STUDIES ON DECREASING THE REACTION OF NORMAL SKIN TO DESTRUCTIVE DOSES OF X-RAYS BY PHARMACOLOGICAL MEANS AND ON THE MECHANISM INVOLVED.*

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Plates 100 to 102.

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In an earlier investigation evidence was presented to show that an organism sensitized by a foreign protein could locally autoinoculate itself with the same protein when certain conditions were fulfilled. As this mechanism would serve to explain a number of abnormal reactions of hitherto cryptogenetic origin, it was desirable to advance still more proof. For this reason work was undertaken in which x-rays were the local irritating agent which caused the autoinoculation. During the preliminary stages of this research, however, an impression gradually developed that a certain group of rabbits seemed to show an increased resistance to doses of ordinarily destructive x-rays. Since this would be of considerable theoretical as well as practical value, if true, we abandoned our original object temporarily in order to study this point. The result demonstrated the correctness of the impression that the skin after the systemic incorporation of serum could be rendered resistant to doses of x-rays which are lethal to the tissues of non-prepared animals.


REACTION OF NORMAL SKIN TO X-RAYS

Method.

An interruptorless, 10 kilowatt, 220 direct current machine with a medium focus Coolidge tube was used. After preliminary trials 30 skin units (Witherbee-Remer formula) were chosen as the standard test dose of x-rays. This was produced by a 3 inch spark-gap, 10 milliamper current, 6 inch distance from target, and 20 minute exposure. All these factors were constantly controlled throughout the period of treatment of all the animals. No filter was employed, except that a disc of ordinary filing card was placed between the tube and the skin surface in order to reduce the heat effect.

Rabbits only were used. The area x-rayed was always 4 sq. cm. of the upper half of the right ear, the central artery of the ear passing through the middle of this space. The rest of the ear and body was protected by a sheathing of lead. Shifting of the x-rayed area, due to movements of the animal, was minimized by a simple device. The right ear was turned forward, smoothed out upon a small board and a strip of plaster fixed the tip of the ear to the board and the board to the box. A mask of sheet lead provided with an opening of 2 by 2 cm. was carefully placed in position on the right ear and held there by a strip of plaster. After covering the rest of the head and the entire box with lead sheeting, the animal was ready for treatment. Great care was exercised to prevent a circular constriction of the neck. This procedure was quite successful, though a moderate lateral shifting of the ear occurred in some instances.

It should be emphasized that the site chosen for x-raying offers a number of advantages: the ear is always easily available for inspection with no discomfort to the animal; two skin surfaces are affected by the x-rays, the dorsal on entry and the internal surface on exit of the x-rays; no serious systemic effects are to be feared even after massive doses because the x-raying is entirely localized to a comparatively small area; the ear of the rabbit is richly vascularized, and possesses a number of direct arteriovenous anastomoses which guarantee an especially efficient collateral circulation.

All animals except the normal controls were x-rayed on the same day, but the members of no group were x-rayed in succession. The procedure was to take one animal from each group in rotation until all animals had been exposed to the x-rays.

After the rabbits had been x-rayed they were examined at 2 to 4 day intervals or daily when necessary, for a period of over 300 days, at which time the evidence was deemed sufficient to terminate this aspect of the work.

The end-point of the reaction was the appearance of a spot of dry gangrene in the x-rayed area, with subsequent fenestration. The number of days which elapsed between the time of x-raying and the appearance of dry gangrene, or the

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TEXT-FIG. 1. Duration of the reaction from the day of x-ray treatment to the appearance of perforating gangrene.
REACTION OF NORMAL SKIN TO X-RAYS

duration of the inflammatory process up to gangrene, was then plotted. Text-
figs. 1 and 2 bring out well the striking difference between the various groups.
The type of rabbit employed, their feeding, care, method of injection, etc.,
have been described in an earlier paper.¹

Text-Fig. 2. Duration of the reaction from the onset of the first exudate to
the appearance of perforating gangrene.

The experimental animal material was composed of four groups of rabbits:
(1) normal controls, (2) serum controls, (3) sensitized group, and (4) sensitized-
reinjected group.
The normal controls were normal, untreated rabbits in which various doses of
x-rays were tested as described. Both ears were utilized at different times.
There were six rabbits in this group; in three the standard dose was used.
The serum controls, five in number, were normal rabbits which received a single injection of 10 cc. of horse serum intraperitoneally, 13 days after the ear had been x-rayed.

The sensitized group of rabbits, five in number, was sensitized by two subcutaneous and two intramuscular injections of 1 cc. of horse serum each, at 3 to 4 day intervals. 10 days after the last sensitizing dose the right ears were x-rayed locally with the standard dose.

The sensitized-reinjected group, five rabbits, was prepared exactly as has been described for the sensitized group, but 13 days after x-ray treatment this group was reinjected intraperitoneally with 10 cc. of horse serum. This group was thus subjected to an anaphylactic reaction 23 days after the last sensitizing dose of serum. Text-fig. 1 shows the relation of the groups and the various procedures.

During the early stages of the work one rabbit in each of the last three groups died without obvious lesions. These groups therefore now consisted of four rabbits each.

RESULTS.

Before presenting the results in detail we shall first give the main outstanding facts of this work.

The normal control rabbits developed dry gangrene and fenestration in the x-rayed area in 30, 33, and 37 days respectively.

The serum control rabbits showed dry gangrene and fenestration in the x-rayed ears after 36, 47, 50, and 50 days respectively (Figs. 1 and 2).

