THE INHIBITION OF DNA SYNTHESIS BY CALF-THYMUS POLYMERASE BY X-IRRADIATION OF THE PRIMER DNA.[1]

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Among the many effects produced by ionizing radiations in cells, one of the most characteristic is the inhibition of DNA synthesis, whether temporary or permanent. This statement and its exceptions has recently been reviewed by Gouthier (1961). Two obvious possible explanations of this effect are that the block occurs in enzymes essential to DNA synthesis or that the DNA is damaged in such a way that it cannot be replicated. Though Okada and Hempelman (1959) found evidence for the first of these hypotheses, it seems likely from the experiments of Bollum, Anderegg, McElya and Potter (1960) that regenerating rat-liver can still fully synthesize thymidylic kinase and DNA polymerase in tissues in which DNA synthesis is blocked by X-irradiation. The object of the experiments reported here was to test an alternative possibility, that DNA damaged by X-irradiation may not be so easily replicated by the DNA

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synthetic mechanism. Preliminary results show that the ability of X-irradiated DNA to "prime" the action of polymerase preparations from calf thymus (Bollum, 1960) is indeed reduced, but only by relatively high doses. Similar experiments, using an extract from regenerating rat-liver have been reported by Wheeler and Okada (1961).

METHODS

The method described by Bollum (1960) for the partial purification of DNA polymerase from calf thymus glands was followed exactly to the end of the third stage, his Fraction C, and the experiments described here were performed with this preparation. The specific activity was 3 mμM of labelled nucleotide incorporated per mg. of protein per hour. Acting on a suggestion by Dr. J. Krakow of Yale University, it was found that cysteine activated the system somewhat more than mercaptoethanol. The radioactive 5'-triphosphates of deoxycytidine and thymidine were prepared in the laboratory of Dr. E.S. Canellakis. The monophosphates were made by incubating the nucleoside with radioactive polyphosphoric acid (Chambers, 1959). After isolation, the monophosphates were raised to the triphosphates with a preparation rich in kinases made from E. coli and these were then purified by column chromatography (Canellakis, Gottesman and Kammen, 1960). The non-radioactive triphosphates were obtained from Pabst Laboratories. The rapid technique using filter paper discs devised by Bollum (1959) was used to determine the acid insoluble radioactivity. Calf thymus DNA, (molecular weight 4.5×10^6 by light scattering) prepared by the detergent method was used for the primer. The irradiated primers were produced by delivering varying doses of 250 kVp X-rays at a dose rate of 3,750 rads/min. to 0.4% solutions of

DNA, which were then diluted to 0.2%, heated in a boiling water-bath for 10 mins. and chilled rapidly in an ice-bath. The almost absolute requirement for some form of denaturation of the DNA before it manifested a capacity to prime this enzyme was confirmed; no radioactivity was incorporated into unheated DNA. The reaction mixture contained 200 μg. of heated DNA, 100 μg. of protein, 2 μmoles cysteine, 2.5 μmoles of Mg^{++} and 7.5 or 30 mμmoles each of dCTP, dGTP, dTTP, dATP, with either the dCTP or the d-TTP labelled with P^{32} such that the activity was about 10^6 c.p.m. per micromole. In each experiment the incorporation of $dTMP^{32}$ and $dCMP^{32}$ into the acid insoluble material was measured in parallel.

RESULTS AND DISCUSSION

The time course of the incorporation of radioactivity into DNA, under the conditions described above, is shown in Fig. 1 using, as primer, DNA that had been subjected to various doses of X-irradiation. From this, and Table 1 it can be seen that the synthesis of new material is strongly inhibited by large doses of X-rays and that within the experimental error there is no great difference in the amounts of incorporation of d-TMP and d-CMP at any dose level so far examined. This contrasts strongly with the results obtained by Okada (1960) who, using an unfractionated preparation from regenerating rat liver (Bollum and Potter, 1958), found a striking change in the ratio of thymidine to cytidine incorporation (both tritium-labelled) as the result of X-irradiation of the primer. These results do however support the findings of Wheeler and Okada (1961) who found a similar decline with dose in the priming efficiency of irradiated DNA, in the rat-liver system.

