On the Effect of Roentgen Rays on Cellular Division

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AMONG numerous problems in which Niels Bohr has taken a promoting interest is the biological effect of radiation. This fact induced the writer in this paper to give a survey of some aspects of the effect of Roentgen radiation on cellular division.

Cellular division was found to show a pronounced susceptibility to the action of Roentgen rays. Figure 1 shows clearly that dosages which hardly influence fundamental metabolic processes such as respiration or glycolysis, very strongly influence the growth, thus cellular division. The figure reveals that the growth of the chick embryo[1] is completely inhibited by a Roentgen ray dosage of 1800 r, while respiration and glycolysis continue at an unchanged rate.

In the study of the action of Roentgen rays on cell division,[2] much attention has been paid to the behavior of unicellular systems as, for example, *Bacterium Coli*. When making use of the γ-rays of radium, the dose necessary to block the division of these bacteria, the half-lethal dose, was found to be 4000 r, increasing with decreasing hardness of the rays up to 9000 r. When using α-rays, 24,000 r have to be administered to obtain such an effect.

From the point of view of their biological action, the difference between hard and soft Roentgen rays lies mainly in the fact that hard rays through their path produce ions at a comparatively large distance, expending their energy within a wide range. Soft rays, on the other hand, "live and die" within a comparatively small range, expending all their energy in this range and, thus, producing ions at very close distances. This property of the soft rays is shown most conspicuously when, instead of soft Roentgen rays, another type of "short range" rays is considered, *viz.*, the α-rays. It is seen from Table I that a hard γ-ray quantum within 1 micron of its path in tissue produces 15 pairs of ions, while the corresponding figure for a 200 kev Roentgen ray quantum is 60 and for an α-ray particle it is 4000.[3]

TABLE I. Average number of pairs of ions per micron of trace in tissue.

Secondary electrons produced by γ-rays	Secondary electrons produced by 200 kev Rontgen rays	Recoil protons produced by 2.7 Mev neutrons	α-particles
15	60	1200	4000

The ionization produced by neutrons is of a similar type to that produced by α-rays. While, however, the α-rays cannot penetrate the tissue deeper than about 0.1 mm, the neutrons may reach a depth of many centimeters. Mainly upon this fact is based the hope of a possible application of neutrons in therapy where a dense ionization along the path of the rays may prove to be advantageous.

The lethal effect of radiation is a consequence of the ionization produced. If a single ion or pair of ions suffices to kill the cell, it is unnecessary to employ a radiation producing many ions within the sensitive part of the cell. If, however, the cell is refractory and several ions have to be produced within its sensitive part or parts to obtain a lethal action, the application of soft rays producing a dense ionization might be of great advantage. The fact that the application of soft rays is no advantage to killing *Bacterium Coli*, such rays having proved to be decidedly uneconomical, indicates that *Bacterium Coli* is killed under the action of few, possibly one pair of ions only. The mathematical analysis of the relation between the dose applied and the extent of lethal action leads in fact to the result that a

[1] R. Hubert, Archiv f. ges. Physiol. **223**, 333 (1929).
[2] Cf. C. M. Scott, *Some Quantitative Aspects of the Biological Action of X- and γ-Rays* (London, 1937).
[3] L. H. Gray and J. Read, Brit. J. Rad. **15**, 72 (1942).

single hit suffices if it only reaches the sensitive part of the bacterium.

Yeast cells, on the other hand, are killed more easily by soft rays. When the Roentgen ray tube is run with 60 kv, 16,000 r kill half of the yeast cells, while appreciably higher doses are necessary when hard rays are used. From this observation we can conclude that the sensitive part or parts of a yeast cell need several hits to yield a lethal effect. A few hits do not suffice. When using soft rays, however, the probability of several hits meeting within the sensitive part or parts of the yeast cell increases considerably. Soft rays prove thus to be more effective in the killing of yeast cells than hard ones.

The inactivation of viruses and the production of gene mutations are occurrences of a similar type to the killing of bacteria, while the breakage of chromosomes shows analogies to the behavior of yeast cells. More than one ionization is necessary for a break, and hence the densely ionizing radiations are the more efficient. It is characteristic of these examples that the effect obtained in no way depends on the time of the irradiation. A dose of 1000 r administered within one minute has the same effect on the mutation rate or on the killing of bacteria, etc., as 1000 r administered in the course of several days.

