LETHAL AND DISSOCIATIVE EFFECTS OF X-RAYS ON BACTERIA

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Since the discovery of x-rays by Roentgen in 1895 their use in experimental biology has assumed increasing importance. As early as 1898 Rieder showed that the rays from an anti-cathode could kill *Vibrio cholerae*. Work reported since then has served to establish the lethal action of the rays on bacteria, but most of the studies have been of a qualitative nature and have dealt with the killing action only. More recently, attempts have been made to determine what particular rays are lethal. However, there are very few studies on the changes which occur in the population of x-rayed cultures.

The rays emitted from x-ray tubes are electromagnetic waves whose energy is distributed in units known as quanta. In general, all rays of wave lengths greater than 1 Å (Ångström Unit) are classified as soft rays and those of wave lengths less than 1 Å as hard rays. The latter, due to their greater energy, are able to penetrate farther into substances, while soft rays are more readily absorbed. It is important that the type of radiation, whether hard or soft, be known when applying x-rays to biologic and physical problems.

For quantitative studies of the biologic effects of x-rays, it is necessary to have some reliable means of measuring their energy or intensity. Unless an ionization chamber is available so that the radiation can be measured directly in Roentgen units, the intensity may be calculated to a fair approximation by the application of the empirical law that, for a given target in an x-ray tube, intensity is proportional to the product of the tube current and the square of the tube voltage.

The type of radiation, signifying the softness or hardness of the

x-rays, depends upon the voltage applied to the tube and upon the kind of material used as the target. For a given voltage there exists a minimum value of the wave length of the emitted x-rays. This relation may be expressed by the formula:

$$\lambda = \frac{12345}{V}$$

where λ is measured in Ångström units and V is measured in volts. Unless a filter is used, the energy of the x-ray beam is distributed among all the wave lengths from this minimum to the very longest x-rays; however, most of the energy is contained in the wave length region close to the minimum value. Superimposed upon this continuous radiation is the characteristic radiation. This is dependent on the material used as the target; the higher the atomic number of the metal used, the shorter the wave length, and hence the harder its characteristic radiation. From these considerations it is evident that x-rays become harder as the tube potential and the atomic number of the target are increased.

The majority of previous studies on lethal effects of hard and soft rays have involved the use of agar surfaces for exposing the bacteria to the rays. By the use of this method, Schepman and Flecke (1926) found that soft rays killed bacteria within a few minutes, while equivalent doses of hard rays had approximately half the killing effects. In the same year, Ponzio (1926) subjected bacteria to the secondary rays from copper, molybdenum, silver, tin, antimony, barium, and lead, and reported that the characteristic radiations from the elements of higher atomic number were more destructive to bacteria than those from elements of lower atomic numbers, thereby inferring that hard rays are more destructive than soft. The work that followed, notably that of Burger (1931) and Pugsley, Oddie and Eddy (1935) substantiated the observations on the lethal action of soft x-rays. Wyckoff (1930) tested the lethal effects of X-rays emitted from copper and tungsten tubes and found that the rays killed *Escherichia coli* and *Salmonella aertrycke* in a linear exponential fashion. The cell parts involved in death were calculated to occupy a volume less than 0.06 that of the cell, and the absorption of a single quantum of x-ray energy was sufficient to cause death.

An important consideration in the use of agar as an exposure surface is the observation by Blank and Kersten (1935) that a toxic substance is formed in agar treated with x-rays. They found that such agar could not be solidified and contained a water-soluble toxic factor which inhibited the germination of spores and the multiplication of vegetative cells. This toxic substance was not found in irradiated beef extract, water, salt, or peptone. In the light of these findings much of the previous work using an agar surface as an exposure medium should be reconsidered.

Some investigators have reported that x-rays affected the physiologic and colonial characteristics of yeast and bacteria. Schneider (1926) treated actively fermenting cultures of yeast and found that 10 per cent of their fermenting ability was lost. However, when yeasts were neither actively fermenting nor in the presence of electrolytes, they resisted the action of the rays. Rice and Guilford (1931) demonstrated that x-ray treatment gave a marked increase in dissociation from "rough" to "smooth" colonies in a rapidly growing bovine strain of *Mycobacterium tuberculosis* which showed a tendency toward dissociation of this type. Stable "R" cultures of the same species and non-acid fast organisms were unaffected. Large doses of x-rays led to lethal effects. On the other hand, Bertrand (1929) found that x-rays of 2 Å had no effect on the virulence or on the quantity, quality, or rapidity of growth of *Staphylococcus aureus* and *Microsporon audouini.*

The success which the geneticists have had in using x-rays as a means of analyzing the genetic mechanisms of multicellular forms suggested the application of x-rays to the study of the hereditary mechanisms of bacteria. The present report is concerned with the lethal effects and the dissociative changes produced by hard and soft rays.

