Effects of Irradiation on Nucleic Acid Formation.* (17950)

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Hevesy and his associates have shown that irradiation of a tissue depresses the formation of desoxypentose nucleic acid in it(1,2,3). The method used by them consists in irradiating animals, injecting a tracer dose of $Na_2HP^{32}O_4$ and sacrificing after a given time interval. The desoxypentose nucleic acid is then isolated and the specific activity of its phosphorus is determined. This is an index of the amount of newly formed nucleic acid during the time interval under investigation. Using this technic Ahlström, Euler and Hevesy(4) also demonstrated an indirect effect of radiation probably due to some humoral agent. They used rats with bilateral transplants of Jensen sarcoma. In each animal one tumor was irradiated with 2000 r and the other tumor was shielded, yet the depression of the nucleic acid turnover rate was similar in both groups. Experiments to be presented in this paper demonstrate the presence of an indirect effect when liver or muscle rather than tumor tissue is irradiated.

Methods. Except for the method of irradiating the animals, the plan and procedures used in these experiments very closely followed those used by Hevesy and his associates. The animals used were female A strain mice bearing bilateral transplants of Strong's mammary carcinoma. They were irradiated on the tenth or eleventh day after transplanting when the average tumor weight was about 0.6 g.

Irradiation method. The easiest way to irradiate specific tissues in a large number of animals is to inject some radioactive substance which localizes in these tissues. We have found colloids containing radio-yttrium very useful for this purpose. The chemical and biological properties of these colloids have been reported: Gofman(5a), and Dobson, Gofman, Jones, Kelly, and Walker(5b). The yttrium-hydroxy-citrate developed by Gofman for liver irradiation was made in the following manner: 0.10 ml of 0.2 M yttrium nitrate solution (incorporating the desired yttrium radioisotope) is mixed with 0.18 ml of 0.05 M sodium citrate solution; 1 drop phenol red indicator is added, and the mixture is titrated to the salmon colored end point with 1 M sodium hydroxide. It is then diluted to 1.0 ml with water. The colloid used was made with Y^{90} which has a half life of 65 hours and emits only beta rays with a maximum energy of 2.2 MEV. It may be separated at frequent intervals from a Sr^{90} parent source.

When the colloid is injected intravenously into mice, 95% of it disappears from the blood stream with a half time of 88 seconds. Twelve mice killed 4 hours after the intravenous injection showed the following average distribution: Liver and spleen 84%, carcass 9%, tumors 0.95%. When the colloid was injected intramuscularly 4 hours later most of it was still at the site of injection. The fraction which had been removed from the site showed the following average distribution for 8 mice: Liver and spleen 2.1%, carcass 4%, tumors 0.2%. Our calculations based on current information on the Y^{90} β spectrum indicate that a large block of tissue in which one microcurie of Y^{90} is distributed uniformly in each gram of tissue absorbs 154 ergs per gram of tissue per hour. In the case of mouse livers one must also take into consideration the fact that the half thickness for Y^{90} beta

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* This work was supported in part by the U. S. Atomic Energy Commission.

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2. Hevesy, G., Radioactive Indicators, 1948, 325.

3. Hevesy, G., *Rev. Mod. Phys.,* 1945, v17, 102.

4. Ahlström, L., Euler, H., and Hevesy, G., *Arkiv. Kemi, Mineral., Geol.,* 1945, 19a, No. 13.

5. (a) Gofman, J. W., *J. Lab. and Clin. Med.,* 1948, v34, 297; (b) Dobson, E. L., Gofman, J. W., Jones, H. B., Kelly, L. S., and Walker, L. A., *J. Lab. and Clin. Med.,* 1948, v34, 305.

radiation is approximately 0.1 cm in tissue and hence not all of the energy will be absorbed in the liver. Jones *et al.*(6) have calculated that for beta rays with the above half thickness only about 73% of the beta radiation originating in the liver is absorbed in that organ. The average liver weight of the mice used was 1.7 g and the average Y^{90} dose administered was 900 microcuries per mouse. During the 5 hour period of the experiment then, approximately 4.25×10^5 ergs total or 2.5×10^5 ergs per g (3000 rep‡) were absorbed by the liver. The tumors of these animals received approximately 5×10^3 ergs (50 rep). In the muscle irradiation experiments the yttrium colloid was injected into the gastrocnemius. It is estimated that approximately 90% of the beta radiation originating in the muscle was absorbed. The average Y^{90} dose administered was 630 microcuries which during the 6 hour period of the experiment delivered about 5.25×10^5 ergs to the muscle. Due to the small amount of Y^{90} which left the muscle, the livers of these animals received 5.9×10^3 ergs per g (71 rep) or 1×10^4 ergs total and the tumors received 8.5×10^2 ergs per g (10 rep) or 1×10^3 ergs total.