A very different behavior is exhibited by the effects produced in the tissues, among which the effect on nuclear division is most conspicuous. The effect of 100 r administered within 1 minute on a tumor differs from the effect of 100 r administered in the course of many days, and the same applies even, for example, to the action of Roentgen rays on *Drosophila* eggs. The death-rate of *Drosophila* eggs was found[4] to be the same if the dose of 165 r was applied at any period of time between 2.7 and 1800 seconds. If, however, the experiment lasted more than 1800 seconds, a very marked decrease in the action of the Roentgen rays was found to occur.

If 10 strokes are necessary to get a pneumatic tire filled with air, these strokes being done within a short period of time, while 11 strokes are indispensable to obtain the same effect if the operation is performed within several hours, this suggests that the wall of the tire leaks and that, consequently, the effect of pumping air into the tire is partly compensated by a "regeneration" of the originally "air-free" state. The same consideration applies to the effect of Roentgen rays on cellular division. The phenomenon that a dose administered in the course of a long period of time is less effective than a dose given within a short time indicates that a regeneration of the tissue affected by Roentgen rays takes place to a certain extent. This is a point of greatest importance both for the understanding of the action of Roentgen rays on cellular division and for the practical use of Roentgen radiation for therapeutic purposes.

In the study of the effect of Roentgen rays on growing tissue, the rates of growth before and after the treatment are compared. When using this method, several days or weeks may pass before the question is answered if and to what extent the growth was influenced by the irradiation. By counting the number of dividing cells, i.e., by mitosis counts, the effect of Roentgen rays on the tissue can be investigated a short time after the irradiation. Many very important results were obtained by making use of the method based on mitosis counts. The method fails, however, if the problem that faces us is the investigation of the effect of Roentgen rays on the normal tissue of fully grown animals. In such tissue as, for example, the liver tissue, the mitosis figure is unknown.

Another method which is suited to the investigation of the effect of Roentgen rays both on

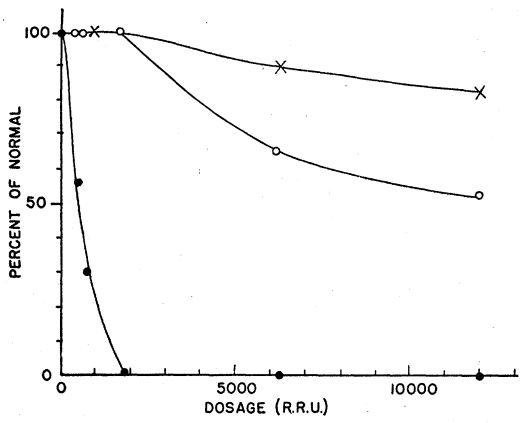

FIG. 1. Effect of x-rays on chick embryo (Hubert). ○ glycolysis, ● growth, × respiration.

[4] R. Sievert and A. Forssberg, Acta Rad. **69**, 181 (1941).

growing and on full-grown tissues is based on the comparison of the amount of nucleic acid molecules formed before and after irradiation.

NUCLEIC ACID

It is the effect of radiation on the cell nucleus which blocks cellular division. This was most directly demonstrated in the following experiment.[5] Agar cultures containing the algea *Zygnema* were irradiated with α-rays emitted by polonium. In one series of experiments, both the cytoplasm and the nucleus were irradiated, while in another series, the range of the α-particles was brought down to such a value as to expose only the cytoplasm to the effect of irradiation. In the latter case the dose necessary to suppress cellular division was found to be several hundred times larger than in the first case.

The main constituents of the nucleus are proteins and nucleic acids.[6] They appear to be chemically combined in the chromosomes which, consequently, consist of salt-like "nucleo-proteins." The specificity of gene action presumably resides in the protein part of the molecule which maintains its continuity throughout the whole nuclear cycle, while the nucleic acids increase and decrease during the various stages of mitosis. This was shown by Caspersson[7] who made use of the method of ultraviolet microphotography.

