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Studies on the Effect of Cortisone on Rat Liver Transfer Ribonucleic Acid*

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ABSTRACT: The effect of cortisone on the population of rat liver transfer ribonucleic acid and aminoacyl transfer ribonucleic acid synthetases was studied. The 20–30% increase in labeling of transfer ribonucleic acid, following cortisone administration, seemed to represent a general increase in most or all types of rat liver transfer ribonucleic acid, rather than a preferential increase in the synthesis of a few transfer ribonucleic acids. No gross differences in relative abundance of amino acid specific transfer ribonucleic acids were apparent in assays of amino acid acceptance capacities of transfer ribonucleic acid obtained from control and cortisone-injected rats. Cortisone did not produce detectable changes in the activities of liver aminoacyl transfer ribonucleic acid synthetases, nor did the hormone form a stable complex with liver transfer ribonucleic acid *in vitro* or *in vivo*. In addition, no changes were found in the isoaccepting species of leucine transfer ribonucleic acid or tyrosine transfer ribonucleic acid, after cortisone administration.

Among the early and most pronounced effects of glucocorticoids is the induction of several hepatic enzyme activities (Knox, 1951) which is due to hormonal stimulation in the rate of their biogenesis (Kenney, 1962; Schimke *et al.*, 1965a,b) leading to elevated levels of these enzyme proteins (Feigelson, and Greengard, 1962; Kenney, 1962) in the hepatic cytosol. Although previous reports have attempted to elucidate the site of action of glucocorticoid hormones at both the translational (Tomkins *et al.*, 1965; Kenney and Albritton, 1965) and the transcriptional (Feigelson and Feigelson, 1965; Koide, 1969) level, the primary target of these hormones remains unknown. Results from several laboratories in the last several years have suggested strongly that increased enzyme synthesis in response to corticosteroids in mammalian liver is secondary to the hormonal stimulation of RNA synthesis (Greengard and Acs, 1962; Kenney and Kull, 1963; Yu and Feigelson, 1969a; Feigelson and Hanoune, 1969). With ^{32}P as a precursor, a two–threefold stimulation in the *de novo* synthesis of all RNA species, including tRNA, has been reported to occur during the first 3–4 hr after injection of corticosteroids (Feigelson *et al.*, 1962; Wicks *et al.*, 1965). This stimulation is also apparent, though less pronounced,

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if radioactive orotic acid is used as the precursor of RNA synthesis (Greenman *et al.*, 1965; Feigelson and Feigelson, 1965). Increased synthesis of tRNA has also been described in the chick oviduct after estrogen administration (O'Malley *et al.*, 1968; Dingman *et al.*, 1969). Furthermore, qualitative differences in tRNA population have been reported to occur in soybean seedling following the administration of 6-benzyladenine, a synthetic plant hormone (Anderson and Cherry, 1969).

The central role of tRNA in translation suggests that changes in the abundance and specificity of individual tRNAs might play an important role in metabolic regulation and hormone action. Alterations in the tRNA population have been demonstrated in bacteria after phage infection, sporulation, and as a consequence of changes in the growth media. Chromatographic differences in the tRNA profile of mammalian cells have been described after Herpes virus infection, in hamster cells transformed with adenovirus 7 or SV 40 virus, during chemical carcinogenesis, and in certain tumors. Such studies suggest that hormones, too, might exert metabolic regulation *via* changes in the tRNA population, thereby altering the capacity of the translational apparatus to translate specific mRNAs. (For a review of this subject, see Baliga *et al.*, 1969, and Weinstein, 1969).

In view of the above considerations, it seemed important to examine in greater detail the effect of cortisone on the population of liver tRNAs. Evidence is presented that cortisone does increase the synthesis of liver tRNA, and that this represents a general increase in most or all types of liver tRNA, rather than a preferential increase in the synthesis or activity of a few tRNAs. Evidence is also presented that cortisone does not produce detectable changes in the activities of liver aminoacyl-tRNA synthetases, nor does the hormone form a stable complex with liver tRNA *in vitro* and *in vivo*.

