimental animals.

Shortly after the discovery of X-ray by Roentgen in 1895, it was noted that pro-longed exposure of the skin to the rays produced erythema, dermatitis and even ulceration. This observation resulted in the introduction of the new ray as a therapeutic agent. It was soon observed that each variety of cells in the body has a specific range of sensitiveness to X-rays. That is when exposed to the same dose of rays under the same conditions some cells are influenced more rapidly and to a greater degree than other types of cells. Some cells such as the leucocytes (particularly the lymphocytes and polymorphonuclear cells) are most susceptible to irradiation, while other cells especially the nerve cells are highly resistant. Between these two extremes the other varieties of cells can be arranged, according to their vulnerability, in a definite order:

1. Lymphoid cells (lymphocytes in the spleen,

lymph nodes, intestinal lymph follicles, circulating blood, bone marrow, thymus, tonsils and other stuctures in which such cells may be present.)

2. Polymorphonuclear leucocytes and Eosinophiles in the blood stream.

3. Epithelial cells: Basal epithelium of certain secretory glands especially salivary glands;

Basal epithelium (spermatogonia cells of the testis and follicular epithelium of ovary.)

Basal epithelium of the skin, mucous membranes and certain organs such as the stomach and intestines;

Alveolar epithelium of the lungs and epithelium of bile ducts;

Epithelium of tubules of the kidney.

4. Endothelial cells of blood vessels, pleura and peritoneum.

5. Connective tissue cells.

6. Muscle cells.

7. Bone cells.

8. Fat cells.

9. Nerve cells.

There is not only a variable response seen in different kinds of cells to the same amount of irradiation, but even cells of the same kind maintained under constant environmental conditions evince a wide variation in sensitiveness to the ray. This variation in effect is correlated first, with the phase of metabolism in which the cell happens to be when irradiated and second, with the degree of differentiation of the cell.

Although there is this wide variation in response by different kinds of cells and even with the same kind of cells, the effect of the rays on all cells appears to follow about the same pattern. The difference in results seems to be one of degree and speed of reaction. Since the effects of irradiation occur most rapidly and to the greatest extent in the lymphocyte, a description of the changes produced in this cell will afford a picture of the effects produced by irradiation on cells in general.

After a longer or shorter latent period, changes begin to take place. The translucent protoplasm becomes turbid and then finely granular and the proteins normally in a finely divided state, form visible aggregates. The cells lose their motility and remain completely quiescent. If the irradiation has not been too severe, the cells resume their activity, return to normal appearance and divide normally.

If the irradiation is sufficiently severe, the cells which are paralyzed never regain motility, but begin to disintegrate, sometimes almost explosively.

In addition to the changes observed in living cells others can be followed in stained preparations. Cytolysis occurs, the chromosomes shrink, clump together, and group themselves irregularly. The chromatin falls in fragments and the nucleus is destroyed. The protoplasm becomes more viscous, and vacuoles are formed in it. Cells in which these changes occur never recover.

In addition to these specific changes in cell structure and behavior, certain physiological reactions are affected. There is an increase in hydrogen-ion concentration, which may be very transitory in some tissues, but which may persist for hours or even days in other kinds of tissues.

With the change in acidity is associated an increase in the permeability of the cell membrane. Such a condition may allow the acid content of the cell to escape into the tissues, increasing the acidity there, and causing the increase in the viscosity of the protoplasm.

And so in general the same destroying effects are seen in all types of cells---the polymorphonuclear leucocytes, epithelial cells, connective tissue cells, muscle, bone, fat and nerve cells---and although some cells respond to a much less degree, no living organism has been found which will not thus be influenced if sufficient radiation is administered.[2]

The earliest biological experiments, within a few months after the discovery of X-ray, were a search for bactericidal effect. Bacteria in pure cultures can be killed with sufficiently large doses of radiation. Most of them are so resistant,

however, that any attempt to destroy them as a clinical measure, would require doses which would be dangerous or even lethal for their hosts.[2]

II

MODE OF ACTION OF RADIATION ON CELLS

It is obvious that these series of changes produced in the cells result from the cells or tissues absorbing the energy from the rays. Therefore, the question is how the absorbed energy acts upon the cell molecules.

The first result of the impact of radiation on matter is the production of secondary radiations in the form of moving electrons. These electrons ionise atoms in their paths. The ionized atom is temporarily in an abnormal condition, and while in this state it lends itself readily to entering into new combinations. Ionisation of the individual molecule lasts only a very short time. If, however, enough of them are ionized simultaneously, a large enough percentage of the cell constituents may undergo transformation so that an apparent change is produced. This theory has been elaborated by Holthusen and Lacassagne.[2]

A different point of view is that held by Dessauer.[2] He considers that the point of actual attack in the cell is the large protein molecule, and that cell changes are brought about not by chemical transformation but by an actual coagulation analogous to the action of heat. The energy absorbed by the tissues even for very large doses of radiation is very small in comparison to the amount necessary to produce complete coagulation of the aggregate of molecules. in the cell. Dessauer avoids this difficulty by his "point heat hypothesis". He assumes

that there must be a certain number of direct impacts of secondary electrons with atoms, which result not in ionization but in actual increase in the velocity of the entire atom, a warming up. (It should be remembered that increase in temperature actually only means increase in atomic or molecular velocities within the solid matter.) He considers that if a sufficiently high percentage of the molecules of a given cell--1/100 to 1/10 are thus warmed up, coagulation will occur.

Failla[2] has offered the most helpful theory for the action of radiation on the cells and its nucleus. It combines features of both above mentioned concepts and builds from them a really workable hypothesis. He suggests that a certain portion of the cell proteins are broken down during the radio-ionization into simpler substances of the type which dissociate, or ionize electrolytically. When this occurs, the ion concentration of the fluids both inside and outside the cell increases. The fluids outside the cell are diluted or carried away by the circulation; those inside cannot be thus affected. In the effort to equalize the concentration inside and outside the semi-permeable cell wall, fluid will enter the cell causing it to swell. This process will be hastened if the membrane has itself been weakened by direct hits. Subsequently a similar effect takes place within the nucleus, as the cell fluids outside the nucleus become more dilute than those inside, by the process just mentioned. The swelling of the cell and of the nucleus are well known radiation effects.

III

THEORIES OF TISSUE RESISTANCE TO BACTERIAL INVASION

Every infectious disease is the result of a struggle between two variables--the pathogenic powers of the bacteria on the one hand and the resistance of the subject on the other. Thus, a microorganism may be capable of causing fatal infection in one individual, but may be moderately virulent or even entirely innocuous for another. Conversely, the same individual may be highly susceptible to one variety of bacteria but highly resistant to others. It is this problem of resistance--what it is, how it is established--that has demanded the best efforts of workers since the eighteenth century and is still not understood in its entirety.

There is no single factor upon which we can say that the resistance of a species against a particular organism or virus depends. In a few instances the unfavorable environmental conditions offered to the bacteria within the host is a possible defense mechanism. For example, some diseases of warm-blooded animals do not affect the cold-blooded species due to the change in temperature. This is clearly revealed by the classical experiments of Pasteur, Joubert and Chamberland[33] who were able to decrease the resistance of chickens to anthrax bacillus by lowering the body temperature that the bacteria were able to invade and kill these normally refractory animals. Conversely, Gibier[34] infected frogs with anthrax by keeping them in water at thirty-five degrees Centigrade, and Nuttall[35] secured a similar result with plague bacilli in lizards by holding the latter at a

temperature of twenty-six degrees Centigrade.