MATERIALS AND METHODS

X-ray apparatus

A self-rectifying water-cooled copper x-ray tube operated at voltages ranging from 15 to 36 kv. and with currents ranging from 5 to 20 ma. was used for the studies on lethal effects. With these voltages, the values of the minimum wave length ranged

from 0.823 Å to 0.344 Å, respectively, as determined by the law previously stated. The Kα radiation has a wave length of 1.54 Å, which comprises most of the intensity of the characteristic radiation. The x-rays produced at 15 kv. may be considered as soft rays, those at 36 kv. as hard rays.

For studies on dissociation, a Coolidge molybdenum tube as well as the copper tube was used. The molybdenum tube was operated on a rectified power supply at a voltage of 35 kv. and at currents ranging from 15 to 25 ma. Under these conditions the minimum wave length was 0.353 Å and the wave length of the maximum characteristic radiation was 0.710 Å. Therefore, these x-rays were decidedly hard, although, due to the presence of the continuous radiation, some soft rays were present.

The bacteria to be treated were exposed at a distance of two inches from the target of the x-ray tube. A fluorescent screen was then placed directly behind the vials so that they could be accurately located in the x-ray beam and also so that an estimate of the degree of penetration of the x-rays through the vials could be made.

Lethal effects

The bacterial suspensions were prepared in the following manner. A culture of *Staphylococcus aureus* was purified by single-cell isolation. Agar slant cultures were incubated for 24 hours and the growth harvested by gentle washings with distilled water. The cells were then thrice washed in water by centrifugation and diluted to give a concentration of approximately one-half to one billion cells per milliliter, as determined by plate counts. The absence of nutriment in the solution used to suspend the cells reduced the chances of multiplication during treatment.

To avoid the source of error introduced by the possible formation of toxic substances, paraffin-cellulose vials were used. Cellulose extraction thimbles, 10 mm. in bore and 50 mm. in length, were used to prepare the containers. The thimbles were infiltrated and coated with melted paraffin, tightly stoppered with corks, placed in cotton-stoppered French bottles, and then autoclaved at 15 pounds pressure for 20 minutes. After sterilization

the vials were cooled rapidly and, when used, 1.5 ml. of cell suspension was pipetted into each. The vials were kept in the sterile bottles until the time of x-ray treatment. Several vials of each series remained untreated to serve as controls.

Immediately after treatment, the vials were agitated to distribute the cells evenly and 0.2 ml. transferred from each to nutrient broth for dissociation studies. One ml. of each of the suspensions was diluted in a tenfold series to a dilution of 1:10 million, using 9 ml. water blanks, and pour plates were prepared from each of the higher dilutions (1:100 thousand, 1:1 million and 1:10 million) for survival counts. All plate counts were prepared in duplicate and incubated for 48 hours. Those plates that contained from 30 to 300 colonies were selected for determining the number of bacteria that survived.

Dissociation effects

In the study of the influence of x-rays on the hereditary mechanisms, purified cultures of *Staphylococcus aureus*, *Staphylococcus albus*, *Serratia marcescens* and *Leuconostoc mesenteroides* were irradiated with the rays from the copper and molybdenum tubes already described. The organisms were treated either as young growing broth cultures or as thrice-washed cell suspensions.

In most cases the tests on washed cells were made in conjunction with the studies on lethal effects. The vials containing the bacteria to be treated were filled with 1.5 ml. amounts of suspension. After treatment, 1 ml. was used to determine the numbers killed and 0.2 ml. was transferred to fresh broth for the studies on dissociation. The plates used in determining the numbers killed also served as material for observations on dissociation immediately after x-ray treatment. The broth cultures of treated bacteria were incubated at their optimum temperatures and daily observations were made on the character of the growth and morphology of the cells. Agar plates were streaked and colonial observations were made after 48 hours of incubation.

In the studies on growing cells, 1.5 ml. of 18-hour broth cultures were transferred to sterile paraffin-cellulose vials and treated with radiations from the molybdenum tube. After exposure, 1 ml.

of the culture was transferred to 9 ml. of nutrient broth and incubated. Random samples were made from day to day to observe any dissociation.

To minimize contaminations preparation of cell suspensions and handling of cultures were performed in a dust-free room. Nutrient agar plates which had been exposed to the air while work was being done were incubated with the test materials and served as controls on air-borne contaminations. Similarity of air-borne contaminants to the colonies found in the treated and control cultures was taken as sufficient reason to discard the suspected plates.

