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Received September 17, 1952. P.S.E.B.M., 1952, v81.

## Effect of Total-Body X-Irradiation on Relative Turnover of Nucleic Acid Phosphorus.* (19992)

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Supplee and Entenman(1) have observed that fasted adult female Sprague Dawley rats, previously subjected to large doses of total body X-irradiation, have larger livers than fasted non-irradiated control rats. This enlargement of the livers was found not to be due to edema or to the excessive accumulation of lipids. The maximum increase in liver mass occurred at approximately 24 hours following 2500r total body X-irradiation. Since the increase in liver size seems to be due to an actual increase in liver tissue, it was believed that this change might be reflected in the synthesis of cytoplasmic pentose nucleic acid (cPNA). It has been postulated by Casperson, Brachet, and Spiegelman(2-4), that the formation of PNA and protein are intimately related. Abrams(5) studied the effect of X-irradiation on the formation of desoxypentose nucleic acid (DNA), PNA, and protein in rat intestine. He observed that with 500r the incorporation of glycine-1-$C^{14}$ into DNA and PNA purines is depressed, while there is no apparent change in the incorporation of glycine into proteins. This study might be interpreted to contradict the earlier observations of Casperson and Brachet. However, since the incorporation of the radioactive precursor was observed at only one time interval (*i.e.*, 48 hours), and since it is not known at which step between precursor and final product inhibition occurs, it cannot be concluded from Abrams' findings that there is no relationship between PNA and protein synthesis.

In the experiment reported in this paper we determined the incorporation of radioactive phosphorus ($P^{32}$) into rat liver DNA, cPNA, and nuclear PNA (nPNA) 24 hours post-irradiation, at which time the maximum effect of total body X-irradiation on liver weight was observed. We also determined the same nucleic acid fractions in mice exposed to 600r at 2½ hours post-irradiation.

*Experimental.* Twenty female *Sprague-Dawley* rats each weighing approximately 200 g were given a total dose of 2500r of 250 kv X-rays filtered through 0.5 mm Cu and 1 mm Al at an average dose rate of 22.7r/min. Food was removed from cages just prior to irradiation. Water was allowed *ad lib.*

* Partially supported by U. S. Atomic Energy Commission.

throughout the experiment. The rats were injected intraperitoneally with approximately 100 $\mu$c of $Na_2HP^{32}O_4$ 24 hours after the completion of irradiation and sacrificed with ether 4½ hours later. Eight female *Long-Evans rats* weighing an average of 280 g were given a total dose of 2440r of 215 kv X-rays filtered through 1.1 mm Cu and 1.2 mm Al at an average dose rate of 12.2r/min. Six female Long-Evans rats were used as controls. Food was removed from the cages just prior to irradiation. Water was allowed *ad lib*. The rats were injected intraperitoneally with approximately 135 $\mu$c of $Na_2HP^{32}O_4$ 18 hours after the end of irradiation and sacrificed 4 hours later. Thirty-six male A strain mice, weighing an average of 25 g were given a total body X-irradiation of 600r of 215 kv X-rays filtered through 1 mm of Cu and 1.2 mm Al at an average dose rate of 16.7r/min. Twenty-four male A strain mice were used as controls. Food was removed from the cages 24 hours before the animals were sacrificed. Water was allowed *ad lib*. throughout the experiment. The mice were injected intraperitoneally with approximately 20 $\mu$c of $Na_2HP^{32}O_4$ 20 minutes after the end of irradiation, and sacrificed 2 hours later.

The livers were removed immediately, weighed and put in iced beakers. The separation of nuclei and cytoplasm and the isolation of the nucleic acids has been described previously(6,7).† Essentially it consists of homogenizing the livers in isotonic saline and separating the nuclei and cytoplasm by differential centrifugation. The nuclei were washed in citric acid. The DNA-protein and nPNA-protein were separated in a sodium bicarbonate-sodium carbonate buffer. The DNA was then isolated by a modified Beck and Klein method(7). The nPNA-protein was obtained as a trichloroacetic acid precipitate, and the protein was split from the nucleic acid in cold sodium hydroxide.