The sensitized-reinjected group exhibited the same lesions after 50, 52, 62, and 85 days respectively (Figs. 5 and 6).

The sensitized group, however, responded quite differently on the whole. Only one rabbit showed gangrene and fenestration of the ear in 46 days. A second rabbit developed the same lesion, but only after 131 days. The two remaining rabbits have developed no gangrene or fenestration even after the lapse of more than 340 days (Text-figs. 1 and 2 and Figs. 3 and 4).

These data demonstrate clearly that rabbits previously sensitized by the parenteral injection of horse serum acquire a remarkably increased resistance in the majority of instances to doses of x-rays which are lethal to the tissues of normal control rabbits or serum control rabbits.

4 The horse serum was kindly furnished by Dr. W. H. Park and Dr. E. J. Banzhaf of the Department of Health of the City of New York.
The results also show convincingly that the protection which serum sensitization previous to x-ray treatment confers is largely abolished when the sensitized and x-rayed animals are subjected to a general anaphylactic reaction (Text-figs. 1 and 2, sensitized-reinjected group).

The main objective details of the investigation are as follows: Within 24 hours after x-ray treatment two to three rabbits out of each group of five showed a slight pinkness of the x-rayed area which disappeared within 2 to 3 days. This pinkness is probably due to a heat effect from the Coolidge tube. Within 4 days the hair of the x-rayed area began to loosen, though there was considerable variation. Thus for example on the 11th day after x-ray treatment some ears showed bald patches, while in others the hair was still firmly fixed. This variation bore no relation apparently to the experimental group. Pigmentation of the x-rayed area was noticeable in 2 to 4 days after x-ray treatment and varied with the different animals. In some it became very marked, while in others the pigmentation was always slight. The degree of pigmentation bore no definite relation to the group to which the animal belonged. A slight thickening of the x-rayed area, without any obvious vascular congestion, was first noticed 9 days after x-ray treatment; it occurred in one to three rabbits of each group. After 11 days these rabbits showed, in addition to the thickening, a slight but definite congestion of the x-rayed area. 13 days after x-ray treatment all rabbits, except two members of the sensitized-reinjected group, showed a definite though slight congestion with slight thickening of the x-rayed area. It should be noted that no rabbit had yet been reinjected with serum at the time of this examination.

The congestion of the x-rayed area increased slowly, but not at an equal rate in all the groups. Thus, 16 days after x-ray treatment the serum control rabbits still exhibited only a slight congestion of the x-rayed area, while the sensitized group showed a moderate to marked congestion, and the sensitized-reinjected animals a fair to moderate congestion. Associated with the increased congestion there was also a slight increase in the thickness of the x-rayed patch. 18 to 25 days generally the x-rayed area developed an exudate on both surfaces which dried into crusts. The healing of this first inflammation was usually complete within 28 to 36 days and the x-rayed areas now appeared like healed superficial wounds. The x-rayed area was absolutely bald and practically free of crusts; the skin was thin, whitish, glistening, and easily crinkled into thin folds; the blood supply was good, though many rabbits showed pearly white spots in the x-rayed areas; there was no gangrene.

This termination of what we shall call the first inflammation did not take place in all rabbits, but occurred in a majority of the serum controls, the sensitized group, and the sensitized-reinjected group (Table I). In the normal controls (three rabbits) this first inflammation with crusts did not clear up but passed at once to a complete perforating gangrene. The same fact was also observed once in the serum control group (No. CX 18) and once in the sensitized-reinjected group (No. HSX 8) (Table I).
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<td></td>
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<td>Day of onset of exudate.</td>
<td>Day of healing.</td>
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<td>Day of onset of exudate.</td>
<td>Day of appearance of gangrene.</td>
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<td>CX 19</td>
<td>22nd</td>
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<td>33rd</td>
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<td>69</td>
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<td></td>
<td>CX 20</td>
<td>16th</td>
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<td>37th</td>
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<td>69</td>
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<tr>
<td></td>
<td>CX 21</td>
<td>16th</td>
<td>“”</td>
<td>30th</td>
<td>—</td>
<td>69</td>
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<td>Serum controls.</td>
<td>CX 14</td>
<td>18th</td>
<td>28th</td>
<td>8</td>
<td>36th</td>
<td>46th-49th</td>
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<td>CX 16</td>
<td>18th</td>
<td>36th</td>
<td>6</td>
<td>42nd</td>
<td>49th-52nd</td>
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<td></td>
<td>CX 17</td>
<td>18th</td>
<td>36th</td>
<td>13</td>
<td>49th</td>
<td>49th-52nd</td>
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<tr>
<td></td>
<td>CX 18</td>
<td>25th</td>
<td>No healing before gangrene.</td>
<td>36th</td>
<td>171st-195th</td>
<td>300+</td>
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<td>Sensitized group.</td>
<td>HSX 9</td>
<td>15th</td>
<td>28th</td>
<td>14</td>
<td>42nd</td>
<td>46th</td>
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<tr>
<td></td>
<td>HSX 11</td>
<td>20th</td>
<td>28th</td>
<td>68</td>
<td>96th</td>
<td>128th-135th</td>
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<td></td>
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<td>36th</td>
<td>At least 340.</td>
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<td>0</td>
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<td></td>
<td>HSX 13</td>
<td>23rd</td>
<td>28th</td>
<td>At least 340.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sensitized and reinjected group.</td>
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<td>31st</td>
<td>42nd</td>
<td>10</td>
<td>52nd</td>
<td>82nd-89th</td>
</tr>
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<td></td>
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<td>16th</td>
<td>36th</td>
<td>13</td>
<td>49th</td>
<td>49th-52nd</td>
</tr>
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<td>3</td>
<td>52nd</td>
<td>61st-64th</td>
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<td>No healing before gangrene.</td>
<td>52nd</td>
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The figures represent the number of days after x-ray treatment unless otherwise stated. In all rabbits 4 sq. cm. of the right ear were x-rayed with 30 skin units.
The recovery from the first inflammation and the disappearance of the crusts were, however, not permanent in all the rabbits. After a period during which the x-rayed areas looked like healed or practically healed surface wounds, another exudate appeared unexpectedly on these x-rayed surfaces. This typical, second inflammation always led to a perforating gangrene (Table I). The interval elapsing between the end of the first inflammation and the onset of the second inflammation varied from 3 to 13 days in the serum controls and the sensitized-reinjected group. In the sensitized group, on the other hand, the interval before the second inflammation appeared was 14 days in one rabbit, 68 days in a second, and in the two remaining rabbits no second inflammation leading to gangrene has developed after more than 340 days (Table I and Text-fig. 1).