LETHAL EFFECTS OF HARD AND SOFT RAYS

The results of preliminary studies demonstrated that destruction of bacteria by x-rays is a function of their wave lengths. Experiments were planned to study the part played by various wave lengths, by exposure time, and by intensity of the x-rays.

The relation between time of exposure and lethal effects

Suspensions of staphylococci were exposed to the x-rays from the copper tube operated at 36 kv. and 20 ma. for 5, 10, 20, 40, and 80 minutes, after which plate counts were made.

It was found that killing was dependent on the time of exposure. The results, when plotted on the basis of the per cent of bacteria killed, yield a sigmoid curve similar to the "death curve." When a semi-logarithmic curve is made from the logarithms of survivals, a straight line results (fig. 1). In other words, the rate of destruction of the organisms is directly dependent upon the number present at any given time.

Relative importance of wave length and intensity

Suspensions of staphylococci were irradiated with x-rays from the copper tube operated at 15, 25, and 36 kv. with a constant exposure time of 15 minutes. At each of these voltages the following tube currents were used: 2, 5, 10, and 20 ma. This gave an ample range of intensities for each type of ray (as determined by the voltage) so that the relative effects of intensity as

compared to wave length could be studied. In order to insure a uniform treatment of all of the cells in the suspensions the vials were agitated after 8 minutes exposure.

A second series of radiations was performed, using the same apparatus with the same range of voltages and intensities, but a longer exposure time. These suspensions were exposed for 30 minutes, the vials being agitated after 15 minutes of exposure in each case. Plate counts were made in the usual manner. The graphs in figure 2 and figure 3 represent these data, in which the

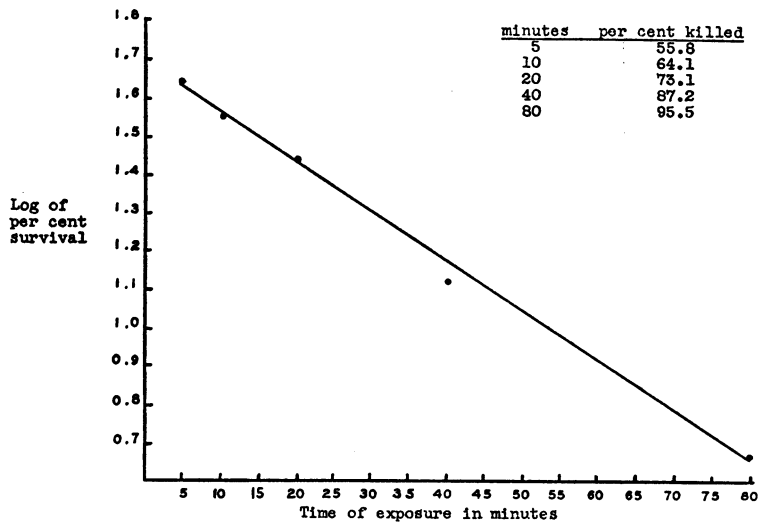
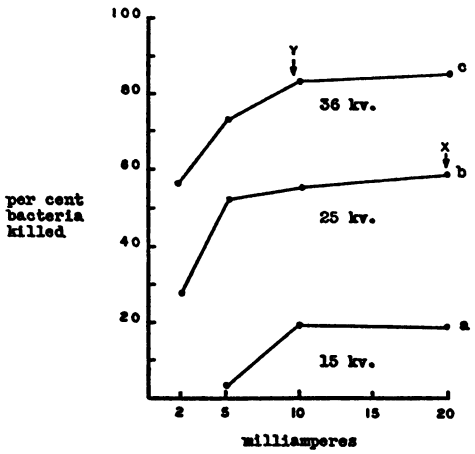


FIG. 1. Semi-logarithmic graph showing the effects of various exposure times on the survival rates of *Straphylococcus aureus*. Copper target—36 kv. at 20 ma.

per cent of bacteria killed is plotted against the milliamperes used, with a fixed value of voltage for each curve. The results of the second series (fig. 3) were essentially the same as those presented in figure 2, but the increase in time served to raise the total killing effects and to lessen the distance between the three curves. The order of magnitude of the curves with respect to each other was unchanged.

