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ON THE SITE OF CORTISOL INHIBITION OF THYMUS RIBONUCLEIC ACID SYNTHESIS*

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Abstract—Cortisol, in doses shown to inhibit RNA synthesis by thymus aggregate enzyme, did not alter maximum template activity of chromatin isolated from thymus glands of steroid-treated rats when assayed in the presence of *E. coli* RNA polymerase. Cortisol was shown, however, to decrease the amount of endogenous thymic RNA polymerase activity extractable from purified nuclear preparations. The magnitude of this effect was similar to the decrease in [^{3}H]-UMP incorporation seen with intact nuclei or aggregate enzyme preparations when assayed without added bacterial RNA polymerase. When aggregate enzyme was obtained from cortisol-treated rats and assayed at low ionic strength (no NH_4Cl added to assay system) in the presence of added *E. coli* RNA polymerase, the usual cortisol-induced inhibition of RNA synthesis disappeared; when this same preparation was assayed in the presence of 0·3 M NH_4Cl, however, the inhibitory effect of cortisol was paradoxically converted to a stimulatory effect. Removal (by extraction) of endogenous RNA polymerase from control aggregate enzyme-like preparations was also shown to cause a stimulation in [^{3}H]-UMP incorporation when the resulting preparation was assayed in the presence of 0·3 M NH_4Cl and added *E. coli* RNA polymerase. The paradoxical stimulation in [^{3}H]-UMP incorporation seen after cortisol treatment was therefore explained on the basis that the presence of less endogenous RNA polymerase might allow greater access of the more efficient *E. coli* RNA polymerase to the template. These results are compatible with the suggestion that cortisol may inhibit thymic RNA synthesis by decreasing the amount, availability or activity of thymic RNA polymerase rather than by an effect on template activity.

AN EARLIER report[1] characterized the inhibitory effects of cortisol injected *in vivo* on the cell-free RNA polymerase system (aggregate enzyme) isolated from rat thymus glands. That study indicated that cortisol inhibits the final polymerization of nucleoside triphosphates into RNA and that this effect is not the result of decreased availability of nucleoside triphosphates. Similar results have been obtained by Nakagawa and White.[2] These investigators also showed that cortisol did not alter either acid or alkaline RNase activity in rat thymus.[3] Since all of these reports dealt with aggregate enzyme, a preparation containing both the repressed template complex and endogenous RNA polymerase, the data obtained did not permit a choice between the two possible sites for steroid action, i.e. the DNA-RNA-protein template complex or the enzyme RNA polymerase.

Data will be presented in this report suggesting that cortisol inhibits thymic DNA-dependent RNA synthesis by reducing the amount, availability or activity of the enzyme RNA polymerase rather than altering chromatin template activity.

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METHODS

Preparation of aggregate enzyme and chromatin. Male Sprague–Dawley rats, 80–100 g at the time of sacrifice, were adrenalectomized on arrival and maintained in our animal quarters for 3 days prior to use. Standard laboratory diet and 1% NaCl in the drinking water were available *ad lib.* to the time of sacrifice.

Rats were injected intramuscularly with cortisol (50 mg/kg) or an equal amount of the appropriate vehicle. Twelve hr later they were sacrificed by decapitation and the thymus glands rapidly excised. This, and all subsequent operations, were carried out in the cold (2°–4°). Intact nuclei and aggregate enzyme were isolated by methods previously described;[1] chromatin was prepared according to the method of Marushige and Bonner.[4] Assays on all preparations were completed within 4 days after isolation. Chemical assays of chromatin and aggregate enzyme were performed as previously described.[1]

