THE INCREASED ABSORPTION OF X-RAYS BY VITALLY STAINED WHITE RATS.*†

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In a previous paper a series of experiments was reported demonstrating the action of x-ray energy upon paramecia which had been stained with certain vital stains; namely, Nile blue sulfate, alizarine blue S, trypan blue, isamine blue, Nile blue hydrochloride, trypan red, dahlia (Grübler), neutral red, aniline red, Sudan III (oil), Janus green (Eimer and Amend), and methylene blue (BX Merck).[1] It was ascertained by this study that a dosage of from 50 to 60 milliampere minutes[2] produced death within 48 hours in those organisms which had not been subjected to the stain. On the other hand, the presence of the stain within the cytoplasm of the paramecia so greatly increased the absorption of x-ray energy that a dosage of from 5 to 10 milliampere minutes given under the same conditions of x-raying was sufficient to produce death within 48 hours. The most plausible explanation of this phenomenon which could be presented at the time was that the permeability of the nuclear membrane to the stain had altered. This alteration was dependent, apparently, upon two factors, the presence of the stain in immediate proximity to the nuclear membrane, and the action of the x-ray energy.

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[1] Baldwin, W. M., A study of the combined action of x-rays and of vital stains upon paramecia, *Biol. Bull.*, 1920, xxxix, 59.

[2] The amount of radiation is calculated as the product of the current strength in milliamperes and the time of exposure in minutes and is expressed as milliampere minutes.

It was difficult to stain the nucleus in the paramecium while the organism was alive. Diffusion of the stain into the nucleus indicated ordinarily the ensuing death of the organism. It was found, on the other hand, in most marked contrast to this condition that the carrying capacity of the cytoplasm for the stain was relatively great. A careful examination of the experimental data demonstrated an increased absorptive property of the nucleus for the stain which resulted invariably in the death of the paramecium.

Since the publication of these results investigation has been prosecuted along the same line, of the chemical sensitization of tissue cells to x-ray energy, by the use of adult white rats. While a wide variation has been noted in the behavior of adult mammalian tissues to the combined action of vital stains and x-rays, the general principles underlying the protoplasmic reactions to this combination of experimental conditions may be illustrated by the results obtained with trypan blue. No effort was made to test out the minimum lethal dose of this stain for rats.

Method.

The dry stain was dissolved in the proportion of 0.25 gm. to 50 cc. of sterile 0.6 per cent sodium chloride solution. Each rat received a hypodermic, subcutaneous injection of from 2 to 4 cc. of stain, depending upon weight, on the morning of the 1st, 3rd, and 5th days. On the afternoon of the 5th day, after the injections of stain had been completed, a dosage of 100 milliampere minutes was given at a distance of 17.5 cm. from the target. The source of the x-ray energy was a large, water-cooled, Coolidge tube, the surface of which was additionally cooled by a strong air blast. A thin layer of pasteboard separated the rats from the target. The compartment of the container holding the rats was adjustable to the extent that once in position the animals could not move about. Consequently, the maximum dose of x-ray energy was received by the skin upon the side of the rat turned toward the tube.

EXPERIMENTAL.

A significant feature of the paramecium experiments obtained likewise with the stained rats. With the former, the absorption capacity for x-ray energy varied within wide limits, depending upon the different strains of paramecia. Similarly, the stained white rats exhibited a wide range of susceptibility. It may be stated in gen-

eral, however, that a dosage of $\frac{100 \text{ MAM}}{17.5 \text{ cm.}^2}$ at 50 kilovolts of x-ray energy induced death in the unstained rats within from 110 to 120 hours. On the other hand, the same amount of energy, acting under the same experimental conditions upon rats which had been vitally stained, shortened the period of viability to from 60 to 70 hours. This fact may be interpreted as indicating, therefore, an increase of almost 100 per cent in the susceptibility of the stained over the unstained rats to this form of energy. Some data upon the point will be given.