Differences in metabolic processes, oxygen tension, carbon dioxide production, water relations may likewise present barriers against infection. Such hypotheses, although logical enough, have at present little or no direct experimental support, so that while interesting they have been considered but as ideas and points of departure for further investigations.

Much more definite is the role of phagocytic cells and natural antibodies in establishing immunity. The researches which demonstrate parallelism between these factors and resistance of species as a whole will be outlined briefly here.

The two lines of investigation were begun almost simultaneously, one centering upon the activities of cells especially as manifest in local inflammations, the other upon the possible protective powers of the body fluids, particularly the blood plasma.

As early as 1870 pathological anatomists observed the presence of microorganisms within the cells of the animals and human tissues. Hayem,[31] Klebs,[32] Waldeyer[33] and others saw leucocytes containing bacteria but failed to interpret this in the sense of possible protection. The process was regarded rather as a means of transportation of the bacteria through the infected body, or it was assumed that possibly the microorganisms entered these cells because of the favorable nutritive environment thus furnished.

The first to suggest that such cell ingestion might

represent a method of defense was Panum[36] who referred to it as a vague possibility.

The significance of cell ingestion as a mode of protection against bacterial invasion was hardly more than a vague suggestion when Metchnikoff[37] began to experiment with a small crustacean, the daphnia, in which he studied the reaction which followed the introduction of yeast cells. He observed the struggle which ensued between the amoboid leucocytes of the crustacean and the infecting agents and determined that complete enclosure of the yeast within the leucocytes assured protection to the daphniz, while a failure of this process, either from fortuitous causes or because of too large a quanity, or too high a virulence of the infecting agents, resulted in disease and death. This early work forms the beginning of a train of investigations to which we owe most of the basic facts we possess concerning the role of the phagocytic cells in the protection of the body against infection.

Many studies have been made to determine which cells of the body of higher animals can take in and digest foreign particles and to classify them according to this power. On the basis of these studies, a large variety of cells distributed widely throughout the body have been grouped together under the heading of the "Reticulo-Endothelial Metabolic Apparatus" or Metchnikoff's Macrophage System.

The following table is a classification of those cells exhibiting definite ability to ingest foreign matter.

Microphages Granulocytic elements of the Circulating blood			Macrophages Mononuclear cells: fixed and wandering cells of the tissues and of the blood				
Neutrophiles (most active)	Eosinophiles (Confliting reports as to activity.)	Basophiles (Slightly active.)	Fixed endothelial cells of the blood and lymph sinuses of certain organs. (Most active.)	Mobile histiocytes of the connective tissue. (Very active.)	Septal cells of the lung. (Very active under certain conditions.)	Splenic pulp cells and blood monocytes. (Moderately active)	Reticular cells of the Spleen and Lymph nodes. (Least active)

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The early works of Denoys,[38] Leishman,[39] Wright,[40] Rosenow[41] and Penn[42] although made in vitro formed a chain of studies which have lead to the present understanding of the mechanism of phagocytosis. Within the living body the various constantly changing environmental circumstances which may influence the phenomena obviously are even more difficult to analyze.

In attempting to elucidate the mechanism of phagocytosis two stages may be recognized: In the first the cell comes in contact with the particle and in the second the particle is engulfed by the cell.

When the animal body represents the theater of phagocytic activity, the first stage——the coming together of foreign particle and phagocytic cell——is not dependent on chance. When a pathogenic bacterium enters the body, it frequently arouses an inflammatory response surrounding the point of entry, characterized by increased capillary permeability permitting the escape of leucocytes and the fluid constituents of the blood into the tissue

spaces. Here these phagocytic cells "crawl" about by means of the pseudopodial extension and retraction of the cytoplasm. These amoboid-like movements follow a very definite pattern which is believed to be due to a chemical attraction and not just to chance. This chemotaxis may be either positive i.e. attractive, or negative, i.e. repellant, depending on the nature of the material. A good example of the influence of the chemical nature of the particle in attracting the phagocyte is again an experiment of Fenn,[43] who allowed a suspension of cells and equal number of particles of MnO_2, and $MnSiO_3$, to run under a coverslip on a slide and observed the ensuing phenomena under the microscope. With MnO_2, 2.4 times as many encounters took place as with $MnSiO_3$, resulting in the phagocytosis of twenty times as many particles of the former substance. This selective motion of the leucocytes toward these substances indicates a reaction on the part of the cell to changes in its environment set up by the particle. Leber,[44] in the course of his studies on inflammation was one of the first to examine the chemotactic properties of certain substances on leucocytes. He found that these cells were actively attracted by powdered gold or iron. Dead bacteria, he also observed, exerted a similar positive chemotactic influence, and Buchner[66] later succeeded in extracting from Friedlander's bacillus a protein exhibiting strong attraction for leucocytes. Moreover, the latter noted that glycine and leucine were definitely chemotactic, whereas tyrosine and trimethylamine were inert in this respect. It appears from these and other investigations that positive chemotaxis is an attribute of all bacteria, equally apparent in bacterial extracts

or living and dead organisms. It is likely, therefore, that the attraction of leucocytes toward the point of invasion is in part, at least, due to the chemical properties of the bacterial aggressors. The work of Massart and Bordet[45] demonstrated that this migration of leucocytes in inflammation was probably not exclusively conditioned by the chemotactic effect of the microorganisms. Their findings showed that even products of leucocytic disintegration might be chemotactic. It would seem therefore, that when many types of tissue injury occur, a stimulus results which attracts leucocytes. This accounts for the migration of these cells into inflamed areas not of bacterial origin, as well as local accumulation following the injection of insoluble inorganic substances. Lately Menkin,[46] reinvestigating this problem, has isolated from sterile inflammatory exudates a crystalline nitrogenous substance revealing distinct chemotactic capacity in extremely small quantities. This material, tentatively termed "leukotaxine", the chemical nature of which has not yet been completely identified but which in the best preparations takes the form of needle-like crystals, also appears to be responsible for increased capillary permeability.

Various explanations have been advanced to account for chemotropism. Thus Maltaner and Hoppe[47] have asserted that osmotic forces govern the movement of phagocytes toward particles from which dissolved substances are diffusing, since they observed that leucocytes enter capillary tubes only when they contain solutions of greater concentrations of certain materials than that of the medium in which the leucocytes are suspended. However, Wolf[48] has presented data which indicate that the chemotactic

properties of a number of salts vary independently of the osmotic pressure of their solutions. Others have considered the possibility that differences in the surface potential between the leucocytes and the particles or injured tissues might be sufficient to exert an attractive force between them. Abramson and Feringa[49] have suggested the possibility that immigration of leucocytes into areas of inflammation depends upon a difference in potential between the phagocytes and the injured tissue. But as Wells[50] points out, relatively high hydrogen-ion concentration of the tissues which could induce a change in potential, cannot be the single determining factor in the migration of leucocytes since in certain conditions where this obtains, such as violent muscular activities or local asphyxia, no significant assemblage of these cells is observed.