Incorporation of [^3H]-UMP. The incubation system for assay of [^{3}H]-UMP incorporation into RNA, using chromatin as template, was carried out as described by Chamberlin and Berg.[5] The assay medium contained 10 μmoles tris–HCl, pH 8·0; 1 μmole $MgCl_2$; 0·25 μmole $MnCl_2$; 3 μmoles 2-mercaptoethanol; 0·1 μmole ATP, GTP and CTP; 0·1 μmole UTP containing 5 μc of [^{3}H]-UTP; RNA polymerase in the amount of 5 units unless otherwise stated and chromatin. The final volume of the assay was 0·25 ml and the ionic strength was 0·045. This system was also used for the assay of extractable thymic RNA polymerase using excess purified DNA as template. *E. coli* RNA polymerase obtained from Biopolymers Research, Inc. had a reported specific activity of 2000 units per milligram. When aggregate enzyme was used for [^{3}H]-UMP incorporation, the incubation system was essentially that used by Goldberg.[6] The assay medium contained 50 μmoles tris–HCl, pH 8·0; 1·5 μmoles $MnCl_2$; 10 μmoles NaF; 5 μmoles 2-mercaptoethylamine; 1 μmole ATP, CTP and GTP; 0·003 μmole UTP containing 5 μc of [^{3}H]-UTP and aggregate enzyme. The final volume was 0·50 ml. Ionic strength in the aggregate enzyme assay system was controlled by the addition of NH_4Cl. No NH_4Cl was added for what is termed "low ionic strength (0·094)" assay, and NH_4Cl was added to a final concentration of 0·75 M for what is termed "high ionic strength (0·844)" assay. In all cases, replicate samples were prepared and incubated at 37° for 15 min. Un-incubated zero-time samples were included. Incubations were terminated by addition of 2 ml cold 6% trichloroacetic acid and the precipitate was then washed and dissolved in 0·5 ml NCS or Soluene solubilizer and assayed for tritium activity in a Tri-Carb liquid scintillation spectrometer. An automatic External Standard System was used to make any necessary corrections for chemical quenching.

Incorporation of [^{3}H]-UMP for both systems was shown to proceed in a linear fashion for at least 15 min and to depend upon the amount of aggregate enzyme or chromatin added (up to 80 μg DNA per incubation tube for aggregate enzyme assayed in the Goldberg system,[6] and up to 8 μg DNA per incubation tube for either chromatin or purified DNA assayed in the Chamberlin–Berg system).[5] All assays of aggregate enzyme activity were done with limiting amounts of the aggregate complex present. Assays using chromatin as template were performed so as to include both template-limiting and enzyme-limiting conditions.

Partial hepatectomies (70 per cent removal) were performed in 90-g male Sprague–Dawley rats, according to the procedure described by Higgins and Anderson.[7] In

addition, sham operations were performed by opening the abdomen and exposing the liver in the same manner used prior to ligation of the lobes in the partially hepatectomized rats. After this manipulation, the livers were inserted back into the peritoneum and the abdomen sutured.

These partially hepatectomized and sham-operated animals were maintained on standard laboratory diet and 20% dextrose in the drinking water until time of sacrifice.

Crude-soluble thymus RNA polymerase was prepared from the thymus glands of male Sprague–Dawley rats, according to the method of Jacob *et al.*[8] The various modifications of this method are indicated in the Tables.

RESULTS

The characteristics of the rat thymus chromatin [^{3}H]-UMP incorporation system were similar to those previously shown for aggregate enzyme.[1] These systems require the presence of all four nucleoside triphosphates for maximum activity. In the case of chromatin, incorporation of [^{3}H]-UMP into RNA was reduced to 21 per cent of control in the absence of ATP, 32 per cent in the absence of CTP and 31 per cent in the absence of GTP. Actinomycin D (5 μg) inhibited incorporation by 95 per cent. Chromatin preparations did not incorporate labeled UMP into RNA unless RNA polymerase was added to the incubation system, indicating that the purification procedures for chromatin isolation had removed most, if not all, of the endogenous RNA polymerase activity. The bacterial enzyme did not incorporate measurable amounts of [^{3}H]-UMP when template was omitted from the incubation mixture.

Thymus chromatin template activity from cortisol-treated and control rats was compared over a wide range in the ratio of RNA polymerase to DNA by varying the amount of DNA (0·2 to 16 μg) in the presence of 5 units (2·5 μg) of added *E. coli* RNA polymerase. As can be seen in Fig. 1, this range in the ratio (RNA polymerase/DNA)