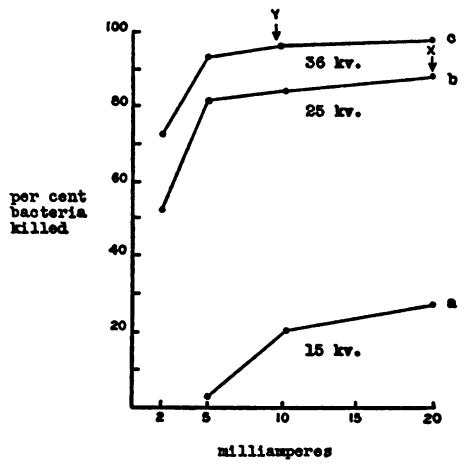
The results presented in figures 2 and 3 show that the destructive power of the rays is increased as the milliamperage is raised up to a certain point. After this point is reached, which in our

experiments, is approximately 10 ma., a further increase has much less effect on the destructive powers of the rays. For example,



kv.	ma.	per cent killed
15	5	3.3
15	10	19.5
15	20	18.8
25	2	28.0
25	5	52.5
25	10	55.3
25	20	59.1
36	2	56.9
36	5	73.2
36	10	85.5
36	20	85.3

FIG. 2. Effects on *Staphylococcus aureus* of varying voltages and currents of the x-ray tube. Copper target. Exposure time 15 minutes.



kv.	ma.	per cent killed
15	5	2.8
15	10	20.4
15	20	27.8
25	2	52.9
25	5	82.4
25	10	83.5
25	20	88.0
36	2	71.2
36	5	92.6
36	10	95.4
36	20	97.2

FIG. 3. Effects on *Staphylococcus aureus* of varying voltages and currents of the x-ray tube. Copper target. Exposure time 30 minutes.

at 5 ma. and 25 kv. (curve "b" of fig. 3), 82.4 per cent of the organisms were killed, while four times this milliamperage resulted

in only 5.6 per cent increase in destruction. The slope of the curve represents the per cent of bacteria killed as the milliamperage, hence the intensity, is increased. The marked decrease in the slope of the curve is explained by the fact that all of the organisms in the suspensions were not completely bathed in the rays until the intensity had become great enough to penetrate the sample completely; at this point, then, the curvature changed. The lack of penetration of the x-rays through the specimen below this point was noted by observing a fluorescent screen which was placed behind the vial.

From these curves, it appears therefore, that the type of ray is more important in the lethal effect than the intensity, and that, accordingly, hard rays are more destructive than soft rays of the same intensity. This is concluded from the following considerations.

If intensity alone were the important factor in killing bacteria, then two rays of the same intensity and capable of equal penetration should show the same lethal effects. In the relation

$$I \alpha iV^2$$
$$\therefore \frac{I_1}{I_2} = \frac{i_1 V_1^2}{i_2 V^2}$$

I_1 and I_2 are the intensities corresponding to the currents i_1 and i_2 and to the voltages V_1 and V_2 respectively. If these intensities are equal, then we have the condition

$$i_1 V_1^2 = i_2 V_2^2$$

Using this formula, one finds that 25 kv. at 20 ma. (point "x") gives an intensity equal to 36 kv. at 9.65 ma. (point "y"). However, the actual lethal effects were quite different (figs. 2 and 3). In figure 3, 25 kv. at 20 ma. gave 88 per cent lethal effects, while 36 kv. at 9.65 ma. had approximately 95 per cent. The reduction of exposure time served to make a significant difference between the points as shown by the results that for 15 minutes exposure, 25 kv. at 20 ma. killed 59 per cent of the cells in the suspension, while 36 kv. at 9.65 ma. killed approximately 82 per cent of the cells.

THE DISSOCIATIVE EFFECTS OF X-RAYS

On the basis of the work of Muller (1927), who experimentally produced mutations in the fruit fly, it is conceivable that if bacteria have an hereditary mechanism which functions in the same manner as that of higher forms of life, this mechanism should be affected by x-rays. Alterations of this mechanism might be detected by studying the characteristics of treated cells or their progeny.

Dissociative effects on young broth cultures

Eighteen-hour broth cultures of *S. aureus*, *S. albus* *S. marcescens*, and *L. mesenteroides* were prepared and transferred to vials, four vials for each culture. Three vials of each culture were treated with x-rays from the Coolidge molybdenum tube operated at 35 kv. and 23 ma. using exposures of 10, 17.5, and 25 minutes. The fourth vial of each set was left untreated and served as a control. After treatment, all of the cultures were transferred to 9 ml. of nutrient broth and incubated, *S. marcescens* and *L. mesenteroides* being incubated at 25°C., and *S. aureus* and *S. albus* at 37°C. Plates were streaked daily from the sixteen cultures and observations were made on the colonies after two days of incubation.

After this period of incubation, two days after x-ray treatment, *S. marcescens* began to yield colonial variants of rough and mucoid types and color variants ranging from red to white. By the tenth day, these variations were quite striking. The control cultures yielded only a few white colonies and the remainder of the colonies were of the parent type in color and form.