The character of the second inflammation occurring in the x-rayed area was interesting. A fairly marked redness and swelling of the x-rayed tissue preceding gangrene were observed only once (No. HSX 11), and in all the other rabbits in which fenestration of the ear developed, the inflammatory signs were a moderate pinkness of the x-rayed tissue with no definite swelling or heat; the most noticeable feature was the appearance of a sticky exudate, often on both ear surfaces. This exudate apparently was poured out at various rates, for the subsequent crusts exhibited a definite lamination in many instances.

The difference between the inflammatory reaction of the x-rayed tissue and the normal surrounding tissue was well illustrated in four animals, one in each group—Nos. CX 21, CX 14, HSX 11, and HSX 8 (Table I). In these rabbits an inflammation of the right ear set in, perhaps due to scratching. This inflammation was most marked around the periphery of the x-rayed area, especially at the upper and lower borders. In the untreated part the tissues were red, swollen, hot, and in two rabbits (Nos. HSX 11 and HSX 8) a tongue of edema ran from the lower border of the x-rayed area towards the base of the ear; the blood vessels of the untreated part of the ear were markedly dilated. The inflamed tissue, however, stopped sharply at the x-rayed area, and this latter tissue, while more or less pink in all, stood out in striking contrast to the inflamed surroundings, which thus framed the comparatively pallid x-rayed area. The central artery, turgid with blood above and below the x-rayed area, was a mere red thread within this space. There was no appreciable thickening of the x-rayed area on palpation except in No. HSX 11, in which a well marked edema of the lower portion set in. The second inflammation of the x-rayed area was therefore in general of a definite subacute character, while the first inflammation resembled a mild, acute inflammation.

Another striking difference between the x-rayed and normal tissues was observed in the development of exudate and crusts in the four rabbits mentioned above. The marked inflammation of the normal ear tissue did not lead to exudate and crust formation, while thick crusts often developed in the x-rayed areas.

This lamination was observed in the secondary exudate; no notes were made on the structure of the crusts in the primary exudate.
The chief sign which heralded the onset of a perforating gangrene was the appearance of a small, brown-black, slightly sunken spot on the internal skin surface of the x-rayed area; occasionally an exceptionally thick crust was the first sign. In the last three groups the sunken, discolored spot was noted seven times in the ten rabbits in which fenestration took place. The appearance of this discoloration, however, did not invariably foretell the onset of a perforating gangrene. Thus No. HSX 13 exhibited a brown discoloration of the internal surface with slight, thin crust formation 64 days after x-ray treatment. This lesion did not progress, but was practically healed on the 86th day. It will be remembered that this rabbit belongs to the sensitized group and showed no gangrene of the x-rayed area within more than 340 days after x-ray treatment (Table I and Text-fig. 1).

The initial point where a perforating gangrene developed was small when first observed, at times not more than 1 to 2 mm. in diameter. This dry spot then increased in size, first rapidly, then slowly until an equilibrium was established between the destructive and reparative factors. Several times two spots of gangrene developed, one on each side of and close to the central artery of the ear. These two spots always fused sooner or later, but the gangrenous process was always more rapid away from the artery than towards it, though finally the intervening section of the artery also dried up.

The amount of tissue lost by gangrene was never equal to the entire area x-rayed; in only two instances did the loss exceed 50 per cent. The gangrenous process began near the center of the area x-rayed and then progressed towards the borders. This spread was usually greater in the lateral direction than towards the root or apex of the ear. In the serum control group the loss of tissue varied between 80 and 130 sq. mm.; in the sensitized group (two rabbits) between 117 and 224 sq. mm.; and in the sensitized-reinjected group the loss fluctuated between 70 and 210 sq. mm.

The measurements given are only rough approximations of the losses, and no effort was made to determine the true areas of the more or less irregular fenestrations. We believe, however, that the figures convey a just impression.

In addition to the second, subacute type of inflammation leading to gangrene, which has been described, a delayed, second form of subacute inflammation also leading to gangrene may be distinguished. This form was observed only once; it occurred in Rabbit CX 18 of the serum control group (Table I). In this rabbit the first inflammation beginning 25 days after x-ray treatment led at once to a perforating gangrene 36 days after treatment. A similar acceleration of the process took place in No. HSX 8 of the sensitized-reinjected group and in all the

6 No measurements were made in the last rabbit, No. CX 18, because the gangrene involved the border of the ear, due to a shift during x-ray treatment. Here also the loss was less than 50 per cent of the x-rayed area. The loss of tissue sustained by the normal controls was not measured, due to an oversight.
normal controls (Table I). In No. CX 18, however, a subacute inflammation leading again to gangrene developed 135 days after the first. During this interval of time the remainder of the x-rayed area did not exhibit any obvious differences from the x-rayed areas of other rabbits.