Table 1

The fraction of surviving priming ability expressed as

$$\frac{\text{amt. of incorporation at 10 mins. using irradiated DNA primer}}{\text{amt. of incorporation at 10 mins. using control DNA primer}} \times 100$$

Dose (rads)	$dCMP^{32}$	$dTMP^{32}$
7,500	128	105
20,000	90	75
94,000	32	34
200,000	14	19.6

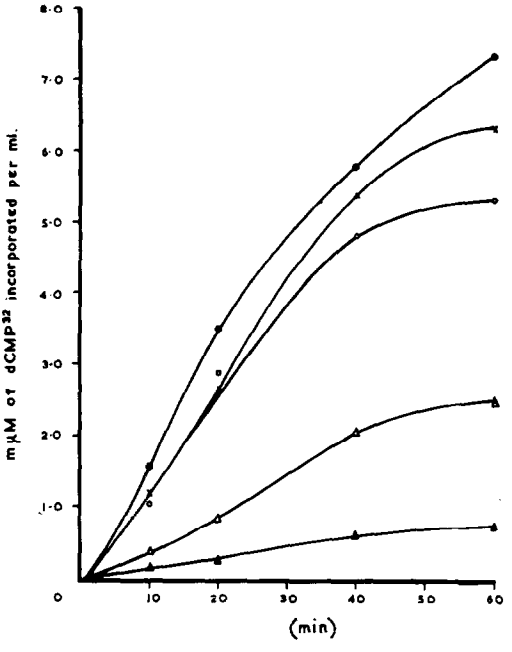

FIG. 1a

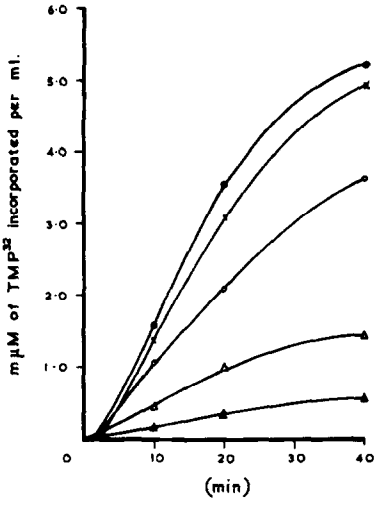

FIG.1b

The time-course of the incorporation of the labelled compound (Fig. 1a $dCMP^{32}$, Fig. 1b. TMP^{32}) in an acid insoluble form, using the conditions described in the text and primer irradiated with these doses of X-rays, x——x control, ●——● 7,500 rads, 0——0 20,000 rads, △——△ 94,000 rads, ▲——▲ 200,000 rads.

These results are preliminary, for the total amount of DNA newly synthesized was only a small fraction (~1%) of the amount

of DNA used as a primer. Since the addition of fresh substrate at the end of the normal incubation time stimulates a comparable amount of synthesis the low degree of conversion is due to the low activity of the enzyme used here. However, it is apparent that X-irradiation does reduce the ability of DNA to stimulate synthesis in this system. Bollum (1960b) has remarked that the priming ability of DNA was not abolished by 100,000 rads, but one would not anticipate total inhibition of synthesis because the damaged regions are distributed randomly along the DNA molecules, and the undamaged ends may still be effective primers. Multiplicity reactivation of X-irradiated bacteriophage (Harm, 1958) does suggest the possibility of the partial synthesis of DNA damaged by X-rays. The difference between these results and those of Okada (1960) emphasizes that the system used for assaying the effects of irradiation may greatly modify the results obtained and experiments with more highly purified polymerase preparations are therefore in hand.

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