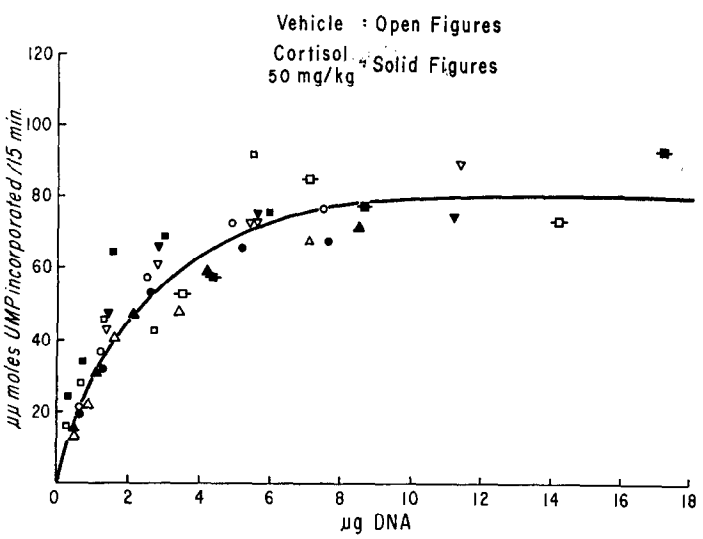

FIG. 1. Effects of cortisol on rat thymus chromatin template activity (prepared according to the procedure of Marushige and Bonner).[4] Results are shown from five separate experiments. Each point represents the average of triplicate determinations for chromatin preparations, each of which was obtained from pooled thymus glands of six rats. Standard error of the mean for replicate assays was always less than 6 per cent of the mean value.

covers conditions where RNA polymerase is limiting, as well as conditions where the amount of incorporation reflects the amount of template added. No significant differences because of cortisol treatment were observed under the conditions studied.

An additional experiment on rat thymus chromatin template activity was performed, using slightly different conditions as described by Dahmus and Bonner[9] for the isolation of chromatin. Similar results were obtained (Fig. 2).

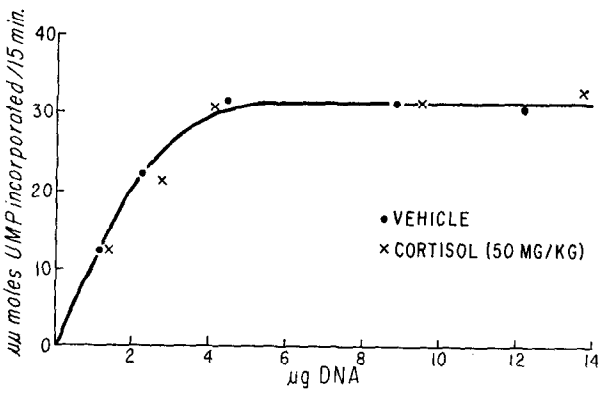

FIG. 2. Effects of cortisol on rat thymus chromatin template activity (prepared according to the procedure of Dahmus and Bonner).[9] Each point represents the average of triplicate determinations for a single experiment where each chromatin preparation was obtained from pooled thymus glands of six rats. The standard error of the mean for replicate assays was always less than 4 per cent of the mean values.

Note: Less RNA polymerase (1·25 units) was used in these assays, accounting for the lower maximum rate of incorporation obtained and the lower amount of DNA required to bind all the enzyme, in comparison to the results reported in Fig. 1.

Assay of chromatin template activity for normal and regenerating rat liver (Table 1) provided evidence that changes could be detected when they did occur. Chromatin template from regenerating liver was found to be 1·7–1·9 times more active than chromatin template obtained from sham-operated animals. These results agreed with those of Thaler and Villee.[10]

The effect of exogenous *E. coli* RNA polymerase on the cortisol-induced inhibition of RNA synthesis by aggregate enzyme was also studied. Table 2 shows the effects of

TABLE 1. EFFECT OF PARTIAL HEPATECTOMY ON ACTIVITY OF RAT LIVER CHROMATIN*

Treatment	DNA (μg)	Incorporated/μg DNA† (μμmoles UMP)	Hepatectomized/ sham-operated
Sham-operated	4·74	15 ± 1	
Hepatectomized	4·24	25 ± 1	1·7
Sham-operated	2·36	19 ± 1	
Hepatectomized	2·12	36 ± 1	1·9

* Twenty-four hr post-operative.