Nucleic acids are polymers of nucleotides, each nucleotide being composed of one molecule phosphoric acid, a pentose sugar, and a purine or pyrimidine ring. Various kinds of nucleotides have different purines or pyrimidines. Apart from this, two main types of nucleotides are observed which differ in the nature of the pentose, *viz.*, the desoxyribose ("thymus") and the ribose ("yeast") nucleic acids. While the desoxyribose nucleic acid is wholly or mainly located in the nucleus, ribose nucleic acid is largely found in the cytoplasm. Nucleotides of the desoxyribose type can polymerize to a much greater extent than the nucleotides of the ribose type. The molecular weight of the first mentioned type was found to lie[8] between 500,000 and 1,000,000. Since the average weight of a sodium nucleotide is about 330, it seems likely that the polymerized nucleic acid molecules in the chromosomes consist of about 2000 nucleotides. From the observation that very large quantities of ribose nucleic acids are localized to the chromosomes during cell division, Caspersson[7] concludes that the cytoplasmic nucleotides serve as a source of nucleic acids from which the chromosomes derive their supply. The preliminary stages of mitosis involve the transfer of nucleotides from the cytoplasm to the chromosomes, their conversion from ribose into desoxyribose nucleotides, and their polymerization into long chains. Thus, we may suppose the existence of a regular cycle of changes which are reversed at the end of each mitosis, when the nucleotides are surrendered by the chromosomes. Since the ionizing radiation blocks cell division, it will influence the said cycle of changes, and a reduction in the number of desoxyribose nucleic acids built up during a given period of time can be expected to take place. Such a reduction in the rate of formation of desoxyribose nucleic acid can easily be determined by making use of an isotopic indicator.

DETERMINATION OF THE AMOUNT OF NEWLY FORMED NUCLEIC ACID MOLECULES IN JENSEN'S SARCOMA OF THE RAT

The amount of newly formed nucleic acid molecules can be determined by making use of radio-phosphorus as an indicator. After administration of labelled sodium phosphate, the phosphate ions penetrate at a fairly rapid rate into the cells of Jensen's rat sarcoma[9] which were chiefly used in the experiments to be described. This results from the observation that, when administering the phosphate by subcutaneous injection, after the lapse of two hours the "free" phosphate extracted from the sarcoma tissue was found to have a specific activity (activity per mg P) of 80 percent of the specific activity of

[5] J. Petrová, Beih. bot. Zbl. **A61**, 399 (1942).

[6] Cf. M. J. D. White in G. Bourne's *Cytology and Cell Physiology* (Oxford University Press, New York, 1942).

[7] T. Caspersson, Skand. Arch. f. Physiol. **73**, Supp. No. 8 (1936); Chromosoma **1**, 562 (1940); Naturwiss. **17**, 33 (1941); T. Caspersson and J. Schultz, Nature **142**, 294 (1938); **143**, 602 (1939). Cf. also A. Brachet, Arch. Biol. **51**, 151 (1940).

[8] R. Signer, T. Caspersson, and E. Hammarsten, Nature **141**, 122 (1938).

[9] H. v. Euler and G. Hevesy, Kgl Danske Vid. Sels. Biol. Medd. **17**, 8 (1942); Svenska Vet. Akad. Arkiv. f. Kemi **17A**, No. 30 (1944).

TABLE II. Ratio of newly formed desoxyribose nucleic acid molecules before and after irradiation in Jensen's sarcoma of the rat. Sarcoma irradiated only.

Dosage in r	Time interval between irradiation and injection	Time elapsed between injection and sacrificing the rat in hours	Ratio of newly formed nucleic acid in the controls and in the irradiated sarcoma
750–1500	Few min.	½	3.2
335–1500	Few min.	1	2.4
450–1500	Few min.	2	2.2
1500	Few min.	4–6	2.8
1230–1500	3–7 days	2	1.7

TABLE III. Ratio of newly formed desoxyribose nucleic acid molecules present before and after irradiation in the organs of adult rats. Dosage applied, 1480–3000 r. Rat irradiated in toto. ^{32}P injected after termination of the irradiation. Rat killed two hours after administration of the ^{32}P (see reference 11).

Organ	Ratio of newly formed nucleic acid in the organs of controls and of irradiated rats
Liver	3.3
Spleen	2.4
Intestinal mucosa	2.3

the "free" plasma phosphate. This figure is an average obtained from several hundred experiments. The "free" phosphate extracted from the tissue is mainly of cellular origin. Now, if new nucleic acid molecules are built up in the sarcoma cells, the phosphate ions present being labelled, these newly formed nucleic acid molecules, in contradistinction to the "old," non-labelled nucleic acid molecules present, will also be labelled due to their ^{32}P content. If, for example, the average value of the specific activity of the cellular free P during the experiment was 100, and the specific activity of the nucleic acid P at the end of the experiment was found to be 1, then 1 percent of the nucleic acid molecules present at the end of the experiment in the sarcoma was built up during the experiment.

The percentage of newly formed desoxyribose nucleic acid in the course of two hours in the growing Jensen sarcoma in most of the several hundred cases investigated was found to be 2–3 percent. Since Roentgen radiation is known to have a blocking effect on cell division, a smaller percentage new-formation of desoxyribose nucleic acid molecules can be expected to take place in the irradiated tissue. From Table II it is seen that this is actually the case.