The cultures exposed for 10 minutes produced several dwarfed colonies, rough types, mucoid colonies, and color variants. Those exposed for 17.5 minutes produced variants of the same types observed in the previous case, but in greater abundance. An occasional hard, pinnacled, rough, red colony was found. This type of colony seemed to have grown into the agar and had to be pried loose from the surface before isolation could be effected. The greatest number of variant types was observed in the cultures irradiated for 25 minutes, more than 50 per cent of all colonies on

streaked plates being variants. All the forms described by Reed (1937) were present in this culture. There were the pinnacled hard colony types, mucoid color varieties, rough mucoids, smooth white colonies, violet tinged colonies, and dwarfed colonies.

Representatives of each of the different types were isolated for the further study of their physiologic and morphologic characteristics. Most of the mucoid forms of *Serratia* consisted of encapsulated bacilli, while those of the non-mucoid forms failed to show capsule formation on plain nutrient agar. The isolated variants were purified by successive plating and selection. Of the purified variants, 38 were selected for further study.

The physiologic characteristics of these *Serratia* variants were tested in glucose, sucrose, lactose, mannitol, glycerol, galactose, and salicin broths containing brom-cresol-purple as the indicator. The liquefaction of gelatin and the growth in litmus milk as well as the production of indole were also tested. The media were inoculated and observed at 24-hour intervals.

The physiologic characteristics of the variants remained similar to that of the parent type, while the colony morphology was widely different. However, the following alterations in physiologic properties were observed: Two of the variants failed to ferment glycerol, three formed indole, and eight produced coagulation and peptonization of milk. The alterations in physiologic properties as compared to the parent culture did not appear to be associated with any particular changes in colony types.

After the twentieth day of incubation, all of the variants of *S. marcescens* found in the irradiated cultures were present in the controls although they were more numerous in the former. The possibility that the results obtained were due to chance is ruled out by the fact that essentially similar results were found on repeated experiments under similar conditions.

In the irradiated broths the growths appeared red with lumpy and granular sediments. In contrast, the shade of red of the control broth cultures was not so deep and the sediment was finely granular. After five days of incubation the differences between the treated series and the controls were very definite.

Cultures of *L. mesenteroides* failed to show any noticeable

effects due to x-ray treatment. The staphylococci showed few changes, although some variant forms were found in the treated cultures several days before they appeared in the controls.

Effects on non-proliferating cells

The studies on lethal effects of x-rays were accompanied by dissociation studies. In the experimental series recorded in

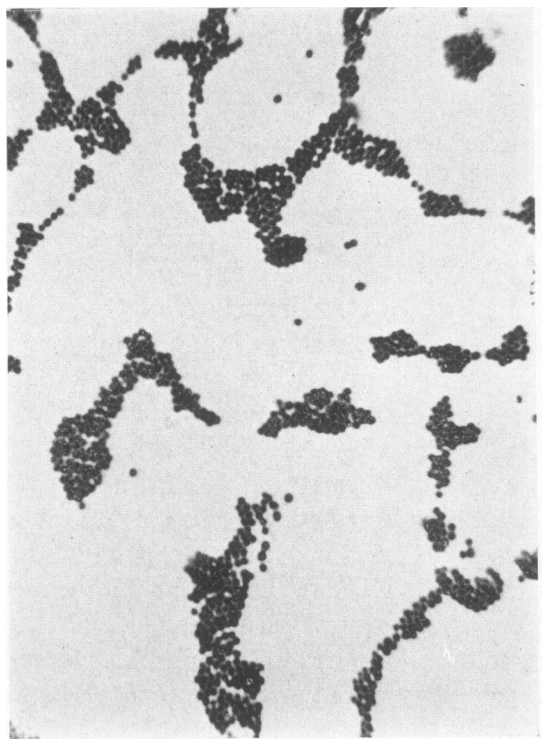


FIG. 4. *Staphylococcus aureus* before x-ray treatment. Gram stained

figure 2, it was found that the broth cultures prepared from cells treated with radiations from the copper tube operated at 15 kv. and currents of 5, 10, and 20 ma. and at 25 kv. with 5 ma. showed a distinct granular growth. This granulation was so marked that most of the growth was in this form and had settled to the bottom of the tubes as granular masses. The cellular morphology of these cultures was studied by means of the gram stain. The

control cultures showed only normal gram-positive cells (fig. 4,) while the experimental cultures containing this marked granulation were found to be composed of an abundance of streptococcus-like forms. The chains, made up of paired cocci, sometimes contained as many as 100 cells. Plates streaked from these cultures yielded only white variants and normal staphylococci. All attempts to isolate the streptococcus-like form failed. Figure 5 shows this form as it occurred after 18 hours of incubation. After 10 days of incubation, long, gram-negative, thread-like forms appeared among the chains (fig. 6). These could not be subcultured and it was conjectured that they were formed by the fusion of cocci in a chain.