† Results are shown for a single experiment. Each value represents the mean ± standard error of triplicate assays on chromatin preparations obtained from pooled livers from six rats in the case of sham-operation and from nine rats in the case of partial hepatectomy.

cortisol on aggregate enzyme when assayed at both high (0·75 M NH_4Cl) and low (no NH_4Cl) ionic strength. The cortisol-induced inhibition was found to be somewhat less, but still demonstrable in the low ionic strength system. When equal amounts of *E. coli* RNA polymerase were added to the low ionic strength system, [^{3}H]-UMP incorporation by aggregate preparations from both control and steroid-treated rats increased markedly to the same value and the steroid-induced inhibition disappeared.

Figure 3 shows the effects of ionic strength on [^{3}H]-UMP incorporation by aggregate enzyme from thymus glands of control or cortisol-treated rats when assayed in

TABLE 2. REVERSAL OF CORTISOL-INDUCED INHIBITION OF AGGREGATE ENZYME BY *E. Coli* RNA POLYMERASE

Treatment	Ionic strength	RNA polymerase	Incorporation ($\mu\mu$moles UMP/mg DNA)*	Per cent of appropriate control	P†
Vehicle	High	0	32·8 ± 3·2		
Steroid	High	0	16·7 ± 1·5	51	< 0·02
Vehicle	Low	0	24·2 ± 1·3		
Steroid	Low	0	15·8 ± 0·9	65	< 0·02
Vehicle	Low	5 units	185·0 ± 19·0		
Steroid	Low	5 units	207·0 ± 27·0	111	> 0·50

* Results are the mean ± standard error for six separate experiments. In each experiment individual aggregate enzyme preparations were isolated from pooled thymus glands of 6 rats. The amount of UMP incorporated by each preparation was taken as the average of triplicate assays. The standard error of the mean for replicate assays was always less than 5 per cent of the mean value.

† Student's *t*-test (two-tail).

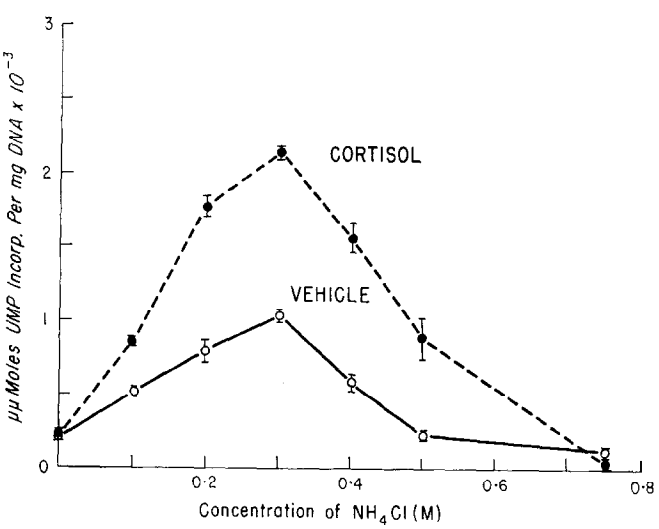

FIG. 3. Effect of NH_4Cl on the incorporation of [^{3}H]-UMP by aggregate enzyme prepared from thymus glands of vehicle- and cortisol- (50 mg/kg) treated rats assayed in the presence of 10 units of *E. coli* RNA polymerase. Assays were performed using the Goldberg system (Methods).[6] The bars indicate the standard error of the mean of 3 essays.

the presence of 10 units of bacterial enzyme. Data obtained in the absence of NH_4Cl confirmed those given in Table 2. As the concentration of NH_4Cl in the assay system was increased, however, aggregate enzyme from thymus of cortisol-treated rats became more active in making RNA than the identical preparation from control rats. This paradoxical stimulation of [^{3}H]-UMP incorporation by aggregate enzyme from cortisol-treated animals was maximal when NH_4Cl was present in the assay system at a final concentration of 0·3 M.

The effect of varying the amount of *E. coli* RNA polymerase was studied in the presence of 0·3 M NH_4Cl and the results given in Table 3. In the range of 0–6 units of added *E. coli* RNA polymerase, the amount of stimulation (steroid–vehicle ratio) increased rapidly. At levels above 6 units of *E. coli* RNA polymerase, the increase in the amount of stimulation occurred more slowly, reaching an asymptotic value of 2·5–2·6.

