EFFECT OF ACTINOMYCIN D ON THE SYNTHESIS OF DNA POLYMERASE IN HEPATECTOMIZED RATS

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The existence of a DNA polymerase in the soluble cytoplasm of regenerating rat liver has been described (Bollum and Potter, 1958). This enzymatic activity is very low in normal liver and begins to increase at 18 hours after hepatectomy, following an exponential curve until about 24 hours and a slower rise until about 30 hours (Bollum and Potter, 1959).

Bollum et al. (1960) have shown that whole-body irradiation is effective in suppressing this rise in DNA polymerase activity only if given in early periods of the regeneration. Somewhat similar results were reported by Van Lancker (1963).

The hypothesis can be made that irradiation interferes with the synthesis of a specific RNA which is made during the first stages of regeneration. Welling and Cohen (1960) showed that X-irradiation is mostly effective in suppressing the synthesis of nuclear RNA if administered 2 to 4 hours after hepatectomy.

The present paper will report the effect of the injection of actinomycin in rats on DNA polymerase activity at various times after hepatectomy. This antibiotic is known to inhibit the DNA-dependent RNA synthesis (Hurwitz et al., 1962; Goldberg et al., 1962, 1963). A brief account of the effect of actinomycin on amino acid incorporation in vivo is also given.

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MATERIALS AND METHODS

Male Sprague-Dawley albino rats, weighing from 140 to 180 g, were subjected to 70% hepatectomy under ether anesthesia. A suspension of 70 μg of Actinomycin D in 0.1 ml of 1,2-propanediol was injected into each rat intraperitoneally at the times indicated in the Figure. The livers were separately collected at 28-29 hours after hepatectomy, and the DNA polymerase activity determined as described by Bollum and Potter (1958, Method III). In short, a regenerating normal rat liver postmicrosomal supernatant was used as a kinase preparation to make the deoxyribonucleoside triphosphates from the monophosphates, including H^3-thymidine. The boiled supernatant of this reaction mixture was added as a substrate source to the postmicrosomal supernatant to be tested. One mg of highly polymerized DNA preparation (California Foundation) from salmon sperm was used as primer in each sample, which contained about 4 mg of postmicrosomal supernatant protein. The incorporation of thymidine into DNA after 30 minutes of incubation at 37° C was measured by use of the filter-paper disk method described by Bollum (1959). The same method was used for the measurements of the amino acid incorporation into proteins as modified by Mans and Novelli (1961).

RESULTS AND DISCUSSION

The data reported in the Figure show the DNA polymerase activity determined at 28-29 hours after hepatectomy in the animals injected at the indicated times. The activity is given as per cent of the control animals. When actinomycin is injected 3 hours after hepatectomy, an almost complete inhibition of the rise in DNA polymerase activity is constantly observed. The inhibition is still complete in most cases when actinomycin is given at 8 hours. Actinomycin injection 12 hours or longer after hepatectomy resulted in much lower inhibition. Even though a precise curve cannot be reported owing to great scattering of the data, there is a general decrease in inhibition if actinomycin injection is delayed from 12 hours to 20 hours after hepatectomy. Sometimes when actinomycin is injected at 20 hours following hepatectomy, no inhibition is observed.

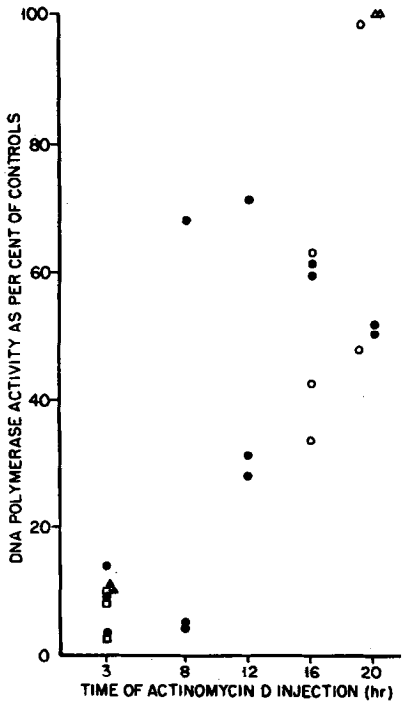

Figure. DNA polymerase activity of regenerating rat liver at 28-29 hours following hepatectomy after injection of actinomycin at various times. On the ordinate are reported the values of DNA polymerase activity as per cent of controls. On the abscissa the times of the actinomycin injection are reported. Each symbol represents a different experiment.

As in the case of the X-ray irradiation, therefore, for the Actinomycin D a period of sensitivity also exists which corresponds to the early stages of regeneration. The injection of lethal doses of Actinomycin D into rats has been shown to completely inhibit RNA turnover (Meritis, 1963).

Our results may suggest the existence of a period of intense specific RNA synthesis up to 12 hours after hepatectomy. The administration of actinomycin during this period prevents this synthesis and therefore the subsequent synthesis of DNA polymerase.

A somewhat different explanation could be that the ribosomes lose their ability to synthesize any kind of proteins after suppression of the supply of messenger RNA. Total protein synthesis could, therefore, stop at a certain time after actinomycin injection, permitting the accumulation of the amount of DNA polymerase synthesized by that time. Such a kind of ribosomal damage after injection of actinomycin into rats has been recently reported by Staehelin and others (Staehelin et al., 1963). These authors reported, however, that with doses similar to those used in our work, very low ribosomal damage is detectable after 4 hours. With 10 times higher doses, after 13 hours 20-50% of the "ergosomes" appear still unchanged. Furthermore, evidence has been recently presented (Korner and Munro, 1963) for inhibition of in vitro protein synthesis in rat liver from 6 hours after actinomycin injection in rats. Again, however, higher doses of antibiotic were employed.

To detect whether actinomycin, in the conditions used in this work, had any effect on total protein synthesis, a short C^{14}-leucine pulse was given to normal rats and to hepatectomized rats at 24 hours after hepatectomy. The latter received an actinomycin injection at 3 hours after hepatectomy, i.e., in a period in which the injection completely prevents the rise in DNA polymerase. The radioactivity of the protein of the total homogenates was measured 15 minutes after the injection. The results, reported in the Table, show no practical differences between the controls and the treated animals; thus suggesting that, at the time when the synthesis of the polymerase should occur (or already occurred), the treated animals are still able to accomplish the usual protein synthesis of the resting liver. On the other hand, they seem unable to synthesize a new protein-like DNA polymerase. Therefore the suggestion of a long half-life of some of the messenger RNA appears justified.

Another proof of the relative stability of the messenger RNA in animal cells comes from the work of Gross et al. (1963) on sea urchins; they find a relative insensitivity of the protein synthesis to actinomycin doses which inhibit RNA turnover until the synthesis of a new kind of protein seems to be required. Here, as our results also suggest, actinomycin seems to inhibit only the synthesis of new proteins, which requires the synthesis of a new kind of messenger RNA.

TABLE

Incorporation of C^{14}-leucine into liver protein of rats hepatectomized and treated with actinomycin

Controls	Counts/min/ mg protein	Actinomycin-treated rats	Counts/min/ mg protein
1	1,315	1	1,338
2	1,294	2	1,050
3	1,428	3	1,085

Normal rats and hepatectomized rats were injected intraperitoneally with 5 μc of L-leucine-U-C^{14} (specific activity = 240 μc/μmole) 24 hours after hepatectomy. The hepatectomized rats received 70 μg of actinomycin at 3 hours. Specific activity of the total liver proteins was determined 15 minutes after the injection.

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