**Final Changes in the X-Rayed Area.**

When the x-rayed areas of all the rabbits are examined some months after the last inflammation, all of them, including Rabbits HSX 12 and HSX 13 which never developed a perforating gangrene, show a number of changes in common. In all, the x-rayed area is hairless, the skin covering this area is smooth with perhaps a slight branny desquamation on the dorsal surface, and this skin wrinkles readily into thin folds. Occasionally, especially on the internal surface, numerous small, oval, yellowish brown thickenings of the outer skin are observable. These are less than 1 mm. in diameter and still less in thickness. They are seated in cup-shaped depressions of the skin, and probably represent keratoses.

The borders of the fenestrations generally show little or no thickening, but at or near the fenestration one or more red or reddish brown, slightly elevated papules are observable. These papules are formed by a number of dilated, small blood vessels. Occasionally, a slight hemorrhage proceeds from the angiectasias and the blood may burrow under the outer layers of the skin epithelium. These small masses of dilated blood vessels were also observed in Rabbit HSX 13 in which no perforating gangrene occurred; they were not seen in Rabbit HSX 12 of the same group.

In addition to these angiectasias the x-rayed areas show a number of tortuous blood vessels; often they are especially evident upon the internal surface. In the two rabbits of the sensitized group, Nos. HSX 12 and HSX 13, in which gangrene of the x-rayed area did not develop, these tortuous blood vessels are especially noticeable about the neighborhood of the central artery, where they form a delicate tracery of blood channels which are apparently superficial. The central artery itself in the x-rayed area of these two rabbits is narrow, slightly irregular in outline, and looks blurred in that portion of its course where the angiectasias are most marked.

The blood vessels of the healed, x-rayed areas do not react normally. In the normal rabbit the ear vessels respond by an initial blanching when the animal is sharply tapped, or a moderate struggle is induced; this blanching is followed by a marked vasodilatation if the original stimulus was sufficiently strong and if the room is not too cold. In the x-rayed areas of the experimental rabbits, however, this test causes at first some increase in the pallor, which later is replaced by only a slight dilatation of both arteries and veins. This striking difference in the vasmotor response of the x-rayed and untreated ears is well illustrated by Figs. 1 to 6, which were obtained by photographing the ears of two members of each group of rabbits during the stage of vasodilatation. It will be noticed that
both arteries and veins show a definite narrowing of caliber on entry of the x-rayed area, and that the original caliber is largely if not entirely regained when these vessels issue from the x-rayed area.

DISCUSSION.

From the experimental facts described above and summarized in the table and charts, it is clearly evident that the skin of rabbits under certain conditions may acquire a remarkably increased resistance to doses of x-rays which are surely destructive to control animals. These conditions are that the animal whose skin tolerance to x-rays is to be increased must be sensitized with horse serum and this sensitization must take place before the rabbit is exposed to the x-rays.

The evidence for these conclusions is summarized in Text-figs. 1 and 2. In these charts it is shown that the standard dose of 30 skin units of x-rays causes a perforating gangrene of the ear in normal controls within 37 days after x-ray treatment. The same dose of x-rays administered to the ear of rabbits previously sensitized with horse serum (sensitized group in the chart) was, however, remarkably weakened in its effect upon the tissues exposed to the x-rays. Two animals showed no gangrene at all during the period of examination (over 340 days); one exhibited a perforating gangrene after the lapse of 131 days, and only one member of the group of four rabbits reacted fairly like the normal controls by developing a perforating gangrene in 46 days. That sensitization must be present before the animal is exposed to the standard test dose of x-rays, if protection from the ordinarily destructive effects of this dose is desired, is shown by the serum control group. These rabbits were normal animals and were injected with horse serum for the first time, but this injection took place 13 days after exposure to the x-rays. In this group all rabbits developed fenestration of the ears subsequent to dry gangrene within 50 days after exposure to the x-rays. The serum injection after x-ray treatment therefore conferred no marked trace of protection to the x-rayed areas of the ears.

Additional evidence to establish this point, that sensitization previous to x-ray treatment confers a marked increase in resistance, is furnished by the behavior of the x-rayed area in Rabbit HSX 13 of the sensitized group. In this animal the x-rayed area a number of times exhibited some crust formation with moderate congestion of the surrounding vessels. In addition, the internal surface presented a brownish, sunken discoloration such as frequently preceded the appearance of a perforating gangrene in the x-rayed areas of the control rabbits. Yet healing was fairly prompt and no perforating gangrene resulted. The recuperative power of this x-rayed area, therefore, was greater than that existing in the x-rayed areas of the controls.
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Another observation which points to the same conclusion is the inflammatory reaction which occurred previous to fenestration within a portion of the x-rayed area of a sensitized rabbit, No. HSX 11. This inflammatory reaction was accompanied by a fair degree of redness and swelling and was much more pronounced than that observed in the x-rayed area of any other rabbit, though it was considerably less than the inflammation which the same rabbit showed in the adjoining untreated portion of the ear. This increased inflammatory response can only be interpreted as an expression of a more vigorous state of this x-rayed area when compared to that of rabbits of other groups.

