LIMITED ACTION OF ACTINOMYCIN D ON PROTEIN SYNTHESIS IN ADRENALECTOMIZED RATS*

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The action of the actinomycins in the inhibition of RNA synthesis is well known. A secondary effect upon protein synthesis results from actinomycin inducted inhibition of messenger RNA. This inhibition has been shown in rat liver systems by Korner and Munro (1963) and Staehelin, Wettstein and Noll (1963).

Korner (1960) has shown that for several days after adrenalectomy rat liver microsomes have an increased capacity for protein synthesis which declines to subnormal levels only after many days. We have used an assay system of purified polyribosomes and transfer enzymes to study the effect of actinomycin D on protein synthesis in the liver of recently adrenalectomized rats. In contrast to its effect on intact rats, actinomycin D has little effect on the incorporation of amino acids into polyribosomal protein in four-day adrenalectomized rats.

Adrenalectomized male rats were injected intraperitoneally for three successive days following operation with actinomycin D, or with actinomycin plus triamcinolone acetonide (TA), a fluorine-substituted prednisolone of potent glucocorticoid activity (Ringler et al., 1959). Unoperated rats were injected in a similar manner; unoperated control rats were diluent-injected. Sixteen hours after the last injection the animals were sacri-

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ficed and the livers were excised and used to prepare purified "heavy" polyribosomes, essentially according to Wettstein, Staehelin and Noll (1963). The postmitochondrial fraction of liver homogenate was treated with sodium deoxycholate and Lubrol WX (Rendi and Hultin, 1960) and layered into centrifuge tubes which held a bottom layer of medium containing 2.0 M sucrose and a middle layer containing 0.5 M sucrose. Centrifugation was performed for four hours at 78,000 x g at 0°. The pellet was resuspended in light medium. Transfer enzymes were prepared from livers of control (diluent-injected) rats by a modification of the method of Nathans and Lipmann (1961), to be described (Breuer and Davis, in preparation). Transfer RNA was also prepared from control rat livers, and was charged with a spectrum of radioactive amino acids (Rosenbaum and Brown, 1961).

Table 1 shows that the polyribosomes of four-day adrenalectomized rats exhibit greater amino acid incorporating ability than unoperated controls, and injection of 5 µg actinomycin D per day is without effect. The same dose injected into unoperated rats decreases the ability of liver polyribosomes to synthesize protein by about one-half.

The absence of actinomycin effect in the adrenalectomized group is emphasized by the results obtained by injection of TA in conjunction with actinomycin. In this case, the capacity to incorporate amino acids into protein is reduced as compared to the group which received actinomycin alone, although values are still above those of the controls.

The slight decrease in amino acid incorporating ability produced by treatment with TA for 3 days after adrenalectomy is in direct contrast with the effect produced by treatment for 8 days after this operation. In the latter case, TA at a level of 25 µg per day results in polyribosomes having 182 per cent of the amino acid incorporating ability of the unoperated control. During this same period, the activity of the liver ribosomes in adrenalectomized rats not subjected to TA treatment drops to 71 per cent of the control value (Breuer and Davis, 1963).

TABLE 1

The effect of actinomycin D upon the amino acid incorporating ability of rat liver polyribosomes from four day-postadrenalectomized rats

Ribosome source	Percentwise incorporating ability of ribosomes
Unoperated, diluent-injected control	100
Unoperated, 5 μg actinomycin per day	54
Adrenalectomized, no actinomycin	132
Adrenalectomized, 5 μg actinomycin per day	132
Adrenalectomized, 5 μg actinomycin plus 25 μg TA per day	118

Control transfer enzymes (0.9 mg) were incubated for 15 min. at 37° with 2.5 mg of experimental ribosomes, 0.09 mg of transfer RNA charged with ^{14}C-amino acids, 50 μmoles tris-HCl, pH 7.6, 120 μmoles KCl, 2.5 μmoles $MgCl_2$, 0.3 μmoles GTP, 2.5 μmoles ATP, and 10 μmoles GSH in a total volume of 1.2 ml. The reaction was stopped by addition of an equal volume of cold 0.5 N $HClO_4$. The precipitate was washed with $HClO_4$, and RNA was hydrolyzed by heating in acid for 15 min. at 70°. The washed residue was plated and counted. Results are expressed in terms of relative incorporation of amino acids into protein per unit of ribosomal RNA. Incorporation into control ribosomes is set at 100 per cent.