The cPNA protein was directly precipitated from the cytoplasm and the cPNA was obtained in the same manner as the nPNA. After obtaining the crude DNA, it was purified in the following manner. The DNA was dissolved in 0.1 M sodium hydroxide, and 0.2 ml of a saturated solution of disodium phosphate were added. The DNA was then reprecipitated five times with hydrochloric acid and methyl alcohol. At this point the specific activity remained constant and did not change upon further reprecipitation. The specific activity of the PNA-P was found not to change upon reprecipitation, therefore it was reprecipitated only once. To test whether there was any contamination of nPNA with DNA, or cPNA with DNA, or DNA with PNA, the nucleic acids were hydrolized and paper chromatographed. No uracil was found in the DNA fraction and no thymine in either one of the PNA fractions. To verify further the purity of the nucleic acids, the PNA was determined by the orcinol reaction and the DNA by the diphenylamine reaction. The density of the color developed per microgram of phosphorus agreed well with that of commercial nucleic acid preparations(6). The $P^{32}$ samples were counted with a Victoreen-Geiger tube. The samples were counted with an accuracy of at least 2%, except the DNA samples from the irradiated rat livers which counted as low as 25% above background, and therefore their counting error was as high as 14%. The specific activity is expressed as counts per minute per milligram of nucleic acid phosphorus divided by the counts per minute injected, corrected for the weight of the animal.

*Results and discussion.* As can be seen in Tables I, II, and III the specific activity of the cPNA-P of the livers of irradiated rats and mice is increased in all cases. On the other hand the specific activity of nPNA-P and DNA-P is decreased. The liver weights, taken as per cent of total body weight, are increased 29% in the irradiated Sprague-Dawley rats, while there is no significant difference in the weights of the livers from the Long-Evans rats. At present the difference between the two strains is not understood. The two sets of rats came from different colonies, received the radiation dose at a different rate, and were not quite the same age.

† The mouse livers were homogenized in 0.25 M sucrose according to Schneider and Hogeboom's(8) method. The rest of the isolation procedure for the mouse livers followed the method of Barnum *et al.*(9).

TABLE I. Averages of Specific Activity × $10^5$ of Nucleic Acid-Phosphorus and Liver Weights of Sprague-Dawley Rats.*

| | Controls | Irradiated | Critical ratio† |
|---|---|---|---|
| cPNA-P | 36.4 ±1.4 | 48.2 ±2.6 | 4 |
| nPNA-P | 97.7 ±4.6 | 78 ±3.8 | 3.3 |
| DNA-P | 1.90± .23 | .28± .026 | 7.2 |
| Liver wt as % of total body wt | 4.12± .07 | 5.32± .12 | 8.5 |

* 20 control rats; 20 irradiated rats.

† Critical ratio $= \frac{M_1 - M_2}{\sqrt{(\sigma M_1)^2 \pm (\sigma M_2)^2}}$

M = mean; $\sigma M$ = stand. error of mean.

TABLE II. Averages of Specific Activity × $10^5$ of Nucleic Acid-Phosphorus and Liver Weights of Long-Evans Rats.*

| | Controls | Irradiated | Critical ratio |
|---|---|---|---|
| cPNA-P | 43.7 ±1.34 | 55.4 ±2.2 | 4.5 |
| DNA-P | 3.35± .69 | .57± .05 | 4 |
| Liver wt as % of total body wt | 4.16± .29 | 4.01± .34 | .33 |

* 6 control rats; 8 irradiated rats.

TABLE III. Averages of Specific Activity × $10^4$ of Nucleic Acid-Phosphorus and Livers* of Male A Strain Mice.

| | Controls | Irradiated | Critical ratio |
|---|---|---|---|
| cPNA-P | 6.8 ± .24 | 7.4 ± .32 | 1.5 |
| nPNA-P | 76.3 ±16.2 | 54.2 ±8.6 | 1.2 |
| DNA-P | .607± .11 | .405± .078 | 2 |

* 9 livers from irradiated mice were pooled, and 8 livers of the control mice.

Whether these factors have any influence on the weights of the livers is not known.

In preliminary experiments total nitrogen determinations were carried out on liver cytoplasm from four fasted irradiated and four fasted control male Sprague-Dawley rats that were sacrificed 24 hours after exposure to 2500r. The average nitrogen values obtained (corrected for a 200 g rat) were 178 mg ± 7.4 mg for the irradiated rats and 148 mg ± 4.2 mg for the control rats. This represents an increase of 20%. Under the same conditions the incorporation of $C^{14}$ labeled formate into liver cytoplasmic proteins (fat extracted TCA precipitate) was also determined. Formate was given intraperitoneally to 4 irradiated and 4 control rats 2 hours before sacrificing the animals. An increase of 34% above the control values was found in the specific activity (counts per milligram of protein). These preliminary findings suggest that the increase in liver weight is due to an increased protein synthesis in the irradiated animals. The simultaneous increase of cPNA-P specific activity is compatible with the concept of an interrelationship of protein and PNA synthesis.

