EFFECT OF HYDROCORTISONE ON THE SIZE OF RAT THYMUS POLYSOMES*

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Recent studies on the inhibition of protein synthesis in lymphoid tissue by hydrocortisone have described the effects of this steroid on the microsomal incorporation of H^3-Leucine into an acid insoluble form (Gabourel and Comstock, 1964; Peña et al, 1964). Microsomes isolated from cells (either Mouse Lymphoma ML-388 growing in vitro or rat thymus) pre-exposed to hydrocortisone for a 12-hour period had a depressed ability to incorporate the label. In addition, Gabourel and Comstock (1964) showed that no effect was seen if hydrocortisone was added directly to the incubation vessel during the amino acid incorporation period. Further, using a crossover type experiment in which microsomes from steroid-treated cells were incubated with cell sap from control cells and vice versa, these investigators were able to show that the biochemical lesion was associated with the particulate (messenger RNA-ribosomal RNA complex) and not with the soluble enzyme systems, transfer RNA or availability of amino acids.

The work reported here extends these observations by demonstrating an effect of hydrocortisone on distribution of mixed ribosome populations isolated from rat thymus in a sucrose density gradient.

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Methods and Materials

Sprague-Dawley rats, weighing 90 to 110 grams at the time of sacrifice, were maintained in our animal colony at least one week prior to use. They were then injected with hydrocortisone (Cortef®) (15 mg/kg) or the appropriate vehicle; 6 or 12 hours later they were sacrificed by decapitation and the thymus glands rapidly excised. This and all subsequent operations were carried out in the cold. Thymus glands from three animals were combined for each sample. The thymic tissue was then homogenized in four volumes of homogenizing solution containing 0.01 M Tris buffer (pH = 7.6), 0.01 M KCl and 0.001 M $MgCl_2$. The resulting suspension was then centrifuged at 8,000 x g for 10 minutes. The supernatant was removed and diluted with 3 parts homogenizing buffer. Sodium deoxycholate was then added to each sample (25 λ of a 20% solution per ml diluted homogenate) and 0.7 ml of the resulting solution layered on top of a 5% - 25% sucrose gradient. Three samples (A, B and C in Fig. 1) were centrifuged simultaneously. All gradients contained 0.01 M Tris buffer, (pH = 7.6), 0.01 M KCl and 0.001 M $MgCl_2$. The samples were centrifuged for 90 minutes in a S.W. 25.1 rotor at 25,000 RPM. The gradients were collected fractionally and the distribution of material absorbing at 260 mμ determined spectrophotometrically.

Results and Discussion

The distribution of 260 mμ absorbing material in the sucrose gradients from a typical experiment is shown in Fig. 1. Fig. 1A shows the OD_{260} pattern obtained from the 8,000 x g supernatant of rat thymus homogenate of control animals. The typical single ribosomes (73S particles*) are seen in fractions 38 to 44. Polysomes (dimers (96S) or larger aggregates ($>$ 100S)) are seen in fractions 6 to 38. The poly-

*Sedimentation coefficients were determined, using a Model E Analytical Ultracentrifuge, through the courtesy of Dr. Demetrius Rigas, Department of Experimental Medicine, University of Oregon Medical School.

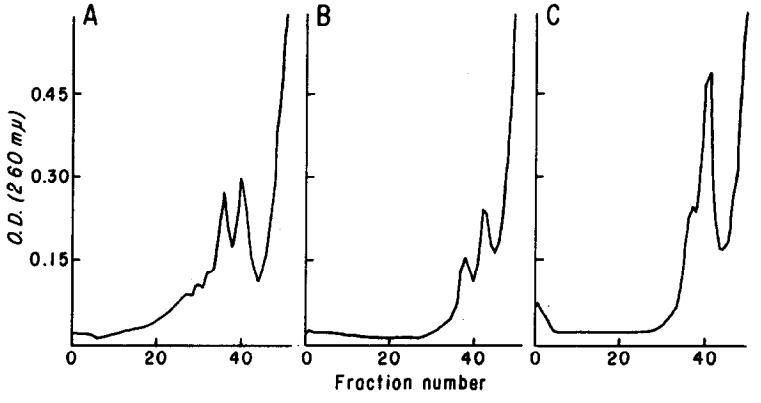

Figure 1. A. Sedimentation pattern of mixed ribosome population from thymus of control rats. B. Sedimentation pattern of mixed ribosome population from thymus of rats treated with hydrocortisone (15 mg/kg) 12 hours prior to sacrifice. C. Sedimentation pattern of mixed ribosome population from thymus of control rats treated in vitro with RNase (0.5 γ ml) for 10 minutes at 0° C.

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some unit is known to be resistant to sodium deoxycholate, a salt used here to dissolve away microsomal lipid membranes, but readily subject to reduction in size by RNase (J. R. Warner et al, 1964 and Wettstein et al, 1963). Fig. 1C shows the reduction in polysome size by RNase obtained when the 8,000 x g supernatant from control rat thymus homogenate was subjected to RNase for 10 minutes at 0° C prior to centrifugation. Fig. 1B shows the distribution of 260 mμ absorbing material obtained from rats pretreated with hydrocortisone 12 hours prior to sacrifice and removal of thymus glands.