There is, however, evidence from the studies of Menkin[51] that the hydrogen-ion concentration plays a definite role in determining the prevailing type of cell in an inflamed area. The developing local acidosis conditions the predominating cell in the exudate, but apparently not through a chemotactic mechanism. Thus this author found that when the pH of the tissue, such as in the earlier stages of inflammatory processes, is above 7, polymorphonuclear leucocytes are the most numerous among the cellular elements. With increasing hydrogen-ion concentration attendant upon the progress of the lesion, the macrophage supplants the multi-nucleated cell. These researches indicate that the viability of leucocytes in exudates is a function of the hydrogen-ion concentration. The polymorphonuclear cells are incapable of

surviving in an acid medium. Mononuclear phagocytes, on the other hand, display perfect resistance in media at pH ranging from 7 to about 6.8. At greater hydrogen ion concentration these cells likewise succumb and frank suppuration results.

The hypothesis concerning chemotropism that most nearly seems to coincide with the majority of the facts would regard changes in the surface tension of the leucocyte brought about by soluble substances emanating from the particle or injured cells as the fundamental factors in the mechanism of the phenomenon. In considering the possible effect of surface tension in determining the motions of unicellular organisms, attempts have been made to imitate these by means of various life-less systems. Bernstein,[52] Rhumbler[53] and others have produced "artificial amebae" which in almost all respects behaved like the living organisms by placing globules of mercury in acidified water containing crystals of potassium dichromate. As the latter begins to dissolve, diffuses toward the mercury and touches it, the mercury globule will begin to become elongated and often move in the direction of the remaining undissolved dichromate. In addition, Rhumbler showed that a drop of clove oil in the presence of alcohol and glycerin becomes motile, and that a globule of chloroform in water will move toward a particle of shellac, flow about it, and dissolve it.

m The similarity between these phenomena referable to surface tension alone and those taking place in living cells is therefore very striking. Wells[50] has considered them at length and emphasizes the possibility of their relationship. He points out

that positively chemotactic substances diffusing from a given direction and reaching the leucocyte will lower its surface tension on the side at which they come in contact, and there in consequence pseudopodia will be thrown out. The leucocyte will then move toward the point where these substances originate. Motion in a given direction will continue as long as the concentration of the chemotactic material is greater on this same side. When the concentration becomes equal on all sides, motion will cease.

This theory that surface changes determine the tropism of leucocytes, although conforming to many of the observed facts, has not been universally accepted by all investigators. Furthermore, certain experimental findings more directly concerned with the actual ingestion of particles, which will be considered immediately, are admittedly difficult to account for on the basis of alteration in surface tension alone. We cannot conclude, therefor, that all the elements in the mechanism of leucocytic chemotropism have been entirely revealed, although there can be little doubt that surface relationships frequently play a major role.

The condition underlying the second stage of phagocytosis, i.e., ingestion, are also in part due to the forces of surface tension. In addition, other factors enter into and modify this process. Of these the viscosity of the protoplasm of the cell is perhaps of foremost importance. This varies with temperature changes---At low temperature Ledingham found that particles stick to the cell but do not enter it, but as the temperature is raised changes in the internal state of the protoplasm occur

accompanied by the ingestion of the particles.

A few observations have been recorded on the changes induced in phagocytic systems by variations in osmotic pressure relationships. Hypotonic solutions increase the ingestion while hypertonic tends to inhibit the reaction.

The experiments of Hamburger,[55] Evans[56] and Fenn[57] indicate that optimum phagocytosis occurs at about neutrality or in some cases slightly on the acid side. Any marked increase or decrease in the pH brings about definite diminution in the phagocytic index. This may be due to injury of the leucocytes by increases in the hydrogen ions or as Mudd has suggested that changes in the pH may affect the combining properties of serum constituents and particles.

Bacteriophage, which according to the latest statement of Northrop[58] probably is of the nature of a nucleoprotein, was found by d'Herelle[59] to increase the susceptibility of bacteria to phagocytosis. Gerards[71] has shown that following the injection of phage into a rabbit the phagocytic index for the leucocytes and serum of that animal is markedly increased even after a period of seven days. The phage seems to affect both the bacteria and the cells, so that the former become more susceptible to phagocytic attack and the latter more active in ingestion.

In those instances where complete ingestion does not occur, the fibroblasts are stimulated to form a barrier around the point of infection, thus walling off and preventing the spread of the germs.

Others turned to the blodd plasma as possibly responsible for the protective mechanism, largely because of observations

like those of John Hunter,[61] of Traube[61] and Gscheidlen,[61] and of Lord Lister,[61] who had noted that shed blood did not putrefy as rapidly as did many other organic substances. In 1884, Grohmann[62] determined the inhibitory action of cell-free blood plasma upon bacteria; Fodor,[63] in 1887, and Nuttall,[64] in 1888, showed that fresh normal blood possessed the power of actually killing bacteria (bactericidal power). Nuttall repeated Metchnikoff's experiments on anthrax, in which he had shown the phagocytic destruction of these organisms in rabbits, and confirmed the observations, but interpreted them as indicating that the phagocytosis merely removed the bacteria killed by the blood plasma. Nuttall detected similar bactericidal properties in pleural exudates, pericardial fluids, and aqueous humor, and determined that this property was "inactivated" or destroyed when the fluids were heated to fifty-five degrees Centigrade for ten minutes or longer. Buchner[65] confirmed Nuttall's results and showed further that the bactericidal property resided, not only in defibrinated blood, peptone blood, and plasma, but was present also in the serum obtained after clotting. He applied the term "alexin" to this active constituent of the blood---likening its action to that of a ferment. Ehrlich later applied the name "compliment" to this substance.

These findings stimulated the investigation of the blood serum of immunized animals of all kinds for constituents that would react in the test tube with bacteria and their products or which would protect normal animals by passive transfer. The result was the discovery of the antibodies, the study of which forms

the basis of most of our immunological knowledge.

The first of these factors in the blood serum was found by Pfeiffer,[66] spoken of as the "Pfeiffer Phenomenon" definitely proved that active immunization with bacteria incites in the serum of the treated animal a potent increase of bactericidal properties--an increase which is entirely specific in that the bactericidal power toward bacteria other than those employed in the immunization does not exceed the normal. The immunity in these cases, then, is not antitoxic, but rather "antibacterial," and depends on the development, in the immune sera, of antibodies, quite distinct from the "antitoxines," which act directly upon the bacteria themselves. These immune serum constituents were spoken of by Pfeiffer as "bacteriolysins" or "specific bactericidal substances".

Not long after the discovery of the specific bacteriolysins another property of immune sera was described by Gruber and Durham.[67] They had been studying bacteriolytic phenomena with colon and cholera organisms, and noticed that these bacteria were agglomerated and gathered in small clumps when emulsified in homologous immune serum. Similar clumping had been described before but had not been recognized as a specific property of immune serum. Gruber and Durham determined that it was present to a degree roughly proportionate to the degree of immunization attained, and that its specificity was such that it would be utilized for bacterial differentiation. They believed that the substances in the immune serum responsible for this agglutination were independent of other serum constituents and applied to them the term "agglutinins."