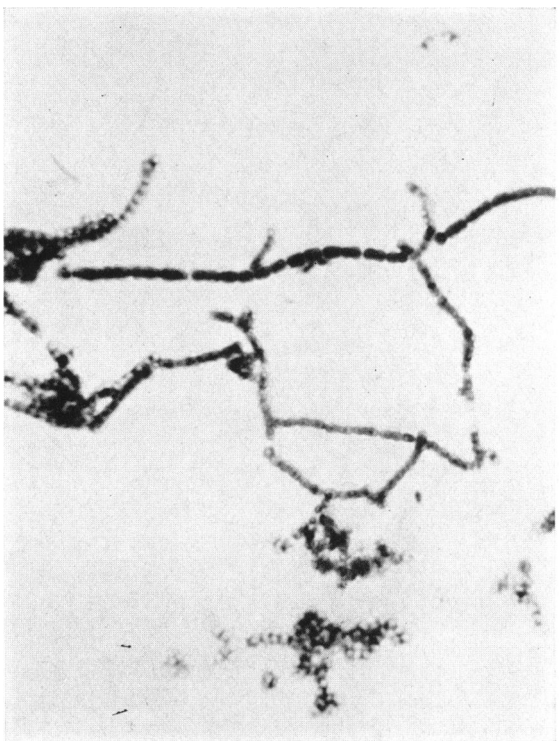


FIG. 5. The streptococcus-like form occasionally appearing in *Staphylococcus aureus* cultures after x-ray treatment. Gram stained.

The experiment was repeated several times and in only one in-

stance were similar forms obtained. In this case it occurred in cells treated with the hard rays from copper (36 kv. and 20 ma.), while in the previous case they were found in cells treated with the soft rays from copper. The production of these forms could not be attributed, therefore, to any definite type of radiation.

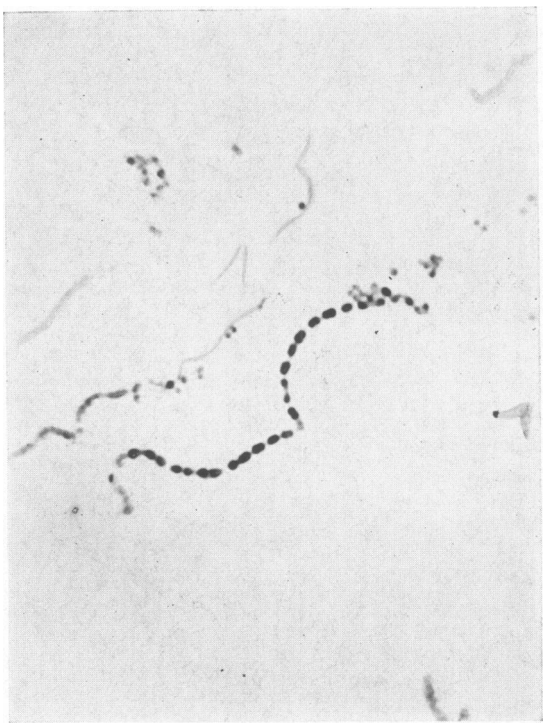


FIG. 6. The streptococcus-like form of figure 5 after 10 days ageing in broth Note the tendency to chain formation. Filamentous forms can also be seen. The chain forms are gram-positive and the filaments are gram-negative.

Streaked and poured plates of x-ray-treated and washed staphylococcus cells showed greater colonial alterations with the harder rays. Variation was stimulated by soft rays, but not to the same extent as by the short wave lenghts. The examination of plates prepared immediately after irradiation showed one or two variant colonies for each several hundred parent type colonies, while the controls of untreated cells remained pure.

Effects of filtered and unfiltered rays of molybdenum

An attempt was made to determine which types of rays emitted from molybdenum were effective in stimulating dissociation in the cultures under study. A zirconium oxide filter was used to absorb the soft radiations, allowing the transmission mainly of the $K\alpha$ rays of molybdenum which have an average wave length of 0.710 Å. In this series, the time of exposure was doubled to compensate for the resulting decrease of intensity due to the absorption by the filter. In all cases, the exposures were made by the methods previously described.

Young broth cultures of *S. marcescens* and *S. aureus*, which had been previously purified by single-cell isolation, were prepared and 1 ml. of each was transferred to each of 3 paraffin-cellulose vials. One set of vials received 30 minutes exposure to unfiltered rays of molybdenum at 35 kv. and 20 ma. A second set of vials was exposed to the zirconium-oxide-filtered rays for 60 minutes, while a third set remained untreated and served as controls. After treatment the cells were transferred to nutrient broth and incubated at their optimum temperatures.