Another fact which seems clear is that the protection to x-rays which sensitization previous to x-ray treatment gives, is largely abolished if these animals are reinjected with serum after being x-rayed; in other words, if they are subjected to an anaphylactic reaction.

The evidence for this statement is summarized in Text-figs. 1 and 2. The sensitized and reinjected group, it will be seen, was treated exactly like the sensitized group with but one exception: 13 days after being x-rayed and 23 days after the last sensitizing dose of serum, this group was reinjected with horse serum, and in consequence a mild, general anaphylactic reaction resulted, from which all promptly recovered. Nevertheless, the further course of the experiment showed that these reinjected rabbits had largely lost the protection which mere sensitization gives (see the sensitized group, Text-figs. 1 and 2), and dry gangrene with fenestration took place in due time. That some protection had still remained, however, is indicated by the fact that the interval between x-ray treatment and gangrene is appreciably longer in two animals (62 and 85 days respectively) than in any of the controls (see also Text-fig. 2).

The increased resistance of skin-covered tissues to unfiltered x-rays which results from previous sensitization with an undenatured protein may be roughly estimated from our data. In preliminary experiments we tested the effects of 15, 18, and 22$\frac{1}{3}$ skin units of x-rays on areas of rabbits' ears 4 sq. cm. in size. With 22$\frac{1}{3}$ units, perforating gangrene occurred in the two rabbits tested within 37 to 43 days. With 18 skin units, perforating gangrene took place 58 days after x-ray treatment in one rabbit, and incomplete gangrene in two others after 72 days, when observations were discontinued. With 15 skin units complete gangrene occurred in one rabbit after 91 days, incomplete gangrene in a second after 91 days, and no gangrene at all in a third animal after the same interval, when, unfortunately, all these rabbits were discarded.
From these incomplete data we may nevertheless conclude that sensitization with horse serum previous to x-ray treatment can reduce, at least in some animals, the destructive value of 30 skin units of x-rays to a level of 15 to 18 skin units.

It must not be forgotten that the conclusions which we have drawn so far rest upon experimental evidence gained under specific conditions which have been described in detail above. Further work must show whether modifications of these conditions entail significant changes in the result.

Our knowledge concerning the various factors involved is limited. We do not know fully what influence the degree of sensitization plays; whether or not a phase of increased susceptibility to the action of x-rays precedes the establishment of a heightened resistance; how long this increased resistance persists; what the maximum resistance is which can be attained by this procedure, and other questions.

To some of these questions a partial answer can be given. As far as the degree of sensitization is concerned, one may state that the rabbits employed were highly sensitized. In earlier series of experiments, the same sensitizing procedure, dose, and period of incubation had been used by one of us, and in these animals the intravenous reinjection test had yielded a high mortality rate. It must always be remembered, however, that the degree of sensitization which a certain fixed method achieves, fluctuates more widely in rabbits than in guinea pigs. This may explain why we failed to produce any sign of protection in one rabbit of the sensitized group (Text-figs. 1 and 2).

As far as the maximum amount of x-rays is concerned, our results with 30 skin units indicate that this dose is fairly close to the limit of tolerance with the experimental procedure employed.

Specificity.

The increased resistance to ordinarily lethal doses of x-rays which tissues may gain after a preliminary treatment with an undenatured foreign protein must be classed as a non-specific reaction, because the altered, abnormal response is called forth not by the sensitizing substance but by an utterly unrelated, physical agent. Such non-specific reactions after sensitization have been described and recognized for years. Heilner\(^7\) in 1908 observed that serum-sensitized rabbits succumbed to an injection of 4 per cent sodium chloride which was practically harmless to normal controls. Davidsohn and

\(^7\) Heilner, E., Z. Biol., 1908, 1, 487.
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Friedemann\(^8\) showed that rabbits sensitized with bovine serum react with temperature elevations to doses of sodium chloride given subcutaneously or intravenously, which produce no such effect in normal rabbits. Richet\(^9\) noted that dogs sensitized by actino-congestine or crépito-congestine vomited after smaller doses of apomorphine hydrochloride, injected intraperitoneally, than normal dogs.

Non-specific reactions have also been utilized therapeutically,\(^10\) but in this respect great caution is advisable. It must be realized that the incorporation of an undenatured foreign protein entails consequences of whose manifestations we are largely ignorant, and therefore often no intelligent balance can be struck between the harm and benefit which the procedure affords the patient. This deficiency in our knowledge should theoretically be lessened by laboratory work on the lower animals. For these reasons the irrational use of vaccines and sera is to be discouraged. Such powerful drugs should be used only when nothing else suffices to gain the desired therapeutic end. A conscious distinction should be drawn between drugs whose single injection exerts a comparatively short effect and those whose single injection releases reactions which are often masked and persist for months and even years. Sera and vaccines therefore may not be employed with the same careless freedom, which, for example, characterizes the use of various synthetic compounds.

**Mechanism of Protection.**

From the experimental data already presented and from the more obvious conclusions which we have so far drawn, no understanding of the underlying mechanism which brings about this increased resistance of the tissues to x-ray destruction can be reached. Some such


basis can be obtained, however, if the results are considered in the light of a broad generalization of anaphylaxis. Such a generalization is the experimentally founded view\textsuperscript{11} that an anaphylactic reaction is initiated when the anaphylactic antibody comes into contact with its antigen, during which process both antigen and antibody largely if not entirely disappear. If Text-figs. 1 and 2 are examined it will be observed that the only difference existing between the sensitized group and the sensitized-reinjected group is that the latter was subjected to an anaphylactic reaction 13 days after the x-ray treatment. In the sensitized-reinjected group, therefore, the anaphylactic antibodies had been removed more or less, while they were still present abundantly in the sensitized group. Since a majority of the sensitized group showed the marked resistance to massive doses of x-rays, while in the sensitized-reinjected group gangrene took place in the x-rayed area, it may be inferred that this protection is attributable to the anaphylactic antibodies which are present in the rabbits of the sensitized group, but which are not present, or at least not to the same functional degree, in the sensitized-reinjected group.