Figure 1 presents sucrose gradient profiles of the various polyribosomal preparations. The control preparation (1A) consists of a population of particle sizes ranging from heavier aggregates at the left to a peak corresponding to the 73S monomer at the right of the graph. Adrenalectomized or unoperated rats, each injected with 5 μg actinomycin D for 3 days, exhibit rather similar profiles (1B and 1C). In each instance the major change occurs in the concentrations of smaller ($(73S)_2$ and $(73S)_3$) aggregates of the 73S ribosome. The 2 to 3-fold difference in amino acid incorporating activities of 1B and 1C (Table 1) is not reflected in the polyribosomal profiles. The most striking effect is seen when TA is administered in conjunction with actinomycin, as shown in 1D. Ribosomal aggregates are drastically decreased. This profile appears similar to the pattern that

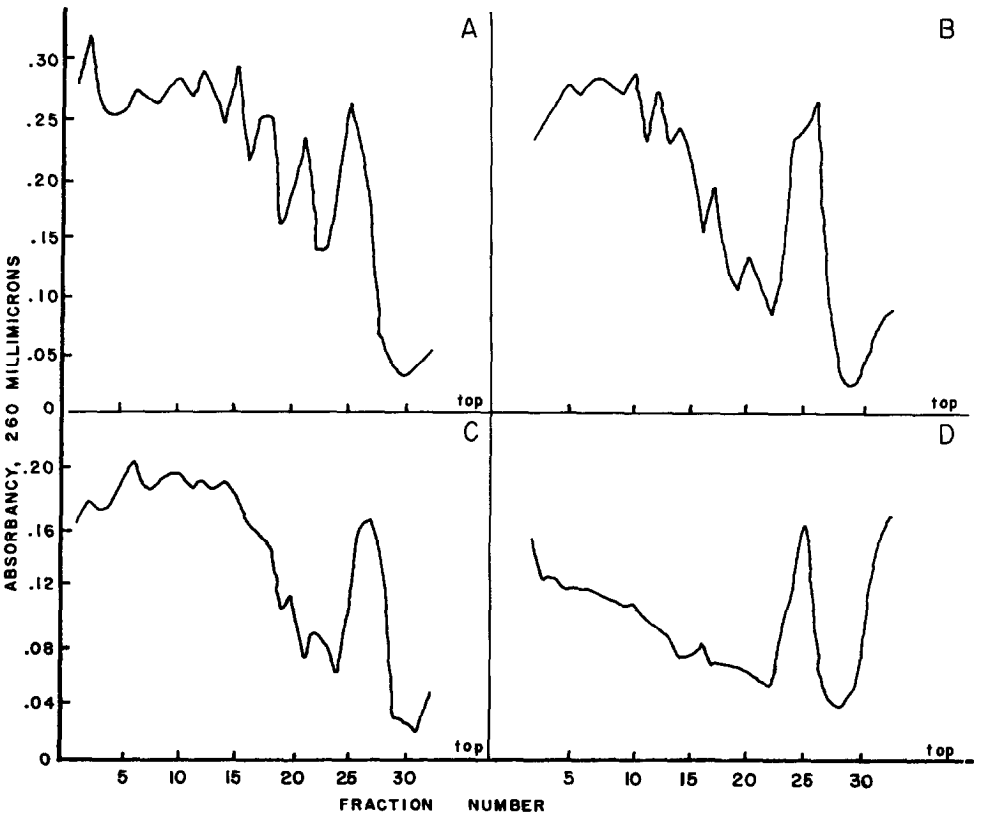

Figure 1. The effect of actinomycin D upon density gradient profiles of ribosomal preparations. 0.5 mg of ribosomes from each group was layered over a linear sucrose gradient from 0.3 to 1.0 molar containing 0.01 M $MgCl_2$. Tubes were centrifuged for 2 hours at 25,000 RPM at 0°C in the Spinco SW-25 rotor. Thirty-two 14 drop fractions were collected. Ribosomes from: A, diluent injected, unoperated rats; B, adrenalectomized, 5 µg actinomycin D daily for 3 days; C, unoperated, 5 µg actinomycin D daily for 3 days; D, adrenalectomized, 5 µg actinomycin D plus 25 µg TA daily for 3 days.

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was observed when a normal rat was given a single large injection of actinomycin C_3 (Staehelin, Wettstein and Noll, 1963).

The reason for the failure of actinomycin D to elicit a reduction in protein synthesizing capacity in livers of adrenalectomized rats remains

obscure, especially since similar decreases in ribosomal aggregates were seen in the ribosomes of this group as in those of unoperated rats. One possibility is that the short term hyperactivity of liver polyribosomes in protein synthesis that is created by removal of the adrenals is not involved with the messenger RNA portion of polyribosomal structure. The observation that corticosteroid injection lowers amino acid incorporating ability in livers of newly adrenalectomized rats while actinomycin is without effect leads to the suggestion that the steroid does not act at the same locus as actinomycin, and that regulation of protein synthesis in ribosomes can occur by mechanisms not related to messenger RNA production.

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