On Dec. 2, 1916, at 11 a.m., six adult white rats were x-rayed. Two were given a dosage of $\frac{75 \text{ MAM}}{17.5^2}$ at 50 kilovolts, two a dosage of 50 milliampere minutes, and two a dosage of 25 milliampere minutes, all under the same conditions. On Dec. 6, one rat which had received a dose of 75 milliampere minutes died, 90 hours after the initial x-raying. On Dec. 10, one rat which had received 75 milliampere minutes died, 195 hours after x-raying. The observation was made on Dec. 30 that both of the rats that had received 50 milliampere minutes and both that had received 25 milliampere minutes were alive and well. One adult white rat was x-rayed on May 1, 1917, at 10.30 a.m., with a dosage of $\frac{100 \text{ MAM}}{17.5^2}$ at 50 kilovolts. This rat died on May 5, 97 hours after the x-raying.

Another experiment was conducted on Sept. 8, 1916. Two adult white rats were x-rayed at 2.15 p.m. They were given a dosage of $\frac{125 \text{ MAM}}{22.5^2}$ at 50 kilovolts. One died on Sept. 14, 115 hours after x-raying; the other died on Sept. 15, 139 hours after x-raying. Furthermore, on Sept. 8, two adult white rats were given a dosage of $\frac{500 \text{ MAM}}{22.5^2}$ at 50 kilovolts, and two a dosage of $\frac{250 \text{ MAM}}{22.5^2}$ at 50 kilovolts. The rats which were given the larger dosage of 500 milliampere minutes died on Sept. 12, 80 hours after x-raying; the two which had been given 250 died on Sept. 12, 84 hours after x-raying.

Another experiment was conducted on July 11, 1916, at 1.30 p.m. Four rats were x-rayed under the same conditions, with a dosage of $\frac{50 \text{ MAM}}{17.5^2}$ at 50 kilovolts. One died on July 14, 96 hours after x-raying; three died on July 15, 108 hours after x-raying.

It was ascertained from experiments similar to the above that the period of viability of the average adult white rat, after a dosage of x-ray energy of $\frac{100 \text{ MAM}}{17.5^2}$ at 50 kilovolts, varied between 110 and 120

hours. A greater dosage, as has been seen in the instance of 125, 250, and 500 milliampere minutes, shortened this period. This standard dosage of 100 milliampere minutes was selected for the purpose of the experiment as what might be termed provisionally the minimal lethal dosage for the period.

This period of viability of x-rayed rats was considerably shortened, however, when injections of trypan blue in aqueous solution were administered to the rats in dosages varying between 2 and 4 cc. of a 0.5 per cent solution. This will be seen by the following extracts from the experimental notes.

Two rats were x-rayed on Sept. 28, 1916, at 2.15 p.m., at $\frac{100 \text{ MAM}}{17.5^2}$ at 50 kilovolts. One rat died on Sept. 30, 60 hours after x-raying, and the other on Sept. 31, 70 hours after x-raying. On Oct. 2, one rat was x-rayed at 5 p.m., under the same experimental conditions, and died 73 hours later. Still another rat was x-rayed on Oct. 3, at 11.30 a.m., and died on Oct. 5, 40 hours after x-raying. On Jan. 9, 1917, one rat was x-rayed at 9.45 a.m., after the standard injections of trypan blue had been administered. This rat died 75 hours after x-raying. On Apr. 24, one rat was x-rayed at 10.15 a.m., at $\frac{100 \text{ MAM}}{17.5^2}$ at 50 kilovolts after two injections of trypan blue had been administered, 1 day intervening between the injections. This rat died on Apr. 27, 70 hours after x-raying. On Apr. 25, one rat was x-rayed, under the same conditions, after three injections of trypan blue had been administered, 1 day likewise intervening between injections. This rat died on Apr. 28, 67 hours after x-raying. On Apr. 27, another was x-rayed under the same conditions, after two injections of trypan blue, 1 day intervening between injections. This rat died on Apr. 29, 60 hours after x-raying.

These are merely typical examples of the results encountered with more than 50 stained rats utilized for the purpose of the investigation. The extreme experimental variations are well illustrated by the few examples given. The average results indicate, however, a marked increase in the susceptibility of the rats to the lethal effects of x-ray energy in the presence of the stain.