Primarily the phenomenon of agglutination was regarded as a part of the struggle of the body against the living bacteria and Gruber himself believed that it depended upon a swelling or "klebrig werden" of the microorganisms which tended to cause their sticking together, and rendered them more readily amenable to the action of the bactericidal powers of the serum. Bordet[68], however, early conceived the process as a physical phenomenon in which the bacteria themselves were entirely passive, and, indeed, Widal and Sicard[69] soon demonstrated that bacteria killed by heat were equally as agglutinalbe as the living germs. Moreover, living bacteria agglutinated by means of specific antiserum in the test tube are not killed, but are simply massed together making it easier for the phagocytic cells to engulf them.

These observations naturally suggested that the reaction between specific agglutinating serum and bacteria was based on individual peculiarities of the bacterial proteins, and it occurred to Kraus[70] to investigate whether or not the immune sera would cause any sort of reaction when mixed with the dissolved body substances of homol gous bacteria. Working at first with cholera and plague he obtained soluble constituents of the bacteria, both by allowing broth cultures to stand for varying periods and by emulsifying agar cultures in alkaline broth. The extracts were then filtered through Pukal filters to remove the insoluble materials. When the sera of immunized animals were added to these clear filtrates---cholera serum to cholera filtrate, and plague serum to plague filtrate---slight turbidity developed, followed within a few hours by the formation of small flakes.

In other words, it was found that the mixture of a clear filtrate of a bacterial culture with the serum of an animal immunized against these bacteria resulted in the formation of a precipitate. This reaction was due to strictly specific antibodies in the immune sera which Kraus called "precipitins".

It was not until relatively late in the development of immunology that the relationship between the two opposing schools, "cellular" and "humoral", was understood. The work of Deneys,[29] Gruber,[29] Futaki,[29] Wright,[29] Neufeld[29] and others made it clear that the degree of phagocytosis depended upon specific constituents of the serum which united with the bacteria and thereby rendered them more easily engulfed by the cells. Thus a bridge was formed between the two opposing views.

Since bacteria are not destroyed by X-ray, since healing is the function of the specific antibodies of the plasma and the phagocytic cells, and finally since it has been observed in clinical practice that certain infections respond favorably when exposed to X-ray, the secret of this favorable reaction must be in the action of the rays on the resistant forces of the body, namely, the antibodies and the phagocytic cells.

On this basis a study was undertaken to determine in experimental animals the tissue reactions in areas previously inoculated with a culture of staphylococci, giving special attention to the types of cells present at various intervals and proportion of cell types after treatment. Untreated lesions produced in the same manner were studied as controls.

IV

PLAN OF EXPERIMENT

In order to select a satisfactory form of staphylococcus, four different kinds were injected into one rabbit, the fur having been removed with a depilatory, and twenty-four hours allowed to elapse to allow any irritation thus caused to subside to normal. From these resultant boils, one was selected to carry out the experiment proper. Six albino rabbits were used as experimental animals. Four lesions were set up in each of the six rabbits by intra-dermal injections of 0.1 to 0.2 cc. of a twenty-four hour broth culture of the previously determined strain of staphylococcus. In each rabbit two of the four lesions were left untreated to serve as controls and the remaining two were irradiated with X-ray employing the following factors: 100 K.V.P. (kilovolt peaks), 5 ma. (millampers), 15 inch distance, through a portal 1 inch in diameter, using 1 mm. (millimeter) of aluminum filter for 8 minutes. These factors when applied to the equipment used in this experiment resulted in a dose of 250 Roentgens measured in air, for each field so treated. To insure against a spread of the effect of the X-ray treatment to the untreated or control lesions, the rabbit was covered with a lead plate in which was cut an opening or portal large enough to expose only the boil to the rays. The treatments were given at intervals of 4, 8, 16, 24, 30, and 48 hours after the injections were made. That is, in rabbit #1, two of the four lesions were treated four hours following inoculation; in rabbit #2, eight hours elapsed between

the time of injection and X-ray treatment. This variation in the time element was made to learn of the changes affected by the X-ray treatment at successive levels of development of the boil, that is, to determine at what period of evolution in the boil the most gratifying results were obtained.

Immediately following treatment, biopsies were performed and the lesions placed in Formal-Zenker. In order to facilitate the giving of the treatment, Nembutal was administered intraperitoneally at the time of irradiation as well as at biopsy. The dosage was determined by the weight of the animal, 1 cc. being given for each five pound of weight. The tissues were then embedded in paraffin, cut 4.5 microns thick and at various levels through the boil, that is through the surrounding tissue, through the rim and through the heart of the boil. Three different stains were used on the tissues cut from the same region of the boil.

Harris's Hematoxylin and Eosin

Dissolve paraffin in xyol	5 to 10 min.
100% alcohol	5 min.
95% alcohol	1 min.
80% alcohol	1 min.
70% alcohol	1 min.
50% alcohol	1 min.
Rinse in water before staining	
Filter all stains	
Harris's Hematoxylin	5 to 10 min.
Rinse in water	
Place in acid alcohol for destaining	
Rinse in tap water for	10 to 20 min.
Eosin	20" to 1 '.
95% alcohol	1 min.
95% alcohol repeated for	1 min.
Absolute alcohol	1 min.
Xylol for clearing	
Mount in damar	

Azure II Eosin Hematoxylin.....Maximow's Stain

The
The Delafield's stain, not artificially ripened, was about six weeks old. A very dilute solution of it was made by adding 2 to 3 drops to 100 cc. distilled water. Stain 24 hours. Wash in distilled water for 24 hours. Result: Chromatin is blue, cytoplasm is either colorless or very light gray. Place in azure II-eosin for 24 hours.

Preparation as follows:

Stock solution A

Eosin, water soluble	.5 gm.
Distilled water	500. cc.

Stock solution B

Azure II	.5 gm.
Distilled water	500. cc.

Stain:

Solution A	10-12 cc.
Solution B	9-10 cc.
Distilled water	100 cc.

Mix the eosin solution and water first, then add the azure solution. A noticeable precipitate should not form for several hours. Leave slides upright in stain for 24 hours. Transfere from stain to 95% alcohol, then to absolute alcohol to differentiate and dehydrate. Finish dehydration in second 100% alcohol, clear and mount. (Differentiation takes place rapidly in 95%alcohol, more slowly in 100%)

Result: Chromatin of the nuclei is dark blue, nucleoli are purple and leucocyte granules are differentially stained in colors corresponding to those given by Giemsa.

Bacterial Stain

Stain 10 to 30 min. in Goodpasture fuchsen solution

30% alcohol	100.--cc.
Basic Fuchsen	.59gm.
Analine	11. gm.
Phenol	1. gm.

Wash in water
Differentiate in formalin---Only a few seconds required
Bright red color changes to rose.
Wash in water
Counter-stain in saturated aquaous solution of picric acid three to five minutes until section becomes purplish yellow
Wash in water

Bacterial stain continued.

Differentiate in 95% alcohol---Red reappears and some of it is washed out.
Wash in water
Stain in Sterlings gentian violet 5 minutes or more.

Crystal violet (85%dye content)--------	44.cc.
Analin oil----------	1.cc.
Alcohol (95%)----------	6.cc.

Wash in water
Grams iodine for 1 minute
Blot dry without washing
Clear in a mixture of equal parts of analine and xylol until no more collar comes away.
Clear in 2 changes of xylol.
Mount in damar.