The whole body x-irradiation experiments were done with 180 KV X-rays filtered through 0.45 g per cm^2 of copper and 0.3 g per cm^2 of aluminum. The dose, as measured by a Victoreen meter was 60 roentgens which is equivalent to 5.6×10^3 ergs per g of tissue or 1.4×10^5 ergs total.

Plan of experiments. The procedure followed for the liver irradiation experiments was as follows: The yttrium colloid was injected into the tail veins of the mice and three hours later the tracer dose of sodium phosphate was given intraperitoneally. The mice were sacrificed exactly 2 hours after the phosphate injection. In order to obtain enough purified desoxypentose nucleic acid the tumors from four animals had to be pooled. The average tracer dose used per mouse was about 20 μc which is equivalent to 1.46×10^7 counts per minute on the counter used for the assay of the nucleic acid phosphorus. The material injected contained less than 0.01 mg Na_2HPO_4 in isotonic saline so that it did not upset the phosphate balance of the mice. In the experiments where the muscle rather than the liver was irradiated, the yttrium colloid was injected into the hind leg in the general region of the gastrocnemius. The tracer dose of phosphate was given four hours later but in all other respects the procedure was identical with that used for the liver irradiation experiments. The liver desoxypentose nucleic acid was also isolated. In the experiments with the low total body x-irradiation, approximately half the dose was given in one hour before the intraperitoneal injection of tracer phosphate and the other half in one hour immediately following it. In all other respects it was like the experiments above.

Nucleic acid isolation procedure. The method used for the isolation of the desoxypentose nucleic acid was essentially Levene's as modified by Klein and Beck(7). Some changes were necessary in order to eliminate the Y^{90} and to make the method suitable for a tracer experiment. Five to 10 g of tissue were ground with sand in mortar and pestle and mixed with 10 ml of 5% sodium chloride. The tissue was then boiled in a water bath for a few minutes, 0.25 ml glacial acetic acid added and then made basic with 0.5 g sodium hydroxide and 0.1 g sodium acetate. The basic mixture was boiled for about one hour or until the organs were almost completely dissolved. One ml of glacial acetic acid and 0.7 ml of a 5% dialyzed ferric hydroxide solution were then added. After standing a short time, another cc of acetic acid was added and the solution centrifuged. The supernatant was treated with an equal volume of methyl alcohol and the crude nucleic acid was centrifuged off. In order to purify the desoxypentose nucleic acid it was dissolved in 5 ml of 1 M sodium hydroxide and the following solutions added: 0.2 ml of a saturated solution of disodium phosphate, 0.2 ml 1 M sodium fluoride, 0.2 ml 0.2 M yttrium nitrate, and an equal volume of methyl alcohol. After heating in a water bath of 65°C

6. Jones, H. B., Wrobel, C. J., and Lyons, W. R., *J. Clin. Invest.*, 1944, v23, 783.

‡ 1 rep = 83 ergs per g.

7. Klein, D., and Beck, Z., *Z. f. Krebsforsch.*, 1935, v42, 163.

TABLE I.
Averages of Tumor Desoxypentose Nucleic Acid Specific Activities.

	No. of animals	
Controls	320	$2.50 \pm 0.045 \times 10^{-3}$
Livers irradiated with 4.25×10^5 ergs	136	$1.65 \pm 0.052 \times 10^{-3}$
Muscles irradiated with 5.25×10^5 ergs	160	$2.10 \pm 0.042 \times 10^{-3}$
60 r total body X-irradiation equivalent to 1.4×10^5 ergs	92	$2.23 \pm 0.041 \times 10^{-3}$

TABLE II.
Averages of Liver Desoxypentose Nucleic Acid Specific Activities.