The increase in cPNA-P specific activity and liver weights may be akin to an attempt of the liver to regenerate, after having been damaged by the exposure to X-irradiation, but which cannot be carried to completion due to an inhibition of cell division. From these data it is not known whether the observed increase in the cPNA-P specific activity is due to an increase in the turnover of all the cPNA fractions or whether it is due to a relative increase in the amount of "supernate PNA" which normally has a higher turnover than the other fractions(10,11).

*Summary.* The incorporation of $P^{32}$ into DNA, nuclear PNA (nPNA), and cytoplasmic PNA (cPNA) of liver was measured in rats which had been exposed to 2500r total body X-irradiation and in mice which had been exposed to 600r total body X-irradiation. It was found that the incorporation of $P^{32}$ into cPNA was increased in all cases, while into DNA and nPNA it was depressed. In the Sprague-Dawley rats an increase in the weights of the liver was observed concurrent with the increase in cPNA specific activity. A possible interrelationship between protein and cPNA synthesis has been discussed.

We are grateful to Dr. Hardin B. Jones for his interest and advice throughout this study. We also wish to thank Mr. Roy W. Melbye for carrying out the paper chromatography.

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Received September 23, 1952. P.S.E.B.M., 1952, v81.

## Experimental Study on Ectopic Beats Following Intravenous Injection of Veratrine.* (19993)

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Subepicardial injection of veratrum alkaloids (veratrine Merck) in the dog's ventricle causes an ectopic rhythm originating in the area of injection. The activity of the ectopic center, firing regular stimuli, is not disturbed by other activation waves spreading over the heart; the center is protected from other stimuli ("protective" block). Thus, a parasystole with simple interference appears regularly(1). On the other hand, true extrasystoles, that is, premature beats with constant coupling to the preceding "initiating" beat(2) were never seen in these experiments. The occurrence of "extrasystoles" following parenteral administration of veratrine has frequently been reported. Since, in general, arrhythmias caused by topical administration of different compounds or electrolytes closely resemble those which appear following the intravenous administration of these substances, we decided to investigate the occurrence of extrasystoles with fixed coupling following intravenous injection of veratridine, cevadine, and a mixture of veratrum alkaloids in the dog.

*Method.* Dogs weighing 20-25 kg were anesthetized with nembutal and the heart was exposed as described in previous papers by the authors. The right vagus was severed in the neck in each experiment. The electrocardiogram was continuously recorded in Lead II from the time an arrhythmia appeared to the final onset of ventricular fibrillation. Registration was only interrupted when a regular sinus rhythm or a regular tachycardia appeared. A 0.025% solution of the alkaloids was used in all experiments. The solubility of the alkaloids was enhanced by the addition of alcohol. One-tenth of 1 cc of the solution was injected intravenously and if no response appeared within 2 to 4 minutes the injection was repeated in increasing doses up to 1 cc until an arrhythmia was observed. Arrhythmias were seen after injection of about 1.3 mg of veratridine or veratrine Merck. Double doses of cevadine had to be administered in order to obtain the same effect. If no change of the existing arrhythmia was seen after 2 to 4 minutes an additional intravenous injection of the veratrine solution was given. Finally, after 30-50 minutes ventricular fibrillation appeared in all 14 experiments.

*Results.* In no experiment did we observe extrasystoles with fixed coupling. There were only ectopic ventricular tachycardias with changing form of the ventricular complexes.

Fig. 1a shows the irregularly formed and timed ectopic beats, which—in the last third of the tracing—change into ventricular flutter and fibrillation. Sometimes a bigeminy was suspected as in Fig. 1b and c but closer analysis showed that we are dealing here with other disturbances. A regular sinus tachycardia exists throughout Fig. 1b. The first few complexes show an elevated RS-T segment, presumably due to the appearance of block areas in the myocardium. The same

* This investigation was supported (in part) by a research grant from the National Heart Institute, U. S. Public Health Service.