This was done in order to gain the three different pictures each of the stains would give of the same level or part of the lesion. The variously stained tissues were then studied and the parts of the picture afforded by the three different stains were correlated and fitted together yielding the results described in the following pages.

Rabbit #	Weight and Dosage	Date and Time treated	Lesion Treated	Condition of Lesion	First Bi-opsy	Second Bi-opsy
#I. (4 h'r.)	6 lb. 1¼ cc. of Nembutal Success-ful.	11:30 a.m. 7/11/38	Ant. dor-sal. Ant. ven-tral.	Ant. dor. well developed--no visible liquifaction Ant. ven. smaller--pus present Post. dor. No definite pus Post ven. Well organized and pus present	3:30 p.m. 7/11/38 4 h'rs. after treatment	
#2. (30 h'r.) and (48 h'r.)	5¼ lb. lcc. of Nembutal ½ cc. in 30 min.	12:15 p.m. 7/11/38	Ant. dor-sal. Ant. ven-tral.	Ant. dor. early lesion no visible pus. Ant. ven. early lesion no visible pus. Post. dor. early lesion--no pus Post ven. developing----no pus	6:00 p.m. 7/12/38 29 h'rs. after treatment	12:00 p.m. 7/13/38 48 h'rs. after treatment
#3. (16 h'r.)	6 1/5 lb. 1 cc. of Nembutal Success-ful.	5:00 p.m. 7/11/38	Ant. dor-sal. Ant. ven-tral.	Ant. dor. Well developed--Slight signs of pus forming Ant. ven. Well developed--Slight signs of pus forming Post.dor. and Post. ven. appear similar to ant. lesions	9:00 a.m. 7/12/38 16 h'rs. after treatment	
#4 (8 h'r.)	9 lb. 1.8 cc. of Nembutal Success-ful.	9:45 a.m. 7/12/38	Ant. dor-sal. Ant. ven-tral.	Ant. dor. Well developed--early supperation Ant. ven. Well developed--no pus Post. ven. Appears to be subsiding Post. dor. Well developed--no supperation.	5:00p.m. 7/12/38 Ether re-quired and lcc. Nembu-tal.	
#5. (24 h'r.)	5½ lb. 1.1cc. of Nembutal Successful	10:45 a.m. 7/12/38	Ant. dor-sal. Ant. ven-tral.	Ant. dor. Poorly developed--appears to be subsiding Ant. ven. Same as ant. dor. lesion Post. dor. Well developed--slightly flucuant Post. ven. Same as post. dor. boil	11:00 a.m. 7/13/38 24 h'rs. after treatment	

V

RESULTS OF EXPERIMENT

Rabbit #1 Experimental and Control at 4 hour Interval

Control or Untreated:

In the untreated or control lesion examined four hours following the intradermal injection of staphylococci, a well developed boil has formed. Lymphocytes are numerous as also are eosinophiles. A very few polyblasts are present, located peripherally, while few if any macrophages are observed.

Treated:

In the lesion treated four hours following injection, the boil was poorly developed in comparison with the untreated lesion at the same stage of development. The cytoplasm of the lymphocytes is broken down leaving many bare nuclei and fragments. Eosinophiles are more numerous here than in the untreated boil. In contrast with the few polyblasts present in the untreated lesion, here they are found to be very abundant at the periphery of the boil. Also a few very pale-staining macrophages having finger-like projections occur.

A histological comparison of the treated and untreated lesions is presented more graphically in the following table.

TREATED	UNTREATED
1. The cytoplasm of the lymphocyte has broken down leaving many bare nuclei and fragments.	1. Lymphocytes are numerous.
2. Boil is not so well developed.	2. Boil is nicely developed.
3. Eosinophiles very abundant.	3. Eosinophiles numerous, but not so abundant as in the treated slide.
4. Polyblasts (Maximow's non-granular having an oval shaped nucleus) are very abundant at periphery of boil.	4. Polyblasts are not so abundant and located more peripherally.
5. Few macrophages which are very pale staining and have finger-like projections.	5. Few if any macrophages present.

Rabbit #4 Experimental and Control at 8 hour Interval

Control or Untreated:

At the eight-hour interval, the boil is the chief point of interest. Within the lesion proper lymphocytes are scattered very thickly, giving somewhat the appearance of the lymphocytes in a node. Near the margin of the lesion are large numbers of pale-staining polyblasts which resemble large monocytes. Bacteria are found within the boil itself and also in the surrounding connective tissue which is somewhat frayed and swollen.

Treated:

Here, instead of the boil itself being the chief part of the picture it is poorly developed. None of the lymphocytes are intact. The cell wall has been broken liberating the cytoplasm leaving many dark staining nuclei and much debris. Bacteria are present in the surrounding connective tissue which is damaged severly leaving the tissues dissembled and far apart. There are not so many polyblasts here as in the untreated boil, but instead more macrophages whose cytoplasm is beginning to stain darker giving the appearance of already ingesting bacteria and debris from the damaged cells.

A review of the microscopic picture presented in the treated and untreated lesion is given in the following table.

TREATED	UNTREATED
1. Boil is poorly developed.	1. Boil is well developed.
2. Connective tissue seems to be damaged severly leaving tissues far apart.	2. Definite damage to surrounding connective tissues.
3. Not so many of Maximow's polyblasts but instead more macrophages whose cytoplasm stains quite dark and gives the appearance of being full and distended with foreign particles.	3. Many, many polyblasts---These look like enlarged monocytes Their cytoplasm is fairly clear.
4. Bacteria quite numerous in surrounding connective tissue.	4. Bacteria not so numerous.
5. No lymphocytes but lots of dark staining structures which look like nuclei and debris.	5. Lymphocytes thickly scattered in boil proper---Give somewhat of the appearance of the lymphocytes in a node.
6. Instead of boil itself being t the chief part of the picture here you find cells scattered and not nearly so dark, but to the periphery of boil are many dark staining macrophages The boil itself is small.	6. Boil is chief point of interest A mass of cells and not nearly so broken up. To the periphery of boil are pale polyblasts developing, but not nearly so many macrophages.

The following table shows the changes which have taken place from the 4 to 8 hour interval.

4 Hour	8Hour
1. Many eosinophiles	1. Eosinophiles far below count of 4 hour rabbit.
2. Surrounding connective tissue is not affected very much.	2. Small amount of edema---Tissues beginning to deteriorate.
3. Few macrophages---pale staining	3. Many macrophages which appear rough and full, staining dark.
4. Very little cell destruction.	4. More cells breaking down leaving more debris.

Rabbit #3 Experimental and Control at 16 hour Interval

Control or Untreated:

Within the boil proper are many lymphocytes. In the surrounding tissues are quite a few macrophages and histiocytes and to the extreme margin there are a few scattered fibroblasts. Here in the untreated lesion, bare nuclei are in evidence but the amount of karyorrhexis and karyolysis is not as great as in the treated tissues. However, there is more debris and at the same time the macrophages are not so dark or large as in the treated tissue for there the phagocytic cells have already begun the process of clearing the area of bacteria and damaged tissues.