	No. of animals	
Controls	188	$3.83 \pm 0.19 \times 10^{-4}$
Muscles irradiated with 5.25×10^5 ergs	148	$2.83 \pm 0.115 \times 10^{-4}$
60 r total body X-irradiation equivalent to 1.4×10^5 ergs	84	$3.16 \pm 0.26 \times 10^{-4}$

for 15 minutes, the impurities were centrifuged off. The supernatant solution was placed in an ice bath, acidified with 3 M hydrochloric acid, and diluted with an equal volume of methyl alcohol. The nucleic acid was then centrifuged off. The purification was repeated 6 times. After this the nucleic acid was dissolved in sodium hydroxide and reprecipitated with hydrochloric acid and methyl alcohol 4 more times and finally dissolved in about 5 ml of 0.1 M sodium hydroxide.

Klein and Beck found that the nucleic acid was pure by chemical criteria after only 3 repurifications. However, as can be seen from the values given below, 8 to 10 precipitations were found necessary in this experiment in order that the specific activity of the nucleic acid remain constant upon successive reprecipitation. In a typical liver sample the following values were obtained on aliquots taken after the stated number of precipitations:

No. of precipitations	Specific activity
3	27.1×10^{-4}
6	4.9×10^{-4}
8	3.7×10^{-4}
10	3.9×10^{-4}

Determination of specific activity. The specific activity of the purified desoxypentose nucleic acid was determined in the following manner. One aliquot of the sodium nucleate solution was used for the determination of the phosphate concentration by the method of Fiske and Subbarow. A second aliquot was using for counting the P^{32}. In order to detect any possible contamination of the P^{32} with Y^{90} the samples were recounted a week later. Since the half life of Y^{90} is only 65 hours compared to the 14 day P^{32} half life, the few percent of Y^{90} occasionally still present could be eliminated. All counting was done with an accuracy of at least 2%.

Results. All the values given represent the number of P^{32} counts per milligram of phosphorus divided by the number of counts injected, normalized for the weight of the mice. Errors quoted are $1\sigma_M$.

Discussion. In this experiment no attempt has been made to measure the absolute turnover rate of the nucleic acid. Hevesy's method of calculating it from measurements of the specific activity of the nucleic acid and the specific activity of the inorganic phosphate is not justified as has been pointed out by Chaikoff(8). In order to arrive at a correct value for the turnover rate one would have to know the intermediates and rate determining steps in the nucleic acid synthesis and this is to date not possible. Hevesy and his associates have shown that the rate of entrance of radioactive phosphate into the cells of normal rat tissues or Jensen sarcoma is not affected by the radiation doses used in the present experiments. In other words the specific activity of the intracellular inorganic phosphate was the same in the control and irradiated animals. Any difference in the specific activity of the nucleic acid phos-

8. Chaikoff, I. L., *Physiol. Rev.*, 1942, v22, 291.

phorus then must have been due to a difference in the rate of incorporation of phosphate into the nucleic acid. The specific activity may be considered equivalent to the relative turnover rate.

The desoxypentose nucleic acid turnover rate as measured by P^{32} is now generally thought to be an index of cell division. Several observations strongly suggest that this nucleic acid is synthesized only during cell division and that phosphate is turned over only during this synthesis. First, the relative turnover rates for various tissues as measured by Hevesy and others closely parallel the known relative mitotic indices for these tissues. Second, Hevesy and Ottesen(9) found that nucleated hen erythrocytes (which are non-dividing cells), in contradistinction to all other tissues tested, formed no active desoxypentose nucleic acid when incubated with radioactive phosphate. Last, Spiegelman and Kamen(10) found that in labeled actively metabolizing yeast cells under conditions where there was no cell division or protein synthesis there was no change in the specific activity of the nucleoprotein phosphorus. Aside from the difficulties still present in the interpretation of nucleic acid specific activities there is the technical difficulty of measuring it. The standard methods of chemical separation are not designed for the degrees of purity necessary in some tracer experiments, particularly when the substance to be measured has a very low turnover rate as is the case with desoxypentose nucleic acid.

In the present experiment the nucleic acid was reprecipitated until the specific activity remained constant on successive precipitations. This seemed the only feasible criterion of purity. However, it is possible that some of the more active phosphate groups which were thus eliminated were actually more labile components of desoxypentose nucleic acid rather than impurities. At the present time not enough is known about the chemistry and structure of nucleic acids to settle this question.