TABLE 3. EFFECT OF VARYING THE AMOUNT OF *E. coli* RNA POLYMERASE ON THE INCORPORATION OF UMP BY AGGREGATE ENZYME PREPARED FROM THYMUS GLANDS OF VEHICLE- AND CORTISOL-TREATED RATS*

Units of *E. coli* RNA polymerase	μμmoles UMP incorporated/mg DNA† Vehicle	Steroid	Steroid/vehicle
0	127 ± 1	71 ± 3	0·59
1·2	162 ± 8	178 ± 9	1·15
2·4	221 ± 19	322 ± 7	1·53
6·0	425 ± 4	882 ± 16	2·19
12·0	856 ± 23	2045 ± 44	2·52
24·0	1870 ± 59	4731 ± 142	2·66

* Rats were injected intramuscularly 12 hr prior to sacrifice with cortisol (50 mg/kg) or an equivalent volume of vehicle. Aggregate enzyme prepared in the usual way was assayed in the Goldberg system[6] (Methods) with addition of varying amounts of *E. coli* RNA polymerase to the assay tube. All assays were carried out in the presence of 0·3 M NH_4Cl.

† Mean of three assays ± standard error.

Addition of large amounts (50 units) of the bacterial enzyme to the incubation system also caused this "paradoxical stimulation" in the absence of NH_4Cl. Under these conditions the aggregate enzyme preparation from thymus of steroid-treated animals incorporated 1·5 times as much [^{3}H]-UMP as did control preparations.

Kinetic data for [^{3}H]-UMP incorporation by aggregate enzyme obtained from control rats in the presence of 5 units of *E. coli* RNA polymerase with no NH_4Cl present and with NH_4Cl at a final concentration of 0·3 M are shown in Fig. 4.

Recent developments in techniques for the extraction of soluble RNA polymerase from mammalian cells provided still another test system for cortisol action. Table 4 shows the effects of cortisol treatment *in vivo* on [^{3}H]-UMP incorporation by intact thymus nuclei and on the level of soluble RNA polymerase activity extractable from this system under controlled conditions. Washed nuclei from thymus of both cortisol-treated and control rats were divided into three aliquots. One aliquot was assayed directly and the other two aliquots subjected to extraction procedures. Two different extraction procedures were used (see legend, Table 4). The extraction procedure used

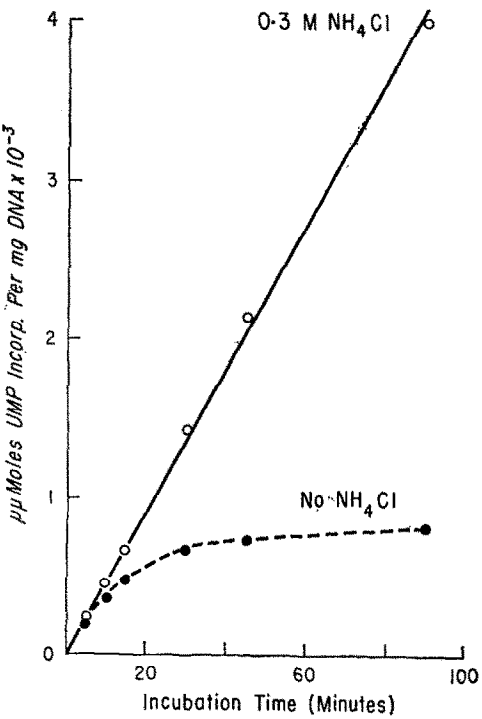

FIG. 4. Effect of ionic strength on time course of [^{3}H]-UMP incorporation by aggregate enzyme from control rats assayed in the presence of 5 units of *E. coli* RNA polymerase. Assays were performed using the Goldberg system (Methods).[6] Each point is the mean of triplicate assays. The standard error of the mean was always less than 5 per cent of each mean value.

to obtain preparation "Soluble-1" removed 50 per cent of the [^{3}H]-UMP incorporating activity from the pellet obtained from lysed nuclei after centrifuging (190,000 *g* for 25 min), whereas the extraction procedure used to obtain preparation "Soluble-2" removed 70 per cent of the activity from this pellet material.