Treated:

Again, there are no intact lymphocytic cells in the treated lesion. There seems to be a definite increase in destruction of cytoplasm of the cells leaving the dark staining nuclei and fragments scattered thickly throughout a mass of broken granules and fibers. Some of these nuclei are from the lymphocytes and others from the polymorphonuclear leucocytes. Macrophages and histiocytes are very abundant, far surpassing the number found in the untreated furuncle at the same period of development. Also the number of fibroblasts has increased under the stimulation of the X-ray.

A confrontation of the treated and untreated lesion at the 16 hour interval is summarized in the following table.

Treated	Untreated
1. No lymphocytes.	1. Many lymphocytes in boil
2. Macrophages..Millions of them	2. Macrophages..Many, but not so numerous as in the treated lesions.
3. Histiocytes..Very, very numerous.	3. Histiocytes.. Very abundant but not so many as in treated lesions.
4. Fibroblasts..Quite a few located along blood vessels.	4. Fibroblasts..Very few scattered through peripheral connective tissue.
5. There seems to be a definite increase in destruction of cytoplasm of cells. This leaves dark staining nuclei and fragments of nuclei scattered thickly throughout a mass of broken granules. Some of the bare nuclei resemble the lymphocytes and others the hetrophile ones.	5. Here in the untreated, bare nuclei are in evidence but the amount of karyorrhexis and karyolises is not as great as in the treated tissues. However, there is more debris and at the same time the macrophages are not so dark or large as in the treated. Perhaps the debris has been ingested by the macrophages in the treated tissues.

Changes which have occured from the 8 to the 16 hour period

8 Hour	16 Hour
1. Cells only beginning to break down.	1. Chief difference is increased destruction of cell cytoplasm.
2. Quite a few of Maximow's polyblasts.	2. Increased number of macrophages of large and dark type.
3. The few macrophages present are smaller and have a clearer cytoplasm.	3. A regular army of macrophages in surrounding connective tissue.
4. Not so many eosinophiles.	4. Eosinophiles are numerous.

Rabbit #5 Experimental and Control at 24 hour Interval

Control or Untreated:

Examination of this untreated lesion shows not only considerable cytoplasmolysis, but also a fine but varying in size spray of debris which proves to be nuclear fragments and eosinophiles granules. Part of this debris is bacteria, sometimes found between the cells and again seen in the cytoplasm of the neutrophiles and macrophages. There is an abundance of eosinophiles, but many are damaged. Neutrophiles are also numerous. There are hematogenous polyblasts arising from lymphocytes and monocytes (Maximow says these are phagocytic and the particles found in their cytoplasm seems to support this idea) Nearer the edge of the boil are a few large monocytes and polyblasts. Still peripheral to these cells are a few eosinophiles and quite a number of fibroblasts setting up a rather broad and loose barrier around the boil. Still external to this in the surrounding connective tissue are the non-granular polyblasts, but the macrophages and histiocytes are not so abundant as in the treated lesion at the same time interval. Here, too, there are many lymphocytes, monocytes and a scattering of fibroblasts.

Rabbit #5 Experimental and Control at 24 hour interval continued

In the treated lesion studied 24 hours after injection, nuclear degeneration is more marked than at the 16 hour interval, but this is relatively slight in comparison to the amount of cytoplasmolysis, which is extreme. There is marked swelling of the collagenous fibers. There seems to be considerably debris scattered throughout the boil. These are probably fragments of nuclei from polymorphs which have been stripped of their cytoplasm, and nuclei from lymphocytes which have degenerated due to the X-ray. Also, many, many bacteria. There are still eosinophiles and neutrophiles intact, but the majority seem to have degenerated. Within the boil proper there are many bacteria already engulfed by histiocytes and macrophages. Then just external to the boil there is a well formed barrier or ring of fibroblasts fencing off the injured area. This was not nearly so complete in the 16 hour stage. Then just to the periphery of this barrier are great numbers of histiocytes and macrophages which stain very dark in many cases, evidently full of bacteria and foreign particles. There are less eosinophiles and neutrophiles and a greater number of the phagocytic cells. The cells then thin out considerably, showing a sprinkling of eosinophiles, neutrophiles, more histiocytes and also a few monocytes. In the spaces between the cells are many bacteria sometimes in clusters and again in chains. The collagenous fibers are very swollen. Instead of the many monocytes as seen in the untreated these seem to have decreased in number with a commensurate increase in the number of histiocytes in the treated tissues.

The histological pictures of the treated and untreated lesion at the 24 hour interval are more graphically collated in the following table.

Treated	Untreatdd
1. Extreme cytoplasmolysis.	1. Cytoplasmolysis present but to a lesser degree.
2. Marked swelling of collagenous fibers.	2. Collagenous fibers swollen.
3. Considerable debris scattered throughout boil..nuclei from polymorphs which have been stripped of their cytoplasm, also nuclei of lymphocytes which have been destroyed by the X-ray.	3. More debris in the untreated lesion, especially eosinophile granules until boil has a very pink appearance. Also many nuclear fragments as nuclear de degeneration of all cells is marked.
4. Many bacteria within boil proper which are already engulfed by histiocytes and macrophages. There are many bacteria in spaces between cells, sometimes in clusters, again in chains.	4. Bacteria in abundance is scattered between cells and in some cases it is seen within cytoplasm of neutrophiles and macrophages.
5. Just external to the boil is a well formed barrier or ring of fibroblasts fencing off the injured area.	5. Here in untreated lesion, fibroblasts are still scattered and without definite barrier formation.
6. To periphery of this barrier are many histiocytes and macrophages which stain very dark, evidently full of bacteria and foreign particles.	6. To periphery are some histiocytes and macrophages, but they are out-numbered by Maximow's polyblasts. There are lymphocytes and monocytes present.
7. At extreme margin, cells thin out considerably showing a sprinkling of eosinophiles, neutrophiles, histiocytes and also a few monocytes.	7. More eosoniphiles here, but many of them are damaged.

Changes which have occured from the 16 to the 24 hour period

16 Hour	24 Hour
1. Cells just beginning to loose cytoplasm.	1. Nearly all cells have lost their cytoplasm.
2. No nuclear degeneration.	2. Slight nuclear degeneration.

Changes which have occured from the 16 to the 24 hour period continued

16 Hour	24 Hour
3. Fibroblasts are few in comparison and are scattered through tissue along capillaries.	3. Outstanding difference is in development of barrier of fibroblasts surrounding the boil....This is a wide but not a very dense band.
4. Histiocytes and macrophages in surrounding tissue less numerous and are not so dark staining.	4. More histiocytes and macrophages in surrounding tissue which stain dark blue as if full of bacteria and debris.
5. More microphages and less macrophages.	5. More macrophages and less microphages.
	6. Within boil proper there are many bacteria already engulfed by histiocytes and macrophages.
	7. Greater swelling and fraying of collagenous fibers.

Rabbit #2, Biopsy #1, Experimental and Control at 30 hour interval

Control or Untreated:

On examing the boil itself, lymphocytes are the outstanding cells. Eosinophiles are not so abundant and the great majority are broken. Macrophages, histiocytes and polyblasts are quite numerous, but when compared with the treated lesion they are not so plentiful, as large or dark staining, but are younger cells. External to the boil in the surrounding connective tissue fibroblasts are quite abundant, but they are scattered and in curved forms rather than packed together to form a solid band.