The effect of irradiation on the formation of new nucleic acid molecules was less pronounced when the labelled phosphate was injected several days after the irradiation. During this time, the inhibiting effect of irradiation on the sarcoma cells was obviously eliminated to some extent. In experiments in which the injection of labelled phosphate was performed before the irradiation was terminated, it became clear that a substantial part of the effect produced by the Roentgen rays decreases within a short time after irradiation. Here, the labelled phosphate was injected 30 minutes after the start of the irradiation which was continued for two more hours, and the rat was killed as soon as the irradiation was finished. In these experiments the irradiation performed with 2250 r was found to reduce desoxyribose nucleic acid formation to 1/7 of the normal value.[10]

DETERMINATION OF THE AMOUNT OF NEWLY FORMED NUCLEIC ACID MOLECULES IN THE NORMAL ORGANS OF THE RAT

a. Adult Animals

Roentgen radiation does not only block the formation of desoxyribose nucleic acid molecules in growing tissue, but also in the normal organs of adult animals. The rate of renewal of the nucleic acid molecules in the intestinal mucosa[11, 12] of the rat was found to be of a similar magnitude to that in the growing Jensen sarcoma, while a still higher rate was ascertained in the thymus gland.[13] In the liver[11, 12, 14] desoxyribose molecules, in contrast to the molecules of most other compounds containing phosphorus, are built up at a low rate only. The ratio of newly formed molecules found in some organs of adult rats before and after irradiation is seen from Table III. A total of 140 rats was used in these determinations.

[10] L. Ahlström, H. v. Euler, and G. Hevesy, Svenska Vet. Akad. Arkiv. f. Kemi, No. 13 (1944).
[11] L. Ahlström, H. v. Euler, and G. Hevesy, Svenska Vet. Akad. Arkiv. f. Kemi **19A**, No. 9 (1944).
[12] G. Hevesy and J. Ottesen, Acta Physiol. Skand. **5**, 237 (1943).
[13] E. Andreasen and J. Ottesen, Acta path. Microbiol. Scand. Supp. 54 (1944).
[14] A. Bruce, M. M. Tracy, and W. E. Cohn, Science **95**, 558 (1942).

TABLE IV. Ratio of newly formed desoxyribose nucleic acid molecules present before and after irradiation in the organs of 3- to 4-day old rats.

Organ	Ratio of newly formed nucleic acid in the organ of controls and irradiated rats. Dose applied, 2000–2250 r. Time of the experiment: 2 hours. ^{32}P administered after irradiation	Irradiated throughout the experiment
Liver	2.3	11
Spleen	—	5.3

Marshak compared the uptake of ^{32}P by the nuclei of the tumor and the liver and found the former to be larger than the latter. He found, furthermore, that the ^{32}P taken up by the nuclei was mostly present in the nucleoprotein fraction.[15]

The percentage effect of the irradiation on the formation of nucleic acid molecules in the normal organs of adult rats is thus of a similar magnitude to the effect of the Roentgen rays on the growing Jensen sarcoma listed in Table II.

b. Rapidly Growing Animals

In the organs of 3- to 4-day old rapidly growing rats a much more rapid rate of renewal of desoxyribose nucleic acid was observed to take place than in the organs of adult animals. In the liver and the spleen of growing rats, the amount of newly formed nucleic acid molecules was found to be 15 and 4 times, respectively, as large as in the corresponding organs of adult rats. Roentgen rays were found to block the formation of nucleic acid in the organs of rapidly growing rats as well, the percentage inhibition being similar to that found in the normal organs of adult animals and in their Jensen's sarcoma. In Table IV it is shown that when ^{32}P was not administered after the irradiation, the rat, however, being exposed to Roentgen rays throughout the experiment, the inhibition of nucleic acid formation was much more pronounced.

Irradiation throughout the experiment with a total dose of about 2000 r reduces the formation of nucleic acid to less than 1/10 of its normal value. *As long as these rapidly growing rats are exposed to the effect of Roentgen rays, nucleic acid formation and, thus, growth practically cease to occur.*

Consequently, the results obtained are as follows.

(a) Roentgen rays inhibit the formation of desoxyribose nucleic acid in growing and full-grown tissues. *The percentage inhibition in both cases does not differ greatly.*

(b) The inhibition of the formation of desoxyribose nucleic acid obtained when the irradiation is continued throughout the experiment, i.e., the animal is killed as soon as the irradiation is terminated, is much more pronounced than the inhibition observed in experiments in which two hours elapse between the termination of the irradiation and the death of the rat. *About 3/4 of the inhibiting effect produced by Roentgen rays thus ceases in the course of the first two hours after irradiation.*

In the following, the conclusions to be drawn from the above results will be discussed.