If the anaphylactic antibody is responsible for the protection to x-rays which the ears of the sensitized group exhibited, another inference may be drawn due to the fact that the x-ray treatment in the experiments was local. It follows that this protection must be assigned to the antibodies which are anchored to the tissue cells exposed to the x-rays and not to the circulating antibodies. This is shown clearly by the animals in the serum control group (Text-figs. 1 and 2). These animals had received one injection of horse serum, but this had been administered 13 days after the local x-raying of the ear. Within a short period an abundance of specific antibodies must have appeared in the circulation, and these necessarily must have traversed the capillary system of the x-rayed area of the ear. Yet practically no protection was conferred.\textsuperscript{13} It appears, therefore, that the cells


\textsuperscript{13} A slight degree of protection is probably present. As shown in Text-fig. 1, the serum controls developed fenestration of the ears in 36 to 50 days while the normal controls attained the same state in a shorter time, 30 to 37 days. See other evidence in Text-fig. 2.
of an x-rayed area are unable to produce anaphylactic antibodies or to fix them, when present in the circulation, in sufficient amount to protect, provided that the x-ray treatment takes place before the injection of the antigen. The sensitized-reinjected group also supports this inference; in this group the antibodies formed as a result of the second injection of serum did not adequately replace those which were originally fixed in the x-rayed area but which were rendered inert by the anaphylactic reaction, though a certain measure of protection was observed (Text-figs. 1 and 2).

Finally, it may be inferred that the locally fixed anaphylactic antibodies (sensitized group) can be functionally removed by an anaphylactic reaction (sensitized-reinjected group) and the local protection which these fixed bodies gave against massive doses of x-rays is then largely abolished.18

It is probable that the increased resistance to x-rays conferred by a previous sensitization to the skin of rabbits will also be obtainable in man, and the procedure may thus be of utility in human therapeutics. Such a contingency will appear when malignant growths must be treated without the scalpel. Under these conditions the applicable dose of x-rays is directly limited by the resistance of the skin overlying the neoplasm for example, and a lethal dose for the cancerous tissue perhaps cannot be applied because it would also destroy the integument. This tentative proposal presupposes that the cancerous tissue does not acquire the same degree of resistance as the skin cells after sensitization, also that the heavy doses of x-rays do not ultimately produce malignant skin alterations. Concerning the first supposition, there is no knowledge available at present, but the experimental test is easily made; concerning the second, it may be said that no malignant changes in the skin of rabbits have been observed after a period of more than 300 days.14 Finally, it may be stated that no objection can be urged against the parenteral employment of an undenatured foreign protein in such cases, because this effort is perhaps a last scientific attempt to help and it is therefore legitimate for the physician to invoke the aid of the protein molecule, fully conscious though he is that some or many of its various effects are not wholly desirable.

18 It is impossible to decide whether the moderate resistance of the sensitized-reinjected group is due to an imperfect removal of the anchored antibodies during the anaphylactic reaction or to anchorage of some antibodies subsequent to the anaphylactic reaction.

14 This period of time in the rabbit is comparable to a much longer interval in the human subject, if we consider the relative length of life in the two species.
That sensitization with a foreign protein protects the skin from the harmful effects of a subsequent x-ray treatment is indicated by studies made by Hektoen. In a series of important observations Hektoen studied the effect of massive doses of x-rays under various conditions upon the production of antibodies, the anaphylactic antibody not being included. His experimental material consisted of white rats, rabbits, and dogs, and the entire body of the animal was always subjected to the action of the x-rays. Hektoen established clearly that the time of x-raying with respect to the injection of antigen exerted a profound effect upon the antibodies. If the antigen was injected immediately after the preparatory x-ray treatment the production of antibodies was practically completely restrained. If, on the other hand, the x-raying was carried out at the height of antibody production (days or weeks after the antigen injection) no effect was noted on the antibody output.

The observations which interested us most, however, were as follows: When young puppies were x-rayed with strong doses of x-rays before they were injected with antigen (10 per cent rat or goat blood suspensions), severe burns of the skin were noted; but if they were x-rayed about 7 days after the antigen injection, Hektoen apparently observed no burns, for he only states that now many dogs showed no disturbances of the general health. If we are correct in this interpretation of Hektoen's work, our observations in this matter accord with his. We have not been able to find any other observations in the literature bearing upon this question.

Inflammation of the X-Rayed Area.

In the objective record of our results we have described three combinations in which inflammation of the x-rayed ear surface may appear. These three combinations, their distribution among the various experimental groups, and the duration of the process can be utilized to give further support to the antibody theory which has already been discussed.