Treated:

In this treated lesion there are no lymphocytes either in the boil or surrounding tissue. Eosinophiles are more numerous than in the untreated furuncles. Macrophages and histiocytes far outnumber those of the untreated. Too, they are larger and distended with ingested matter. Not only do these adult phagocytic cells occur prolifically, but the young polyblasts which are to develop into these scavenger cells are present in great numbers. The most signal feature is the formation of fibroblasts which are long spindle in shape and are closely packed together establishing a barrier about the infection and so preventing its spread. When comparing this feature as seen in the untreated with the treated, the X-ray has hastened the influx, increased the number and established a larger, more compact and hence effecient barrier.

A histological collation of the treated and untreated lesion at the 30 hour period is presented more clearly in the following table.

Treated	Untreated
1. Has no lymphocytes in boil or surrounding tissues.	2. Has many lymphocytes.... They are the outstanding cells when examining the boil itself.
2. Eosinophiles are more numerous than in the untreated furuncles.	3. Eosinophiles are not so abundant and the great majority are broken.
3. Macrophages, histiocytes and even polyblasts are more numerous, larger and stain darker as they have ingested large amounts of bacteria and damaged cells.	3. Here they are quite plentiful but in comparison with treated slide are not nearly so numerous, as large, or as dark staining.
4. The barrier composed of fibroblasts is very definite, the cells are long spindle in shape and are closely packed together. There seems to be even a clear space between the boil and this band of fibroblasts.	4. There are some fibroblasts in surrounding tissue, but they are scattered and in curved forms rather than packed together in a solid band.

Changes which have occured from the 24 to the 30 hour period

24 Hour	30 Hour
1. Barrier of fibroblasts is quite wide and not nearly so dense or compact.	1. Here barrier is much narrower and very heavy. The cells are fitted together closely forming a compact ring.
2. There is more debris here.	2. The boil has broken clear through epidermis of skin, but on each side of this broken area is a definite band of fibroblasts separating boilfrom the underlying connective tissue
3. The swelling and fraying of the tissue is chiefly external to the boil.	3. Swelling and fraying is very marked, leaving wide spaces which appear to be filled with pus. This destruction of tissue is within the boil proper.

Rabbit # 2, Biopsy #2., Experimental and Control at 48 hour interval.

Control or Untreated:

A piece of the epidermis has sloughed out. Leucocytes, both lymphocytes and polymorphs, are packed in the abscess tightly. Many of them are damaged, not only the cytoplasm but nuclear destruction is likewise present. Macrophages and histiocytes are numerous and exhibit a certain degree of ingestion. Many scattered fibroblasts are located peripheral to the lesion but they are not arranged in a compact barrier formation. There is still considerable debris, frayed and damaged tissues.

Treated:

Inflamation appears to be subsiding. The amount of debris, and broken cells are less. That remaining is securely fenced-off from surrounding tissue by a tight barricade of fibroblasts. Both within and external to this fibroblastic barrier are many large macrophages greatly distended with ingested particles which accounts for the gradual clearing of this inflamed area.

A more graphic comparison of the treated and untreated lesion at the 48 hour period of development is given in the following table.

Treated	Untreated
1. Inflamation appears to be subsiding. Much less debris in surrounding tissue than in untreated.	1. Much more debris...frayed and damaged tissues.
2. Boil is much smaller than in untreated lesion	2. Cytoplasmolysis and nuclear degeneration to a marked degree.
3. Large swollen macrophages are myriad in surrounding tissue. Granular fragments are clearing.	3. There are still some intact lymphocytes and polymorphs though the majority are in a state of degeneration.
4. A dense barrier of long spindle shaped fibroblasts securely separates the furuncle from the surrounding tissue.	4. Many macrophages.
	5. Fibroblasts present, but still in scattered arrangement.

Changes which have occured from the 30 to 48 hour period.

30 Hour	48 Hour
1. Swelling and fraying of tissue is very marked.	1. Damaged tissues and injured cells have been cleared from the area to a large degree.
2. Cells are in a state of nearly complete disintegration.	2. Consequently, inflamation appears to be waning and and the boil itself is much smaller.
3. Consequently, lesion appears as a mass of broken cells and debris.	3. Many large phagocytic cells are still present in surrounding tissues.

These sections studied show that the tissues at the abcess site necrose at different times. In order of their necrosis

were noted, (1) lymphocytes, (2) polymorphonuclears, (3) collagen fibers and (4) epidermis. When the necrosis of each type of tissue at the site of injection is analyzed in relation to the influx of phagocytic cells, the one tissue whose damage comes shortly before or parallels the migration of the first scavenger cell, is the cytoplasm of the lymphocyte. Then follows the break down of the polymorphs with a co-influx of the polyblasts. As cytoplasmolysis advances, nearly every type of cell of the entire phagocytic system makes its way to this point of infection. Since the necrosis of the nuclear material of the leucocytes occurs long after the scavenger cells have reached the boil, the observations point to the cytoplasm of the leucocytes as containing the factor which calls forth this army of phagocytic cells. Since the X-ray breaks down the lymphocyte almost immediately, the chain of events in the healing process is "set off" more quickly in the treated lesion than in the untreated and explains the more rapid and complete healing in the irradiated tissue.

VI

DISCUSSION

First, before considering a possible explanation for these effects of irradiation on diseased tissue, it will be well to recall the results of experiments carried out to determine the effects of X-ray on bacteria have proved them to be so resistant to the rays that any attempt to destroy them as a clinical measure would require doses which would be dangerous or even lethal for their hosts.

Since irradiation acts upon so many forms of inflamation in much the same way, it is obvious that the inflamatory lesions must have some common factor. This common factor has been well established by Heineke[5] and further checked by scores of men.[5] Heineke found that when the entire body of animals was exposed to large doses of X-ray, the animals invariably died after an interval which varied according to the dose of rays and size and age of the animals. Regardless of the ability of the animals to tolerate irradiation, however, he observed at necropsy that although the majority of the organs were free from perceptible abnormality, the spleen, mesenteric and other lymph nodes and intestinal lymph follicles showed marked destruction of lymphocytes, and the degree of cellular disintegration varied according to the dose of rays and the interval between irradiation and microscopic examinations. As the number of intact lymphocytes diminished the stroma became more and more prominent. The lymphocytic degeneration in the spleen and lymph nodes was

often so great that most of the Malpighian corpuscles or lymphoid follicles disappeared as such and could be recognized only by the blood vessels and the concentric arrangement of the stroma of these structures. This destruction of the lymphocyte began within fifteen minutes after irradiation, and was characterized by disorganization and fragmentation of the nuclear chromatin of the cells and scattering of the fragments of chromatin between the remaining intact cells and in the spaces of the reticular stroma being complete within twenty-four hours. When the granular leucocytes are exposed to the X-ray, the same disintegrating results take place, but at a slower rate. Since Heineke did his work many others have substantiated these results and so it has been proved quite conclusively that X-ray will destroy the leucocytes.

The next move was to attempt to learn just how the destruction of the leucocytes and especially the lymphocytes was linked with healing of inflammatory lesions which X-ray produced. The importance of the leucocytes in this healing process was further emphasized by the observation that the inflammatory process responds to irradiation in proportion to the degree of leucocytic infiltration, and again, radio-therapy is most beneficial during the suppurative stage.