9. Hevesy, G., and Ottesen, J., *Nature,* 1945, v156, 534.

10. Spiegelman, S., and Kamen, M. D., *Science,* 1946, v104, 581.

The experiments reported here confirm the results of Ahlström *et al.*(4) as to the existence of an indirect effect of radiation on the desoxypentose nucleic acid turnover rate. The tumors in those animals which received 4.25×10^5 ergs to the liver had 66% of the nucleic acid specific activity of the control group. By direct radiation these tumors received 5×10^3 ergs which, according to the total body x-irradiation experiment, would have depressed it to 90% without the indirect effect.

The results in the muscle irradiation experiment are not quite as clear cut. Although the total energy delivered to the muscle was somewhat greater than that delivered to the liver in the earlier experiment, the volume of tissue irradiated was much smaller, which might possibly account for the smaller effect. The tumor nucleic acid specific activity was 84% of the control value and it is felt that nearly all of this depression was due to an indirect effect. These tumors, due to the radioactive colloid contained in them, received one-fifth as much radiation as the total body x-irradiation group so that the direct radiation was negligible. Also, there was no difference between those tumors close to the irradiated muscle and those far removed, indicating that essentially none of the radiation originating in the muscle had reached the tumors.

In this experiment the liver desoxypentose nucleic acid was also measured. However, due to the colloid localized in them these livers received approximately the same dose of radiation as the total body x-ray group and the specific activities do not differ significantly.

Summary. Mice of the A strain bearing bilateral transplants of mammary carcinoma were used to demonstrate an indirect effect of irradiation on the desoxypentose nucleic acid turnover rate. In 2 experiments the animals were irradiated by means of radioyttrium colloids which localize in the liver when injected intravenously and remain at the site of injection when given intramuscularly. The nucleic acid turnover rate was measured by giving a tracer dose of radioactive sodium phosphate, sacrificing the ani-

mals after 2 hours, and isolating the desoxypentose nucleic acid from their tumors and livers. The specific activity of the nucleic acid phosphorus was measured and considered to be an index of the turnover rate. It was found necessary to purify the nucleic acid much more carefully than is done in standard methods in order to obtain a constant specific activity upon successive reprecipitations.

The animals whose livers were irradiated with 4.25×10^5 ergs showed only 66% of the tumor nucleic acid specific activity of the control group. The animals whose muscles were irradiated with 5.25×10^5 ergs showed 84% of the tumor nucleic acid specific activity of the control group. Both these experiments definitely confirm the existence of an indirect effect of radiation on the desoxypentose nucleic acid turnover rate.

One group of animals received 60 r (1.4×10^5 ergs) total body X-irradiation with 180 KV X-rays and both the tumors and livers showed a significant depression in the turnover rate.

Received April 25, 1950. P.S.E.B.M., 1950, v74.

Further Experiments on Influence of Hyaluronidase on Formation of Intraperitoneal Adhesions in the Rat. (17951)

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Chandy and Rhoads reported a reduction in the number of adhesions formed in the peritoneum of the rat in response to standardized trauma when a hyaluronidase preparation was employed locally(1). In this study, hyaluronidase was first applied to crush injuries of the rat cecum on the hypothesis that it might diffuse whatever substances that were responsible for initiating the process of adhesion formation due to its action as a spreading factor(2,3,4). Experiments to extend this series to other types of trauma, and other preparations of hyaluronidase have been carried on in this laboratory since with the results that will be recorded below. The additional experiments that were done in the same way showed somewhat the same trend, but the difference was less marked and was not statistically significant when considered apart from the original series. When the method for producing the trauma was changed, these hyaluronidase preparations were ineffective in inhibiting the formation of adhesions. In all of the experiments the white rat was employed.

Methods. The abdomen was opened under aseptic conditions in 110 rats and the cecum traumatized by the use of one of 3 instruments. An Allis tissue forceps was used in 44 animals, a Payr clamp in 19 animals, and a straight Kelly hemostat in 47 animals. After the injury had been produced, an observer decided whether the animal was to be injected locally with hyaluronidase or used as a control. Schering hyaluronidase was applied to the Allis and Payr clamp injuries and Hydase (Wyeth) and Alidase (Searle) to straight hemostat injuries (see Table).

Discussion. An analysis of the results obtained reveals that adhesions developed in 76% of 55 animals treated with various preparations of hyaluronidase after standardized mechanical methods of producing adhesions were performed. These methods at the same time produced adhesions in 89% of the 55 animals that were used as controls.

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