In both instances, however, the extractable activity (as measured by [^{3}H]-UMP incorporation in the presence of an excess of purified calf thymus DNA) was less for preparations obtained from cortisol-treated animals, compared with controls. The magnitude of the effect was comparable to the cortisol-induced reduction in [^{3}H]-UMP incorporation activity seen with whole nuclei.

Data were also obtained showing that a decrease in the amount of endogenous RNA polymerase in a nuclear lysate pellet (a preparation analogous to aggregate enzyme) could lead to an increase in [^{3}H]-UMP incorporation when the aggregate preparation was assayed in the presence of added *E. coli* RNA polymerase (Table 5). In these experiments the endogenous RNA polymerase levels were decreased by differential extraction at various temperatures under standard conditions.

DISCUSSION

According to Marushige and Bonner,[4] repressed DNA template (chromatin) can be isolated free of its endogenous RNA polymerase and its activity assayed *in vitro*. The purified chromatin template isolated in this laboratory lacked the ability to

TABLE 4. EFFECT OF CORTISOL TREATMENT *in vivo* ON NUCLEAR AND SOLUBLE RAT THYMUS RNA POLYMERASE ACTIVITY

Treatment*	Preparation†	$\mu\mu$moles UMP incorporated per mg of nuclear DNA‡	Per cent of vehicle
Vehicle	Nuclei	40·6 ± 3·7	
Cortisol	Nuclei	14·8 ± 0·4	36
Vehicle	Soluble-1	84·7 ± 0·4	
Cortisol	Soluble-1	36·9 ± 0·6	44
Vehicle	Soluble-2	141·0 ± 2·2	
Cortisol	Soluble-2	45·8 ± 1·7	32

* Rats were injected intramuscularly with vehicle or cortisol (50 mg/kg) 12 hr prior to sacrifice.

† Two different extraction procedures were used to obtain preparations of soluble RNA polymerase. Preparation "Soluble-1" was extracted in 0·05 M tris buffer, pH = 9·1, containing 0·025 M KCl, 0·004 M magnesium acetate, and 0·005 M dithiothreitol for 10 min at 25°; 50 per cent of the initial nuclear activity remained in the pellet after this extraction procedure. Preparation "Soluble-2" was extracted in 0·05 M tris buffer, pH = 8·0, containing 0·025 M KCl, 0·1 M NH_4Cl and 0·005 M dithiothreitol for 10 min at 25°; 30 per cent of the initial nuclear activity remained in the pellet after this extraction procedure.

‡ Soluble RNA polymerase extracts were assayed using the Chamberlain–Berg system[5] (Methods) in the presence of excess purified calf thymus DNA (50 μg per incubation tube). The values reported were calculated on the basis of the amount of nuclear DNA from which they were derived rather than on the basis of the excess DNA added to the incubation tube. These values were corrected for a small amount (< 10 per cent) of incorporation detected when calf thymus DNA was omitted from the incubation mixture. Nuclei were assayed in the same system without added DNA. All values are means of triplicate assays ± standard error.

incorporate measurable amounts of [^{3}H]-UMP into RNA unless RNA polymerase was added to the incubation mixture. In addition, Marushige and Bonner[4] showed that the concentrations of template required to reach one-half maximum rate of RNA synthesis in the presence of a fixed amount of RNA polymerase were similar for rat liver chromatin and purified DNA. Their data also indicated that the ratio of rat liver chromatin template activity to purified rat DNA template activity was the same (approximately 0·20) regardless of the ratio of RNA polymerase to DNA in the assay vessel. Similar data were obtained in our laboratory for rat thymus chromatin except that the ratio of thymus chromatin activity to purified DNA was about 0·05. These findings suggest that excess RNA polymerase is not required for the assay of template activity.

Although no changes in maximum template activity, as measured with *E. coli* RNA polymerase, were detected after cortisol treatment, it is still possible that cortisol might cause an alteration in template activity which could not be detected by the bacterial enzyme but which would be detected by thymus RNA polymerase. Smith *et al.*[11] have concluded that within the limits of sensitivity of the hybridization assay the specificity of transcription is independent of the source of RNA polymerase (mammalian or bacterial). They did note, however, some very slight but consistent differences in the shapes of hybridization competition curves in experiments for RNA made from rat liver chromatin, using the bacterial and mammalian enzyme, and suggested that these slight differences might reflect minor distortions in the relative proportions of the

various RNA molecules synthesized by the two enzymes. The differences were minor, however, and the suggestion only tentative.