As previously discussed in this paper, the natural defense of the organism against infections is leucocytic, and especially lymphocytic infiltration around the site of infection. Therefore, when an inflammation is irradiated, destruction of the infiltrating leucocytes is to be expected. But since

leucocytic infiltration plus the antibodies of the body fluids, particularly the plasma, are the barriers or defenses against infection, it would appear that their destruction by the rays would do more harm than good. This seeming paradox is explained by the fact that the leucocytes and especially the lymphocytes, which the organism mobilizes around the site of inflammation, represents an effort to localize the infection and to get rid of the infectious material by phagocytosis. It must also follow that these infiltrating cells contain or elaborate within themselves the protective substances or other means which enable them to destroy or neutralize the bacterial or other toxic products which give rise to the defensive inflammation. From this premise, it seems not unreasonable to deduce that irradiation, by destroying the infiltrating lymphocytes, causes the protective substances contained by such cells, which Fenn in his experiments has proved to be chemotactic for bacteria, to be liberated and to be made even more readily available for defensive purposes than they were in the intact cell. Also, since the wandering phagocytic cells appear at the point of infection following the influx of leucocytes, and that this migration occurs more rapidly and in greater numbers when the leucocytes are in a state of disintegration, it appears that their contents are chemically attractive for the phagocytic cells.

In review, the X-ray sets off a chain of events--the destruction of the lymphocytes, their consequent liberation of their contents which then are a more available stimuli to attract the granular leucocytes, the polyblasts, histiocytes, macrophages and fibroblasts--thus, the healing of the lesion is more rapid and more complete.

VII

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The untreated lesion at the 8 hour interval: Boil is chief center of interest...Undamaged lymphocytes are myriad within the boil. In the surrounding connective tissue are scattered a few pale-staining polyblasts and macrophages. Compare with photograph of treated lesion on following page.

Treated lesion at 8 hour interval;

Boil is not so well developed as in untreated. Here cytoplasmolysis of lymphocytes is complete. Other cells are deteriorating leaving fragments scattered through out lesion and marginal tissue. There are some macrophages in surrounding tissue.

Untreated lesion at 16 hour interval:

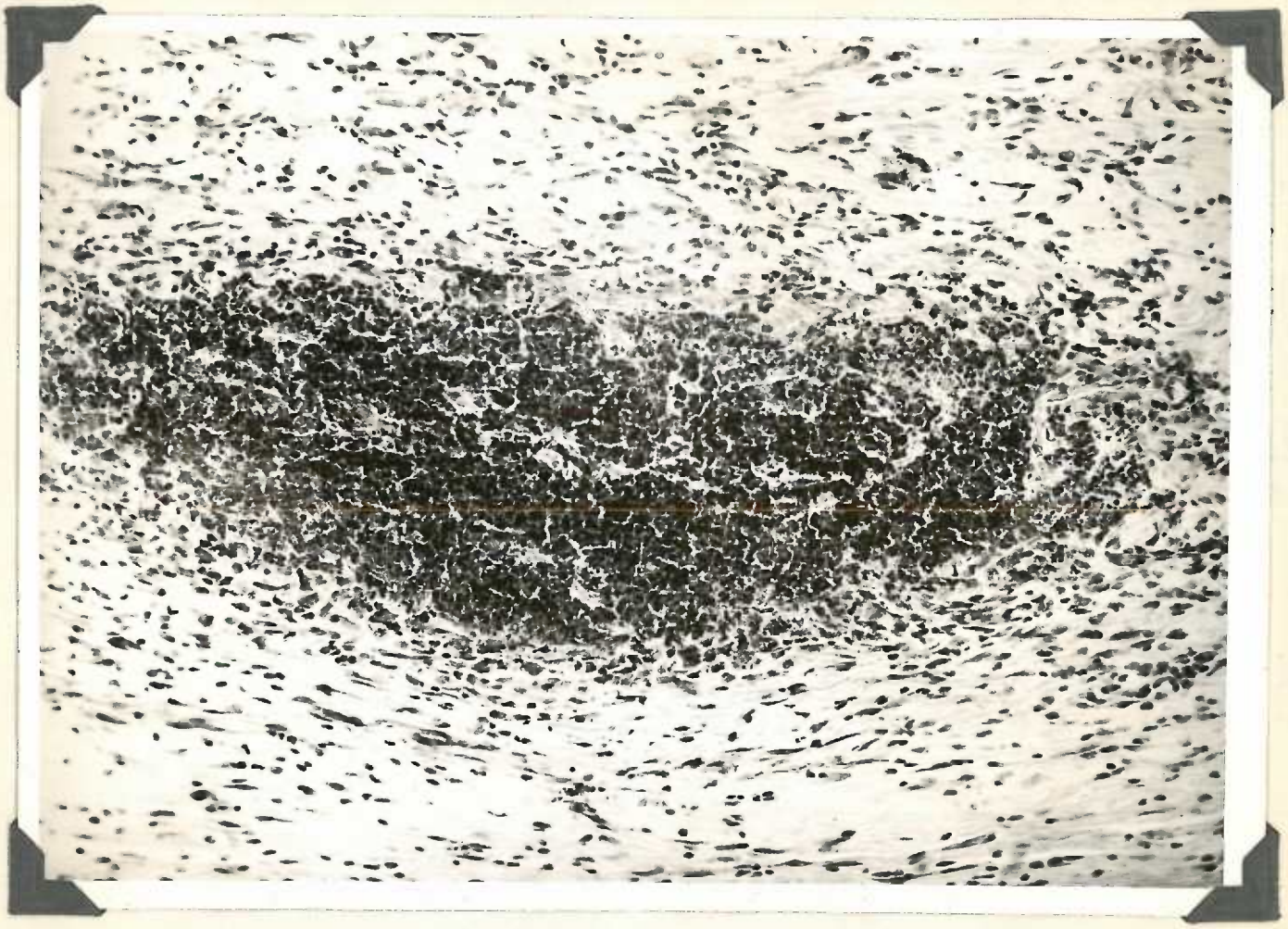
Lower left corner shows margin of boil with many intact lymphocytes External to boil (remainder of photograph) are many pale-staining macrophages, histiocytes and a very few scattered fibroblasts.

Treated lesion at 16 hour interval:

Cytoplasmolysis is extreme leaving dark staining fragments and nuclei scattered thickly throughout a mass of debris. In lower left border of photograph are many large, dark staining macrophages and histiocytes. Compare with untreated on previous page.

Untreated lesion at 24 hour interval:

Within boil proper there is considerable cell break-down, but this is not as marked as in treated at the same stage of development. External to boil are many histiocytes and macrophages but they appear to be very young cells. Compare with treated lesion on following page.



Treated lesion at 24 hour interval:

Within the boil, cytoplasmolysis is extreme. Surrounding the lesion is a large influx of macrophages, histiocytes and even a scattering of fibroblasts. ***Be sure to compare with preceding photograph.***

Untreated lesion at 30 hour interval:

Many intact lymphocytes are found within boil itself. Many Microphages as well as macrophages are present, but they are pale staining. The fibroblasts are still in a scattered formation.

Treated lesion at 30 hour interval:

The epidermis has sloughed off but a very dense barrier of long spindle shaped fibroblasts separates boil from underlying connective tissue. All types of phagocytic cells swollen with particles are numerous. There are no lymphocytes within boil or surrounding tissue.