EFFECT OF ROENTGEN RAYS ON DIVIDING AND NON-DIVIDING CELLS

Overwhelming evidence is available[2] for the fact that, in contrast to growing tissue, full-grown tissue in most cases is hardly sensitive to the effect of a moderate Roentgen ray dose. The results obtained suggest the following explanation of this remarkable difference. The percentage inhibition of the formation of desoxyribose nucleic acid molecules is about the same in dividing and non-dividing cells. While, however, the non-dividing cell has ample time to get rid of the disturbance caused by the Roentgen rays before it is its turn to become a dividing cell, cells in mitosis or cells which will reach this stage within a comparatively short time, have not. The synthetic process going on in the nucleus of these cells is led into abnormal channels and degenerate cells are formed.[16]

The importance of the recovery phenomenon can hardly be overestimated. It is best demonstrated by the fact that 3/4 of the blocking effect of Roentgen rays obtained after application of 2000 r or less was found not to be operative any longer after the lapse of two hours.

In a swiftly growing embryonic tissue, as much as 10 percent of the cells may be in mitotic stage,

[15] A. Marshak, J. Gen. Physiol. 25, 275 (1941).

[16] I. Lasnitzki, Brit. J. Rad. 16, 137 (1943).

in Jensen's sarcoma the corresponding figure amounts to about two. As the mitotic process lasts 1 or 2 hours, all or most cells of such tissues will reach the mitotic stage within a comparatively short time. Recovery from the effect of Roentgen rays will thus take place only to a restricted extent in such rapidly growing tissues. In the normal organs, the mitotic rates are low, and it may take an exceedingly long time until the average cell reaches the mitotic stage. It should be envisaged that each cell is a presumptive mitotic cell, but the time until the state of mitosis is reached may be very different for different types of cells. After the use of very large dosages, the blockage of the formation of nucleic acid even in the cells of the normal organs may be so effective that recovery can no longer take place.[16]

From the above considerations it follows that, by slowing down the speed of growth, the probability of recovery from the effect of Roentgen rays can be expected to increase and thus the detrimental effect of radiation on the tissues can be diminished or eliminated. Ample evidence is available for the assumption that this is the case. Lowering of the body temperature is a very effective means to obtain a temporary suppression of the cellular division. By keeping an organism at low temperature during irradiation it is, in fact, possible to obtain immunity from the detrimental action of the Roentgen rays. It has been shown by several experimenters that this actually is the case.

The effect of lowering of the temperature on the resistance of the organism to the detrimental action of Roentgen rays was shown in a very spectacular way by Lacassagne.[17] This author found that, while the dose of 1500 r given within 1 minute to new-born mice is lethal, the mice continued developing in a normal way if they had been placed in a refrigerator for 10 minutes prior to irradiation. The body temperature obtained by cooling the mice is not stated: we know, however, from similar experiments by Evans *et al.*[18] that, when new-born rats are placed on ice, the heart-beat rate diminishes to 2 per minute while, at 35°C, the count amounts to 350.

In early experiments, Strangeway and Fell[19] exposed six-day chick embryos to Roentgen rays *in ovo*. After a dose of 270 r, followed by incubation *in ovo* at 38°C, mitosis was almost absent and tissues taken from embryos irradiated and incubated for 24 hours showed no trace of growth when explanted *in vitro*. If, after a dose of 270 r, the embryos were kept at 5° for 24 hours, degenerative changes were delayed if not arrested, and tissue fragments showed fair growth with mitosis when cultivated *in vitro*.

Another way of counteracting the detrimental effect of Roentgen rays is the diminution of the blood supply. The marginal cells of a carcinoma having a more abundant oxygen supply were found by Mortram[20] to be more sensitive to the action of the rays than the central cells. The same experimenter found that, when the testicle is radiated, even if a dose given is sufficient to produce permanent sterility, the spermatids which are not near any blood vessel continue to divide and produce viable spermatozoa, whereas the spermatogenic cells close to the intertubular blood vessels die, and thus permanent sterility results. A somewhat similar variation in radio-sensitivity is seen in embryos which, as shown by Spear,[21] become more radio-sensitive as soon as the blood supply is established. More examples could be cited. Among these ranges the diminution of radio-sensitivity of Jensen's sarcoma produced by irradiation of the tumors with sublethal doses.[22] Reduced mitosis, slower growth characterized the pretreated tumors. The average cell thus being further away from the mitotic change, was less influenced by irradiation than was the average cell of the non-pretreated sarcoma. This example illustrates simultaneously the great obstacles encountered in the treatment of cancer by ionizing radiation.