After the second day of incubation *S. aureus* showed no changes while *S. marcescens* yielded an occasional variant colony. By the ninth day there were considerable differences in the character of the sediments of the broth cultures. In the case of *S. aureus* the sediment in the control was light and readily dispersable, that of the filtered-ray-treated cultures was heavy and composed of masses, while that of the culture treated with unfiltered rays was very granular and composed of masses.

S. marcescens presented a similar picture. The control broth culture contained a red pigment with a moderately turbid growth and there was a light red ring on the side of the tube at the broth-air interface. The filtered-ray-treated culture appeared to be the same as the control, while the culture treated with unfiltered rays was deep red and contained very granular growth and a heavy red ring of bacteria on the glass at the broth-air interface. Plates streaked at this time showed that there were more colonial variations in the unfiltered-ray-treated cultures than in the filtered-ray-treated cultures. The controls yielded a few colonial variants. By the fourteenth day of incubation, the differences

between the treated and untreated cultures were very distinct, while the results of filtered and unfiltered x-ray treatments showed the same general changes both qualitatively and quantitatively.

This set of experiments supports those presented in the previous section on the production of dissociants by x-rays, and while they were not conclusive in determining what type of ray was the more effective in producing variations, they did indicate that hard rays seemed to be the more effective.

Effects of x-rays on the broth substrate

The possibility occurred to us that the x-ray treatment of broth might result in the formation of some substance that would influence the dissociation rates of bacteria, and that the results noticed were perhaps not due to the direct action of x-ray energy on the cells. To test this possibility, several vials containing 2 ml. of uninoculated broth were treated for 30 minutes with the rays from the molybdenum tube operated at 35 kv. and 20 ma. The irradiated broth was then transferred to 5 ml. of fresh nutrient broth and the mixture was inoculated with young broth cultures of *S. aureus* and *S. marcescens*. For comparison, young broth cultures were treated with the same rays for 30 minutes and transferred to 5 ml. of fresh nutrient broth. Untreated cultures were used for controls.

After 5 days, the treated cultures showed granulation and masses in the sediment of the tubes. The controls and the culture in treated broth remained normal. Streaked plates, made after 8 days, demonstrated that active dissociation was occurring in the x-ray-treated cultures, as would be expected from the previous experiments, while the treated broth and control cultures showed only an occasional variant. From this it was concluded that the x-rays acted directly on the bacterial cells and not on the broth.

DISCUSSION

As is generally the case with disinfectants, both physical and chemical, destruction of bacteria by x-rays was found to occur in a semi-logarithmic fashion, indicating that the rate of destruction

of organisms was directly dependent on the numbers present at any given time. It is unlikely that the sigmoid curve resulting from plotting the percentage of survivors against the time of exposure is due to normal variability of the bacterial population in sensitivity to the rays. This implies that the individual organisms did not vary greatly in susceptibility to the rays, for if they did, a semi-logarithmic curve could not have been obtained from the results.

The cultures used in this study were purified by single cell isolation and care was taken to reduce the probability of dissociation during the preparation of cell suspensions. Studies on the dissociation rates of the parent strain of staphylococcus demonstrated that the culture purified by single-cell isolation did not show any detectable colonial variants in approximately 2,000 colonies observed on a group of streaked plates prepared by random sampling of a 24-hour-culture. This insurance of a uniform population, together with the physical aspects pointed out by Wyckoff and supported by our results, leads to the conclusion that the lethal effect is proportional to the number of bacteria present at any given time.

The results of the experiments, using tube voltages and milliamperes as variables with the time as the constant, indicate that the types of rays rather than the intensity of the rays is the important factor in killing bacteria, once the rays completely penetrate the sample.

The work of geneticists demonstrates that the treatment of plants and animals with x-rays cause mutations, but these mutations cannot be directed or predicted because of the variety of chromosomal changes produced. Muller (1927) stated that neither the detectable numbers nor types of mutations could be predicted, and found that the mutations may be of a kind already described or entirely different. He concluded that the changes produced were due to the absorption of single quanta of x-ray energy at crucial points on the chromosome.

A similar hypothesis might be adopted to explain the effects of x-rays on bacteria. One might assume that the dissociative effects are accomplished when a quantum of x-ray energy is

absorbed by an exposed cell and happens to affect a crucial point in the genetic mechanism. This effect may result in lethal effects, stable variations, or unstable variations.

No attempt was made to distinguish between lethal mutations and dead cells because of the inherent difficulties. These difficulties arise from the basis upon which cells are classified as living or dead. However, in the treatment of bacteria with x-rays, it is possible that the cells classified as dead were actually alive, but no longer capable of fission because of some effect on the reproductive mechanism of the cell.