The lack of demonstrable effect on thymus template activity after treatment with cortisol led us back to the examination of the aggregate enzyme preparation. It was reasoned that if template activity was not affected by cortisol and the steroid effect seen in the aggregate enzyme system was not because of decreased availability of nucleoside triphosphates[1] or a stimulation of RNase activity,[3] the cortisol-induced inhibition might be because of a reduced amount, availability or activity of endogenous RNA polymerase and therefore might be reversed by the addition of exogenous RNA polymerase to the incubation system.

Since high ionic strength (0·5 M KCl) was known to cause dissociation of *E. coli* RNA polymerase into sub-units and to prevent its binding to DNA template,[12,13] it was decided to omit NH_4Cl from the aggregate enzyme assay system for the initial reversal experiments. Even so, the ionic strength used for these experiments ($T/2 = 0{\cdot}094$) was higher than that used in the chromatin assay system ($T/2 = 0{\cdot}04$). Table 2 shows both [^{3}H]-UMP incorporation and the steroid-induced inhibition were somewhat less but still demonstrable when assayed at low ionic strength instead of high ionic strength. When *E. coli* RNA polymerase (5 units) was added to the low ionic strength preparation, [^{3}H]-UMP incorporation increased in aggregate preparations obtained from both control and cortisol-treated rats and the cortisol inhibition disappeared.

Multiple effects of ionic strength on *E. coli* RNA polymerase and RNA synthesis *in vitro* by this enzyme have been reviewed by several investigators.[14–16] Because ionic strength plays such an important role in cell-free RNA synthesis, the reversal experiments described above were also carried out with various concentrations of NH_4Cl present in the incubation medium. When the ionic strength was increased, unexpected results were obtained: Aggregate preparations obtained from thymus glands of cortisol-treated rats proved to be more active in RNA synthesis than similar preparations obtained from control animals *when assayed in the presence of added E. coli RNA polymerase* (Fig. 3). This paradoxical stimulation of [^{3}H]-UMP was maximal in the presence of 0·3 M NH_4Cl.

The data in Table 3 indicate that the magnitude of the stimulation seen with added bacterial enzyme is dependent upon the amount of RNA polymerase added. The ratio of steroid–control increases rapidly from 0·59 to 2·19 in the range of 0–6 units of added RNA polymerase. Presumably in this range the system is shifting from conditions where most of the RNA synthesized is made by the mammalian enzyme to conditions where the bulk of the RNA synthesized is made by the bacterial enzyme. As more bacterial enzyme is added (range 6–24 units), the ratio steroid/control increases more slowly and appears to reach an asymptote of about 2·5–2·6. Under these conditions, it can be assumed that the rate of RNA synthesis is solely limited by the probability of initiation by the bacterial enzyme.

The only way to reconcile these results with our earlier suggestion that cortisol may inhibit RNA synthesis by reducing the amount, availability or activity of endogenous RNA polymerase is to assume (1) that the bacterial enzyme is more efficient in making RNA than the mammalian enzyme, and (2) that the presence of mammalian enzyme limits access of the bacterial enzyme to the template. Since aggregate enzyme from thymus of steroid-treated rats is postulated to contain less endogenous enzyme, it

TABLE 5. EFFECT OF *E. coli* RNA POLYMERASE ON THE RNA POLYMERASE ACTIVITY OF RAT THYMUS NUCLEAR LYSATES PREINCUBATED AT DIFFERENT TEMPERATURES*

Preincubation temperature	$\mu\mu$moles UMP incorporated per mg DNA† Assayed with 0·75 M NH_4Cl	Assayed with 0·3 M NH_4Cl	Assayed with 0·3 M NH_4Cl and *E. coli* RNA polymerase‡
0°	140 ± 10	68 ± 2	509 ± 11
25°	95 ± 5	37 ± 4	838 ± 10
37°	42 ± 2	16 ± 1	969 ± 23

* Rat thymus nuclei were lysed in buffer containing 0·05 M tris–HCl, pH 8·0; 0·025 M KCl; 0·1 M NH_4Cl and 0·005 M dithiothreitol. The lysates were incubated at the temperatures stated for 60 min, then centrifuged at 190,000 *g* for 25 min. The pellets were suspended in 0·05 M tris–HCl, pH 8·0, by homogenization and centrifuged as before. The washed pellets were resuspended in the tris buffer and filtered through six layers of gauze. The filtered suspensions were assayed for RNA polymerase activity.