Table 1 summarizes the data from a series of six experiments in which thymus glands were excised from rats, either 6 or 12 hours after injection with hydrocortisone. Several facts are apparent from this table: 1) The total amount of particulate RNA was significantly reduced by steroid; this was apparent as early as 6 hours after steroid treatment. 2) All ribosomal fractions (monomer, dimer, and larger aggregates) were reduced by steroid treatment, but the larger aggregates were affected to a greater extent than the monomer or dimer

units. 3) These effects on particulate RNA occurred at a time (6 hours after steroid treatment) before any significant effect on thymus gland weight was seen.

TABLE 1

	Weight Thymus as % Control	OD units in particulate fractions			
		Total	Monomer	Dimer	Larger Aggregates
Control	100 ± 8	4.69 ± .14 (100%)	1.81 ± .07 (100%)	1.20 ± .07 (100%)	1.69 ± .07 (100%)
Steroid 6 hours	98 ± 3	3.13 ± .15 (67%)	1.47 ± .11 (81%)	0.99 ± .02 (83%)	0.67 ± .10 (40%)
Steroid 12 hours	81 ± 6	2.48 ± .13 (53%)	1.42 ± .06 (78%)	0.64 ± .07 (53%)	0.43 ± .05 (25%)

Distribution of mixed ribosome population in a sucrose gradient. Each gradient was layered with rat thymus homogenate equivalent to 0.044 g of the original tissue weight. The number of OD_{260} units for each fraction (monomer, dimer or larger aggregates) was determined by measuring the area under the appropriate portion of the curve as shown in Fig. 1. The data is expressed in absolute OD_{260} units ± standard error of the mean and as percent of control. Each value was obtained from a minimum of three separate experiments. An analysis of variance using a three-way classification shows that the decrease in 260 mμ absorbing material with time was significant ($P < .001$) for the three fractions and that the fractions differ significantly among themselves ($P < .001$).

Fig. 2 is derived from the data in Table 1 and shows the distribution of 260 mμ absorbing material in the various ribosome fractions as percent of the total ribosomal 260 mμ absorbance at various times after steroid treatment. Although the absolute amount of monomer was decreased (Table 1), it became a larger fraction of the total amount of the remaining particulate RNA. The kinetics suggest that the larger aggregates were being broken down into smaller units in the presence of steroid.

It appears that both messenger RNA and ribosomal RNA are being affected by the steroid. Since the larger aggregates are more sensitive than monomer, we conclude that messenger RNA is more sensitive to

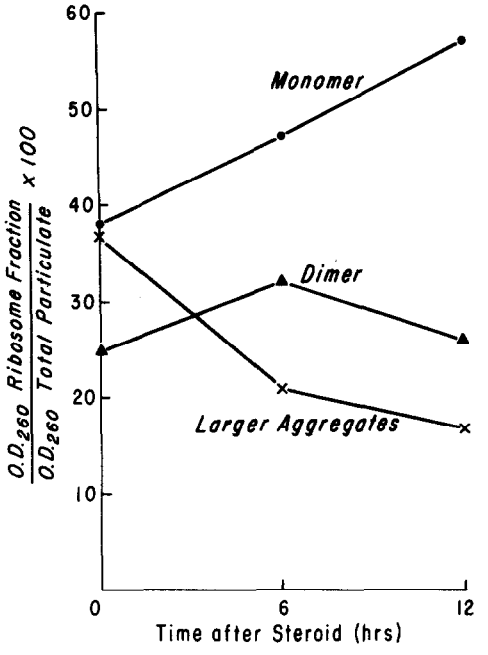

Figure 2. Distribution of 260 mμ absorbing material in ribosome fractions (monomer, dimer and larger aggregates) as percent of total particulate OD_{260} at various times after steroid treatment. These curves were derived from the data presented in Table 1.

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the steroid than is the ribosome. The fact that ribosomes do not accumulate in absolute amount, such as seen when the mixed ribosomal aggregates from control animals are incubated with RNase (Fig. 1C), indicates that a simple interference with the binding of messenger to free 73S-ribosomes is not a major mechanism for the effects observed. At present there is insufficient data available to decide whether the reduction in particulate RNA occurs solely in response to a decrease in the rate of RNA synthesis or is materially aided by an accelerated breakdown of RNA.

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REFERENCES

1. Gabourel, J. D. and Comstock, J. P., Biochemical Pharmacology, In press (1964).
2. Wettstein, F. O., Staehlin, T. and Noll, H. Nature 197: 430, (1963).
3. Warner, J. R., Knopf, P. M. and Rich, A. Proc. Natl. Acad. Sci. U.S. 49: 122 (1963).
4. Peña, A., Dvorkin, B. and White, A., Biochem. Biophys. Res. Comm. 16: 449 (1964).