The results of x-ray experiments conducted upon developing frogs' eggs have been previously reported.[3] In these experiments it was

[3] Baldwin, W. M., The artificial production of monsters conforming to a definite type by means of x-rays, *Anat. Rec.*, 1919–20, xvii, 135; The artificial production of monsters demonstrating localized defects as the result of injury from x-rays, 1920–21, lii, 296; Effects produced by x-ray energy acting upon frogs' ova at early developmental stages, *Science*, 1920, lii, 229.

noted that the developing brain and spinal cord, the epidermis, the enteron, the heart, and the vascular system in general showed a greater specific susceptibility to the destructive action of x-ray energy than most of the other tissues of the embryo. Extended studies gave warrant for the hypothesis that the mitotic activity of the cells in embryonic tissues was in direct ratio to the susceptibility of these cells to x-ray energy. This, in a way, conforms to the general principle previously discovered by other investigators that during the mitotic period the susceptibility of the cell to injury from various forms of physical energy is increased. Moreover, as a direct corollary to this, it was found that the absorptive capacity of tissues most rapidly differentiating greatly exceeded that of the less active tissues. The working hypothesis is not without support that, apart from chemical constitution, the determining factor in specific susceptibility of tissues is very largely, if not entirely, mitotic activity.

The cytoplasmic and nuclear detritus gave evidence that all types of embryonic cells absorbed a certain amount of energy. This contrasted markedly with the relative insusceptibility of these same cell types in adult tissues. Repeated experimentation verified the fact that in the adult the sex glands, bone marrow, leucocytes, spleen, and the epidermis on the side of the body turned towards the tube were most readily affected by x-ray energy. On the other hand, cells of what may be called mitotically inactive types of adult tissues, such as brain and spinal cord, muscle, glandular, and connective tissues, were relatively insusceptible or lacked an absorptive capacity for this form of energy. These findings support the working hypothesis derived from a study of embryonic tissues.

It should be stated that this working hypothesis must conform with the well established physical principle underlying x-ray absorption—the higher the atomic weight of the element, the greater the proportion of x-ray energy absorbed—and, in addition, must allow for those active, soft rays which are almost entirely absorbed by the skin on the side turned towards the tube.

In view of the results with embryos noted above, it was somewhat disappointing that the vitally stained rats which had been x-rayed revealed no departure from the histologic picture presented by the x-rayed but unstained rats. The more actively mitotic tissues,

such as the spleen, the bone marrow, and the sex glands, were deeply stained, and showed wide cytologic variations from the normal both in the number and in the kind of their cellular elements. A considerable proportion of the stain had been taken up by the nuclei of the cells of these organs and of the connective tissues as well.

As in embryos, when the quantity of the x-ray energy was sufficiently great, its injurious action upon both nucleus and cytoplasm was manifested through the presence of both nuclear and cytoplasmic extrusions. In the embryo, all of the body cavities, especially the neurocele, contained relatively large amounts of this detritus, the quantity standing in direct ratio to the amount of energy utilized. It stood in direct proportion, moreover, to the destructive effects upon the cells lining these cavities. With this phenomenon as an indicator, through careful histologic technique it was possible to recognize the same evidences of x-ray energy in the more actively mitotic tissues of the adult rat. Further evidence, belonging in the same category, is to be found in the increase noted in the nitrogen output and uric acid elimination, and in the increased elimination of purine bases and of P_2O_5. It might be inferred that all these effects, which have been observed by others, as well as the author, and are most noticeable in the excretory organs of the body, collectively point to the assumption that such pathologic changes are secondary to, and not the primary effects of, the injurious action of x-ray energy upon cytologic constituents and cytologic metabolism. In both the stained and the unstained rats, the necrotic foci in the mucosa of the small intestine and in the convoluted tubules of the kidney, and the areas of leucocytic infiltration in the liver, observed by Hall and Whipple[4] and by Warren and Whipple,[5] are to be thought of as secondary, not primary, effects of x-ray energy.

[4] Hall, C. C., and Whipple, G. H., Roentgen-ray intoxication: Disturbances in metabolism produced by deep massive doses of the hard roentgen rays, *Am. J. Med. Sc.*, 1919, clvii, 453.

[5] Warren, S. L., and Whipple, G. H., Roentgen ray intoxication. I. Unit dose over thorax negative—over abdomen lethal. Epithelium of small intestine sensitive to x-rays, *J. Exp. Med.*, 1922, xxxv, 187; II. A study of the sequence of clinical, anatomical, and histological changes following a unit dose of x-rays, 203; III. Speed of autolysis of various body tissues after lethal x-ray exposures. The remarkable disturbance in the epithelium of the small intestine, 213.