16 Hektoen, L., J. Infect. Dis., 1918, xxii, 28. This article gives the references to Hektoen's earlier work. See also Hektoen, L., J. Infect. Dis., 1920, xxvii, 23.
19 We have not included among the various types of inflammatory reaction the combination noted in Rabbit CX 18 (Table I). In this serum control rabbit the first inflammation exceptionally led at once to complete gangrene of a section of the x-rayed area. But 135 days later a subacute inflammation with crust formation developed and led to still another loss of tissue. This delayed second inflammation healed in 24 days (195 days after x-ray treatment). This type of reaction is probably allied to the delayed x-ray lesions which at times occur in the human subject months after the last treatment (Pfoerring, S., Review in Am. J. Roentgenol., 1917, iv, 642).
The three combinations of states are as follows:

1. First inflammation ................................... gangrene.
2. First inflammation...healing.....second inflammation.....gangrene.
3. First inflammation......................................healing.

The distribution of these combinations is summarized in Table I. An examination of this table shows that the second type or combination (first inflammation—healing—second inflammation—gangrene) occurs only in the groups which had been subjected at one time or another to the injection of horse serum. It was never observed in normal rabbits treated with a destructive dose of x-rays.

The first combination (first inflammation—gangrene) occurred in all of the three normal controls; it also was observed in two additional normal control animals which had been x-rayed with 22\(\frac{1}{2}\) skin units. But in the serum animals this combination was only noted two times (Rabbits CX 18 and HSX 8).

The third combination (first inflammation—healing) was only observed in the sensitized group, in which the horse serum was administered previous to x-ray treatment. It occurred two times out of four experiments, in Rabbits HSX 12 and HSX 13. A third rabbit of this group (No. HSX 11) shows a very marked prolongation in the interval between recovery from the first inflammation and the onset of the second inflammation which led to gangrene.

From the occurrence of the second type of combination (inflammation—healing—two inflammations—gangrene) in eight out of twelve rabbits which had been treated with horse serum (Table I), and from the failure of this combination to appear in five normal control animals to which no serum had been given, we may infer that the increased resistance of the x-rayed tissue evinced by the second combination of states is definitely ascribable to the serum treatment. In other words, the administration of serum at any time within the limits employed in the experiments changes the reaction of the x-rayed tissue from the first combination (inflammation—gangrene) to the second combination (first inflammation—healing—second inflammation—gangrene) in the majority of the treated rabbits.

It should be remembered that the first inflammation was a mild acute form, while the second inflammation was subacute in character.
We conclude, therefore, that this change was due to a protective antibody action which was produced by the parenterally injected horse serum. From the data given in this section no inference can be drawn concerning the type of antibody which caused this change of reaction to the standard dose of x-rays. Such an inference, however, can readily be drawn if we use the occurrence of gangrene and the duration of the entire process (Text-figs. 1 and 2) as criteria, and in a preceding section evidence has been presented that the anchored, anaphylactic antibody may be considered the protective factor. It is therefore probable that the same anchored anaphylactic antibody is also responsible for the altered character of the local reaction which the serum-treated rabbits exhibit after x-ray treatment. What part, if any, is played by other types of antibodies in this matter cannot be determined by the data at hand.

On the basis of these considerations the various successions of conditions observed in the x-rayed areas of the rabbits may be explained as follows: The inflammation observed in normal control animals which ends in gangrene is the normal slow, destructive action of our standard x-ray dose (30 skin units). The tissues exposed show a mild, acute inflammation which leads to a complete destruction of a portion of the x-rayed area. How these tissue changes are produced by the physical agent, the x-rays, we do not know; vascular changes such as we have described undoubtedly are involved in the process.

If rabbits are treated with horse serum parenterally and exposed to the same standard x-ray dose, the type of reaction changes, due to the presence of anaphylactic reaction bodies anchored in the x-rayed area, the latter factor depending upon the time or times when the serum is administered. If the serum is administered about 2 weeks after x-ray treatment or if it is injected before and after x-ray treatment in such a way that a general anaphylactic reaction results, the second combination of conditions (inflammation—healing—inflammation—gangrene) then appears in the x-rayed areas of a majority of the rabbits (Table I, serum control group, sensitized-reinjected group).

For a good presentation of the various theories concerning the mode of x-ray action upon tissues, see Hall, C. C., and Whipple, G. H., Am. J. Med. Sc., 1919, clvii, 455.
The first inflammation now progresses to healing due to the presence of anaphylactic antibodies anchored in the x-rayed area. But this healing is only temporary, because the amount of locally available antibodies is too small or becomes functionally inert, and the slowly acting destructive forces gain the ascendancy over the reparative agencies. As a consequence the second inflammation appears which leads to a perforating gangrene. The second inflammation is sub-acute in character because the exposed area has been damaged by the x-rays, so that it can no longer react acutely to an inflammatory stimulus.

If, however, the serum is administered to a rabbit about 10 days previous to exposure to the standard dose of x-rays, the anaphylactic antibodies anchored in the x-rayed area may be sufficient in amount to protect that area for a long period or perhaps even indefinitely. The succession of conditions is then inflammation—healing—(Table I, sensitized group).

The explanation which we have given obviously only answers the question why the ordinary process of an x-ray action on tissues should be altered when the organism is treated with serum parenterally; how this alteration is produced we cannot say because it is not known how either the x-rays or the foreign protein exert their effects upon the tissue cells.