† Assays were performed in the Goldberg system (Methods).[6] Values are expressed as the mean of three assays ± standard error.

‡ Five units of *E. coli* RNA polymerase were used per assay.

would then be expected to be more active in the presence of added bacterial RNA polymerase. That this effect was not seen, or seen to a much lesser degree at very low ionic strength (no NH_4Cl added), might be explained by nonspecific (ineffective) binding of a large amount of the added RNA polymerase.

Incorporation of [^{3}H]-UMP by control aggregate enzyme assayed in the presence of added *E. coli* RNA polymerase was linear for at least 90 min when the assay was carried out in the presence of 0·3 M NH_4Cl (Fig. 4). In contrast, when NH_4Cl was omitted from the incubation mixture, incorporation became progressively slower with time so that essentially no [^{3}H]-UMP was incorporated after 30 min. These data are consistent with the suggestion that the termination process in RNA synthesis is dependent on ionic strength and does not occur at low ionic strength.[15,17]

To further test the hypothesis that the endogenous thymus RNA polymerase may be decreased in amount, availability or activity by cortisol, RNA polymerase was extracted from nuclei isolated from thymus glands of cortisol-treated or control animals and assayed in the presence of excess DNA (Table 4). Less RNA polymerase could be extracted by two separate procedures when nuclei were obtained from thymus glands 12 hr after cortisol treatment. Since the inhibition of RNA synthesis by aggregate enzyme or whole isolated nuclei increases progressively with time up to 12 hr after cortisol injection,[1,2] and the degree of inhibition in these systems agrees closely with the magnitude of the decrease in extractable RNA polymerase at 12 hr, it is suggested that this effect reflects events occurring at earlier times after treatment. The observed decrease in extractable nuclear RNA polymerase could be the result of decreased enzyme synthesis, increased enzyme degradation, alterations in existing enzyme leading to decreased activity, or loss of nuclear RNA polymerase to the cytoplasm because of decreased binding in the nucleus (this last possibility is under current study in our laboratory).

The methods for extraction of RNA polymerase also provided an experimental approach to test the assumption stated earlier that the amount of endogenous mammalian enzyme present in the aggregate enzyme preparation limits the access of the

bacterial enzyme to template DNA. If the assumption were true, then an aggregate enzyme preparation from control animals, assayed in the presence of added bacterial RNA polymerase, should be more active after its endogenous mammalian enzyme has been extracted. Table 5 provides data where varying amounts of the endogenous RNA polymerase have been extracted from an aggregate enzyme-like preparation showing that the ability of the system to incorporate [^{3}H]-UMP increases as the content of endogenous enzyme is depleted. These data give support to our two assumptions used to explain the paradoxical stimulation of RNA synthesis by thymus aggregate enzyme when assayed in the presence of added bacterial enzyme.

While the results in this report do not completely rule out an effect of cortisol on thymus template activity, they are consistent with the suggestion that RNA polymerase or the process of its synthesis or degradation may be the site of cortisol action. It is not presently possible to speculate further on the exact mechanisms involved since so little is known about the mammalian RNA polymerase. The bacterial enzyme, however, has been shown to be a complex containing several different peptides,[18] and evidence already exists[12,13,19,20] that purified preparations of *E. coli* RNA polymerase can exhibit heterogeneity with respect to DNA template binding. Burgess *et al.*[18] have demonstrated that a protein component usually associated with *E. coli* RNA polymerase can be separated from the enzyme by chromatography and that the resultant deficient polymerase is unable to transcribe the T4 DNA and has a markedly reduced ability to transcribe calf thymus DNA and ΦX174 DNA unless the factor is added back. It can only be assumed at present that the mammalian enzyme is even more complex and perhaps more susceptible to modification.

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