The assumption by tissue cells of a spherical form and the staining of the nuclei have been considered in this investigation as indicative of the injurious effects of the energy, in accordance with the observations on the embryo. These changes were most marked in the bone marrow, spleen, and sex glands. Towards the end of the life of the x-rayed rat such injured cells almost completely degenerated or disappeared.

DISCUSSION.

The peculiar sensitizing action of the stain as described is comparable to that of an optical sensitizer such as eosin, and in all probability results from the adsorption of the stain on the surface of the nucleus, a physical phenomenon which occurs most actively under the direct influence of an electromagnetic form of energy. Evidence for such a phenomenon was given first by Hertel[6] who, working with the effect of ultra-violet light ray energy upon bacteria in 1905, found that a certain wave-length not injurious to the bacteria was absorbed by them in the presence of eosin with consequent injurious effect, providing the eosin possessed an absorption band within the range of wave-length utilized. Furthermore, the colloidal selenium work of Henri[7] bears out the same theory. The sensitizing effect depends then apparently upon two separate and distinct factors: first, the intimate

[6] Coehn, A., and Barratt, J. O. W., Ueber Galvanotaxis vom Standpunkte der physikalischen Chemie, *Z. allg. Physiol.*, 1905, v, 1. Barratt, J. O. W., Die Addition von Säuren und Alkalien durch lebendes Protoplasma, *Z. allg. Physiol.*, 1905, v, 10. Nagai, H., Erstickung und Narkose des Flimmerepithels, *Z. allg. Physiol.*, 1905, v, 34. Hertel, E., Ueber physiologische Wirkung von Strahlen verschiedener Wellenlänge. Vergleichend-physiologische Untersuchungen. II. Mitteilung, *Z. allg. Physiol.*, 1905, v, 95.

[7] Henri, V., and Wurmser R., Etude de la loi d'absorption photochimique pour les réactions produits par les rayons ultraviolets, *Compt. rend. Acad.*, 1912, clv, 503. Henri, Mme. V., Henri, V., and Wurmser, R., Etude quantitative de l'absorption des rayons ultra-violets par l'albumine d'oeuf et le sérum, *Compt. rend. Soc. biol.*, 1912, lxxiii, 319. Henri, V., Comparaison de l'action des rayons ultra-violets sur les organismes avec les réactions photochimiques simples et complexes, *Compt. rend. Soc. biol.*, 1912, lxxiii, 323. Henri, V., and des Bancels, J. L., L'excitation provoquée par les rayons ultra-violets comparée avec les excitations visuelles et nerveuses, d'une part, et les réactions photochimiques de l'autre. Lois des phénomènes, *Compt. rend. Soc. biol.*, 1912, lxxiii, 328.

association of the foreign chemical with the living protoplasm of the cell; and, secondly, the presence of a form of physical energy. The following evidence must be considered before a rejection of this theory can be entertained. Solutions of the stain that had been subjected to a relatively enormous dosage of x-ray energy produced no lethal effect when afterwards injected into the rats; the period of viability of x-rayed rats was not shortened when they were subsequently injected with the stain. No change in the appearance of the stain or in its distribution within the cytoplasm was demonstrable as the direct result of radiation. Prolonged radiation did not alter the color absorption band or other physical properties of the stain either in the powdered form or in solution.

SUMMARY.

The object of this investigation was to test out the sensitizing effect of various vital stains upon rat tissues in the presence of x-ray energy. For the purposes of the experiment, it had been found that a dosage of $\frac{100 \text{ MAM}}{17.5 \text{ cm.}^2}$ at 50 kilovolts induced death in white rats within 110 to 120 hours. This amount of energy was selected as a tentative standard for the experiments. The preliminary injection of small amounts of such stains as trypan blue in 0.5 per cent aqueous solution sensitized the rat tissues to such an extent that the period of viability of these rats after administration of the standard dosage of x-ray energy, was shortened to from 60 to 70 hours. The cause of death in the stained rats has not been ascertained despite careful histologic study. Considerable variation in the susceptibility of rats to the combined procedure was noted.