The results reported in this paper emphasize a precaution which ought to be observed in all animal experimentation. Since mere sensitization with an alien protein alters the reactivity of an organism not only towards the specific alien protein itself, but also towards an unknown number of other, unrelated substances or even physical agents, it is obvious that sensitized animals cannot serve as normal controls until it has been demonstrated that both the sensitized and normal animals react to the same agent in the same manner and to the same degree. Discarded animals which have been subjected experimentally to the action of undenatured proteins of bacterial, protozoan, metazoan, or vegetable origin should be used in identified groups when they are reemployed for an investigation. Failure to respect this precaution perhaps explains some of the discordant results obtained in diverse studies of the same problem. It is further possible that some of the erratic fluctuations in the degree of a reaction observed in a group of supposedly normal animals have their cause in an unsuspected proteinization of the abnormal reactors. The possibility or even probability of unwittingly employing proteinized mammalian material cannot be denied, for most investigators are compelled to rely upon dealers for their animal stock.
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pensation for this uncertainty, we may perhaps look upon the abnormal reactors among a group of animals as indicators of a possible proteinized state, and thus gain a working hypothesis which may add to our knowledge of non-specific phenomena.

SUMMARY.

When a fixed area of the ears of rabbits is subjected to the action of a standard destructive dose of x-rays (30 skin units) the type of reaction resulting depends upon the previous treatment of the rabbit. (1) In normal rabbits a mild acute inflammation develops in the x-rayed area which leads at once to a perforating gangrene within an average of 15 days. (2) If rabbits are x-rayed and about 2 weeks later injected with horse serum for the first time, a mild acute inflammation appears which heals for a time; then a second, subacute inflammation sets in which leads to a perforating gangrene. The average time of the process from the first inflammation to gangrene is 32 days. (3) If rabbits are sensitized with horse serum and 10 days later are exposed locally to the standard dose of x-rays, the ensuing ear reaction is either similar to the second reaction described above, except that it may last up to 110 days, or the first inflammation leads to a healing which may be apparently permanent (340 + days). (4) If rabbits are first sensitized with horse serum, exposed locally to the standard dose of x-rays 10 days later, and 13 days after the x-ray treatment reinjected with horse serum, the reaction of the x-rayed area of the ears is in general similar to the second reaction described above (inflammation—healing—inflammation—gangrene). The average time of the whole process is about 42 days.

On the basis of the general hypothesis that an anaphylactic reaction is initiated in the body when the specific antibody meets its antigen, and that both antibody and antigen are rendered more or less functionally inert by their interaction, the following inferences may be drawn from our experimental results. (1) The protection from the effects of a standard destructive dose of x-rays which a previous sensitization confers, is referable to the presence of anaphylactic antibodies in the x-rayed area. (2) This protection is largely due to the anaphylactic antibodies which are anchored in the x-rayed area, and not to those which are free in the circulation. (3) An anaphylactic reaction
renders the anchored anaphylactic antibodies largely impotent as protective factors against the standard destructive x-ray dose, even though sensitization preceded exposure to the x-rays. (4) An area treated with the standard destructive dose of x-rays is unable to produce or to anchor a sufficient amount of anaphylactic antibodies for protection from necrosis, when the x-ray treatment precedes the sensitization, or when the locally anchored anaphylactic antibodies are rendered functionally inactive by a general anaphylactic reaction.

It is possible that the procedure of increasing the resistance of the skin to a destructive dose of x-rays by means of a previous sensitization with protein may be applicable in the treatment of certain types of inoperable disease, when it is important to use massive doses of x-rays.

Animals which have been sensitized, or sensitized and reinjected with any undenatured alien protein, should not be reemployed as normal controls in any investigation unless trial has shown that these proteinized animals react quantitatively and qualitatively like normal animals.

The presence of an abnormal reactor in a group of supposedly normal animals may be an indication of a previous proteinization.

EXPLANATION OF PLATES.

The photographs of the rabbit ears were taken by transillumination while the blood vessels were in a dilated state. The time of photographing was 181 days after x-raying. The vessel traversing the middle of the ear is the central artery and in most figures its bifurcation near the upper pole of the ear can be seen. The marginal ear vein is also usually clearly visible. The x-rayed area of the right ear is shown as a bald quadrilateral space. Unfortunately the normal control rabbits were not photographed.

PLATE 100.

FIG. 1. Serum control group; Rabbit CX 16. Perforating gangrene occurred about 50 days after x-ray treatment.

FIG. 2. Serum control group; Rabbit CX 17. Perforating gangrene occurred about 50 days after x-ray treatment, but the process exceptionally is not yet complete although 181 days had passed since the x-raying. This is shown by the slowly healing, superficial ulcer to the right of the perforation, appearing as a black patch in the photograph.
FIG. 3. Sensitized group; Rabbit HSX 12. This figure shows that the bald, x-rayed surface is perfectly smooth with no perforation or crusts. The central artery in the x-rayed area is markedly narrowed. This area has remained in the same state for over 340 days after the date of x-ray treatment.

FIG. 4. Sensitized group; Rabbit HSX 13. The x-rayed area is intact and bald and the central artery shows clearly a partial stenosis. The small black spot represents a slight hemorrhage from a collection of fine, tortuous, superficial vessels at that point. The x-rayed area has remained in this condition now for more than 340 days.

FIG. 5. Sensitized and reinjected group; Rabbit HSX 5. The perforating gangrene took place 85 days after x-ray treatment. This figure shows clearly how both the central artery and the marginal ear vein are narrowed in the x-rayed field.

FIG. 6. Sensitized and reinjected group; Rabbit HSX 7. Perforating gangrene took place 62 days after x-ray treatment. The partial stenosis of a vein in the x-rayed area is shown to the right of the perforation.
Fig. 1.

(Auer and Witherbee: Reaction of normal skin to x-rays.)

Fig. 2.
(Auer and Witherbee: Reaction of normal skin to x-rays.)
(Auer and Witherbee: Reaction of normal skin to x-rays.)