While we possess effective means to diminish the radio-sensitivity of the tissue, it is more difficult to obtain an enhanced susceptibility, the margin between body and permissibly low-

[17] A. Lacassagne, Comptes rendus **215**, 231 (1942).
[18] T. C. Evans, J. P. Goodrich, and J. C. Slaughter, Proc. Soc. Exper. Med. **46**, 602; **47**, 434 (1941).
[19] T. S. P. Strangeway and H. B. Fell, Proc. Roy. Soc. London **102**, 9 (1927).
[20] J. C. Mortram, Brit. J. Rad. **9**, 606 (1936).
[21] F. G. Spear, Brit. J. Rad. **6**, 68 (1935).
[22] B. Snelmann, Acta Rad. **16**, 546 (1935).

ered temperature being much greater than the margin between body and permissibly elevated temperature. The obtainment of an increased radio-sensitivity of tumor cells is of great therapeutic interest and this could presumably to some extent be obtained by increasing the temperature of the tumor and its blood supply while irradiation takes place.

DIRECT AND INDIRECT EFFECTS OF ROENTGEN RAYS

The effect of Roentgen rays on the division of bacteria and on the mutation rate was often interpreted as a direct effect of the radiation, this effect being in no way dependent on the time within which the irradiation takes place. A beautiful example of a direct effect of ionizing radiation is the splitting of the molecules of the blood pigment hemocyanin (mol. weight = 9 million) into two halves.[23] The splitting takes place even at the temperature of liquid air. At such low temperature the rates of diffusion and reaction are exceedingly low. Thus, the splitting of the hemocyanin molecules dissolved in a buffer solution can hardly be caused by an interaction of products produced in the buffer solution and the hemocyanin molecule, but must have resulted from a direct action of the radiation.

In the action of Roentgen rays on tissue, the time factor is very strongly pronounced, a phenomenon which often is interpreted as being due to the fact that we are faced with the interaction of products produced by the Roentgen rays on the cellular constituents involved in cell division. That the action of Roentgen rays on tumors is, at least partly, an indirect effect can be concluded from the following experiment.[24] Rats were inoculated with two sarcomata, one sarcoma was irradiated with up to 2000 r, while the other sarcoma was protected by 5-mm thick lead sheaths. Measurements performed with a small ionization chamber inserted in some of the sarcoma have shown that the shielding was very effective, the shielded sarcoma obtaining less than 3/4 percent of the dose reaching the non-shielded one.

An investigation of nucleic acid formation taking place in both sarcomata led to the result (cf. Table V) that the shielded sarcoma exhibited a reduced formation of nucleic acid as well, the reduction amounting to 4/5 of the reduction occurring in the unshielded sarcoma. The cells of a sarcoma protected from the direct action of Roentgen rays are thus acted upon if only other parts of the body get irradiated. This observation is a conclusive proof of an indirect action of Roentgen rays on cellular division.

When following the growth of the irradiated, shielded, and non-shielded sarcomata for a few weeks, the shielded ones were found to grow at a markedly higher rate than the irradiated sarcomata, which reveals that the indirect effect responsible for the blocking of nucleic acid formation in the shielded sarcomata is more temporary than the effect produced by direct irradiation. The indirect effect is sufficient to block the nucleic acid formation of cells undergoing mitosis, but it influences to a small degree only the cells further away from the mitotic state, as would a non-lethal dose in contrast to a lethal one.

It is quite possible that a direct action of radiation on the tumor cells takes place as well, i.e., some molecules participating in the process of cell division become ionized or split. The probability of such an event is, however, restricted. The chance of an atom being ionized amounts, when applying a dose of 1000 r, to 10^{-8} only.

When discussing the indirect effect of radiation, we have primarily to consider the action of particles produced in the tissue water, as the number of ionizing acts produced in this main constituent of the tissue is much higher than the number produced in other tissue constituents.

TABLE V. Comparison of the formation of desoxyribose nucleic acid in the irradiated and shielded sarcomata of the same rat. Number of irradiated rats investigated: 12. Dosage, 280–2000 r. Time of the experiment: 2 hours.