The methods of detecting variation in this study have inherent weaknesses. For the purpose of the experiments performed, only streaked and poured plates were used to isolate variant forms of colonies. Colonial variations are the easiest to detect, but they are by no means the only changes that occur, and therefore it is quite possible that many variations were overlooked. On this basis, the studies made on colony changes produced by X-ray treatments can be taken only as an index of activity occurring in the culture. It is probably true, however, that this method of detecting variation is reasonably accurate.

Certain objections might be raised to the interpretations placed on the increase of the dissociation rates, in that the variants were not found in the culture for several days after treatment. Such objections can be dealt with in the following manner. As has been found with higher forms of life, the treatment of a group with x-rays does not result in mutation in all of the individual members. Only a small percentage are actually affected in such a manner that detectable mutations can be found. In the case of x-ray treatment of bacteria, the groups treated consisted of populations of several hundred thousand to one billion individuals per milliliter, as determined by plate counts. If the properties of a small part of one per cent of the population are altered so that a detectable variant occurs, the chance of finding such individuals in random sampling of the population by the streaked and poured plate methods is exceedingly small, since before they can be detected they must increase in numbers. If the variant bacterium is of such a nature as to render it capable of competing with the

rest of the population in the culture, it may take several days for the increase of the variant to occur. If the variations in properties are unfavorable to the organism's further growth, it may die or be overgrown by the rest of the culture and not be found.

The increase in the dissociation rates of the cultures seemed to show that the x-ray energy emitted from both the copper and molybdenum tubes affected the hereditary mechanisms of some of the exposed cells. Not all of the experiments performed led to definite increases in colonial variants, for occasional treatments failed to induce any appreciable effects on the rate of dissociation as determined by colony observations. Likewise, the types of variants that appeared in the cultures of treated washed suspensions were unpredictable. This result is consistent with the findings on higher forms of life.

Observations on the colonial and physiologic characteristics of variants isolated from treated material demonstrated that any of the properties might be changed without being accompanied by alterations in the other properties of the cells. This independence of variation suggests that alterations other than colonial were probably missed, due to a lack of adequate methods of detection.

In the studies on lethal effects, pleomorphism was found to be a rare occurrence. No particular importance can be attached to this, other than the fact that it is another case of hereditary change induced by x-rays. A somewhat similar occurrence was found by Lea, Haines and Coulson (1937) using *Escherichia coli* exposed to x-rays. They found that the cells treated with the rays would occasionally form long filamentous rods. This effect was explained as due to interference with the fission mechanism by x-rays. It is quite possible that the same thing occurred in the treated staphylococci of this study. If Figures 5 and 6 are closely inspected, it can be seen that division and complete separation of the cells have not taken place. The fact that the streptococcus-like form was capable of increase for a period of 24 hours indicates that this pleomorphism is an hereditary alteration of the fission mechanism. Somewhat similar observations were made by Spencer (1935) on the pleomorphism of *Eberthella typhosa* and *Streptococcus scarlatinae* after exposure

to emanations from radium. The cultures of *E. typhosa* contained filamentous rods as described by Lea, *et al.*, for *E. coli*, and *S. scarlatinae* showed formations similar to those of the staphylococci of this report.

In a study of the effects of x-rays on dissociation it is difficult to determine which of the variants are attributable to the action of the rays and which are formed in the natural course of multiplication. For this reason, we do not attribute any of the variants, other than the streptococcus-like form of the treated staphyloccus, to x-ray effects until more can be learned. However, it is apparent in this study that x-rays increased the dissociation rates of several strains of *Staphylococcus aureus* and *Serratia marcescens*.

SUMMARY AND CONCLUSIONS

1. When the logarithms of the per cent of cells surviving x-ray treatment were plotted on semi-logarithmic paper against the time of exposure, a straight line was obtained, indicating that the lethal effects of x-rays are dependent on the number of bacteria present at any given time.

2. The results of the experiments using kilovolts and milliamperes as variables, with time as the constant, demonstrated that the lethal action of x-rays is dependent more on the wave length emitted than on the intensity, once the rays completely bathed the suspension.

3. The short wave lengths (hard rays) used in this study were more effective in killing *Staphylococcus aureus* than the long wave lengths (soft rays) of the same intensity.

4. X-rays increased the dissociation rates of *Staphylococcus aureus* and *Serratia marcescens*.

5. Actively proliferating cells showed a greater tendency to form dissociants after irradiation than non-proliferating cells. Occasionally streptococcus-like forms resulted from the treatment of *Staphylococcus aureus* with x-rays.

6. The kind or number of variants that result from x-ray treatment could not be predicted. Often no changes were observed.

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