	Activity of 1 mg nucleic acid P in percentage of the activity of 1 mg cellular free P
Irradiated	0.76
Shielded	0.95
Non-irradiated controls	1.89

[23] The Svedberg and Sv. Brohult, Nature **143**, 938 (1939); Sv. Brohult, Diss. Uppsala 19.

[24] L. Ahlström, H. v. Euler, and G. Hevesy, Svenska Vet. Akad. Arkiv. f. Kemi **19A**, No. 13 (1945).

These products can diffuse to and accumulate in the sensitive parts of the cell.

The primary process in the action of radiation on water consists in detachment of an electron and its subsequent transfer from the ion to one of the neighboring molecules or ions.[25] For pure water, the primary process is

$$(HO)^-H^+ + \text{radiation} \rightarrow HO + H.$$

Although there is a splitting into hydrogen atoms and hydroxyl radicals, generally no appreciable decomposition of the water will be observed. The reason is that the radiochemical primary process mentioned above is always followed by the recombination of the decomposition products according to

$$H + OH = H_2O.$$

Actual decomposition of pure water can only occur insofar as the subsequent reactions, *viz.*,

$$\begin{aligned} &\text{(hydrogen formation)} && 2\,H = H_2 \\ &\text{(oxygen formation)} && 2\,OH = H_2O + O \\ & && 2\,O = O_2, \end{aligned}$$

can compete with the reverse reaction.

The situation is, however, radically changed if substances are present in the water which can interact chemically with the hydrogen atoms and hydroxyl radicals primarily formed. The hydroxyl radical is a strongly oxidizing agent which, by accepting an electron, is transformed into an OH^- ion, whereas the hydrogen atoms are powerful reducing agents. Therefore, there are scarcely any substances which, if dissolved in water, will not be attacked by these powerful reagents and, thus, will act as acceptors towards one or the other radical formed by irradiation.

In the case of more complex organic molecules, there will always be a reaction of the solute with the hydroxyl radicals (possibly also with hydrogen atoms) which will lead to its decomposition and deactivation. If no hydrogen or oxygen gas is evolved, we have to assume that both the radicals primarily formed have reacted with the solute. Arnow[26] has studied the effect of Roentgen rays on the water in the presence of proteins. He found that visible coagulation occurred at the isoelectric point and that oxygen, and in smaller amounts also hydrogen, were utilized by the proteins during the exposure to α-rays, the oxygen production being reduced to 1/7 of its original value while some reduction in the original value of hydrogen was observed as well. A similar effect can be expected to take place in the proteins and other constituents present in the tissue exposed to the action of Roentgen rays.

When two sarcomata are inoculated in the rat, one of which is irradiated, while the other is shielded from the effect of radiation, the product formed under the effect of irradiation will be destroyed to a larger extent before reaching the sensitive parts of the shielded than those of the irradiated sarcoma and, correspondingly, the effect produced in the shielded sarcoma will be less permanent than in the irradiated one. A definite proof that the effect on the shielded sarcoma is owing to the transport of noxious products to the sarcoma is still lacking, however.

That the effect of Roentgen rays on carboxypeptidase and other enzymes is an indirect effect, that the radiation causes some change in the water and the product formed in turn acts on the solute, follows from results obtained by Dale.[27] He found the inactivation of the dose of Roentgen rays of 1200 r or more being applied to decrease with decreasing concentration of the enzyme. He further found that, if there is more than one solute present, each one will share in the reaction with this intermediate product according to its respective amount and affinity, i.e., leaving a larger or smaller share for its partner. When studying the action of Roentgen rays on the conjugated protein *d*-amino-acid oxidase, for example, a "protection" of the dinucleotide part of the molecule was found to take place. This "protective" action of the protein is not confined to it, but was found to be exhibited by a great variety of compounds less inactive than sodium chloride.

A dilute acetylcholine solution, for example, was found to be inactivated by a given dose of Roentgen rays more completely relative to its initial concentration than a more concentrated one, and glucose present in the acetylcholine

[25] Cf. J. Weiss, Nature **153**, 748 (1944).
[26] L. E. Arnow, J. Biol. Chem. **110**, 43 (1944).
[27] W. M. Dale, Biochem. **34**, 1367 (1940); **36**, 80 (1942); cf. also H. Fricke and E. J. Hart, J. Chem. Phys. **3**, 596 (1935).

solution during irradiation was found to inhibit the radiation effect.[28]

In experiments *in vitro* in which only a few solutes were present, a keen competition of these solutes for the decomposition products produced under the action of Roentgen rays, was found to take place. Thus, we can expect to find most intricate conditions in the tissue, where numerous solutes can compete for the inhibiting products. Furthermore, the changes produced by the interaction of the cellular constituents with the decomposition products of water and other inhibiting products need not be permanent, as is shown by the extended occurrence of regeneration processes going on in the tissue acted upon by Roentgen rays.

The so-called refractive tumors (for example lipoma composed of fat tissue) can, in the light of the above considerations, be interpreted as being such in which the inhibiting products are used up by tissue constituents not involved in the process of cell division and thus cannot stop the formation of vital products involved in the mitotic process. An alternative explanation is the greater power of regeneration of such tumors. It would be of great interest to follow the formation of nucleic acid in such refractive tumors by making use of ^{32}P as an indicator.

THE EFFECT OF ROENTGEN RAYS ON THE FORMATION OF DESOXYRIBOSE NUCLEIC ACID

The blocking effect of ionizing radiation on cell division may take place in different ways. It suggests itself, however, to consider first of all the inhibition of the formation of desoxyribose nucleic acid. By Caspersson's work[7] it was made very probable that desoxyribose nucleic acid is formed from ribose nucleic acid. This transformation involves a reduction process. Oxydizing substances produced under the action of Roentgen rays may counteract this reduction process and thus the formation of desoxyribose nucleic acid, which leads to a disturbance and inhibition of the mitotic process.

Mitchell[29] found an increase in the absorption of ultraviolet radiation of wave-length 2537A of the cytoplasm of proliferating and differentiating cells after therapeutic doses of Roentgen radiation. He interprets this increase as due to the accumulation of ribonucleotides in the cytoplasm and as a result of a disturbance of the normal metabolic processes either by increased rate of formation or decreased rate of removal. No change in the concentration of desoxyribose nucleic acid was found to take place by measurement of the absorption of ultraviolet radiation. However, as discussed in the foregoing pages, the inhibition of the formation of such molecules was clearly shown by making use of an isotopic indicator.

It would be of immediate interest to measure the rate of formation of the ribose nucleic acid by the same method and to determine if and to what extent this formation is influenced by ionizing radiation. If, in contradistinction to its effect on the formation of desoxyribose nucleic acid, Roentgen rays would not inhibit the formation of labelled ribose nucleic acid molecules, such a finding would be a strong support to the correctness of the view that an important effect of Roentgen rays on cell division is exercised through the inhibition of the reduction of desoxyribose to ribose nucleic acid. Responsible for the inhibition are presumably the oxydizing products formed in the cells under the action of the ionizing radiation.

An oxidizing action can also be produced by injecting into the circulation oxidizing agencies. There is, however, a fundamental difference between products injected and products produced *in situ* under the effect of ionizing radiation. The products injected reach easily the extracellular space, but they are then faced by the reducing effect of the phase boundary separating the cells from their surroundings. The phase boundary is not permeable or permeable only at a low speed to many products and it thus represents a most effective protection of the organism against the action of noxious agencies. The presence of a phase boundary in no way influences the action of Roentgen rays which give rise to products *in situ* in the cells, products which can thus ignore the protecting barrier. This property makes ionizing radiation in many respects a unique beneficient agency in therapy, but also a most destructive weapon when applied indiscriminately.

[28] W. M. Dale, J. Physiol. **102**, 54 (1943).
[29] J. S. Mitchell, Brit. J. Exper. Path. **23**, 296 (1942).

SUMMARY

A survey of some effects of Roentgen rays on cellular division is given. While mitotic counts supply important informations on the effect of Roentgen rays on growing tissue, this method fails in the investigation of the behavior of full-grown tissue. The effect of ionizing radiation on all types of tissue can be studied by determining the amount of newly formed desoxyribose nucleic acid before and after irradiation. The determination of newly formed nucleic acid is carried out by making use of radio-phosphorus as an indicator.

Roentgen rays (therapeutic and larger doses) are found to inhibit the formation of desoxyribose nucleic acid in the growing and full-grown tissues to about the same extent. A large percentage (about 3/4) of the inhibiting action disappears in the course of two hours following irradiation.

In the light of these results the difference between the action of Roentgen rays on growing and full-grown tissues is interpreted by the assumption that this difference is mainly owing to the very much longer time which elapses for an average cell of the full-grown tissue to reach a mitotic stage and thus to the very much greater chance of regeneration before the detrimental effect of the inhibition of normal desoxyribose synthesis and possibly of other processes becomes effective.

The problem of direct and indirect action of Roentgen rays on cell division is discussed.