Materials

[6-^{14}C]Orotic acid (35 mCi/mmole) and [5-^{3}H]-orotic acid (16 Ci/mmole) were purchased from Schwarz BioResearch, Inc., Orangeburg, N. Y. Radioactive [^{14}C]amino acids were obtained from the same company and had the following specific activities in millicuries per millimole: phenylalanine (530), tyrosine (450), proline (262), alanine (250), threonine (120), leucine (170), serine (89), aspartic acid (111), valine (80), histidine (186), tryptophan (200), glycine (66), arginine (240), glutamic acid (167), and lysine (200). [1,2-^{3}H]cortisone (34.4 Ci/mmole) was obtained from New England Nuclear Corp., Boston, Mass., and [1,2-^{3}H]cortisol (6.8 mCi/mmole from Schwarz BioResearch. BD-cellulose,[1] lot 6802, was obtained from Schwarz BioResearch. Chromosorb W (dimethyldichlorosilane treated and acid washed, 100–200 mesh) was purchased from the Celite Division, Johns-Manville Products Corp. Dimethyldilaurylammonium chloride (Aliquat 204) was a gift from the Chemical Division of General Mills, Inc., Kankakee, Ill. Tetrachlorotetrafluoropropane (Freon 214) was obtained from E. I. Dupont de Nemours and Co., Inc., Wilmington, Del. Phenol (Mallinckrodt) was analytical reagent grade and Na_2ATP was a product of P-L Biochemicals. Cortisone acetate suspension was a preparation from the Upjohn Co., Kalamazoo, Mich.

Methods

Preparation of Rat Liver tRNA. Male, albino Holtzman rats (*ca.* 150 g) were starved overnight and injected intraperitoneally with either saline or cortisone acetate (5 mg/100 g body weight). The animals were killed by decapitation 3 or 4 hr after the hormone injection. The livers were rapidly excised, chilled, weighed, and homogenized in 1 volume (v/w) of cold buffer (30 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 80 mM KCl, 50 mM NaCl, and 0.44 M sucrose) using a Potter-Elvehjem Teflon homogenizer. The homogenate was centrifuged at 15,000*g* for 15 min and the supernatant fraction was recentrifuged at 105,000*g* for 2 hr at 4°. The RNA was isolated from the supernatant fraction following two extractions with an equal volume of water-saturated phenol, and then precipitated by addition of 0.1 volume of 2 M sodium acetate (pH 4.5), and 2 volumes of cold ethanol. After standing overnight at −20°, the tRNA precipitate was collected and resuspended in a small volume of water and in certain cases further purified by sucrose gradient centrifugation as follows: the tRNA solution (0.5–1.0 ml) was layered over 30 ml of a linear sucrose gradient (5–20% w/v), prepared in 1 mM Tris-HCl (pH 7.6) and 50 mM KCl, and centrifuged at 4° for 16 hr at 24,000 rpm in the SW25.1 rotor of a Spinco ultracentrifuge Model L2-65B. The fractions corresponding to the 4S peak were pooled and precipitated with sodium acetate and ethanol.

Assay of Amino Acid Acceptor Activity of tRNA. The procedures for preparing crude rat liver aminoacyl-tRNA synthetase and for determining the amino acid acceptor activity of rat liver tRNA have been previously described (Nishimura and Weinstein, 1969).

Preparation of Aminoacyl-tRNA. For preparing larger amounts of aminoacylated tRNAs obtained from control or cortisone-injected rats, the usual 0.1-ml assay system (Nishimura and Weinstein, 1969) was increased tenfold. The reaction mixture was incubated at 37° for 10 min, diluted with an equal volume (1 ml) of water, and then immediately extracted with 2 ml of phenol. After separation by centrifugation, the aqueous phase was carefully removed and the phenol layer was extracted with an equal volume of water. The combined aqueous layers were reextracted with phenol and the charged tRNA was precipitated with sodium acetate and cold ethanol, as described above. The tRNAs obtained from control and cortisone-treated rats, and charged with either [^{14}C]- or [^{3}H]amino acids, were suspended in column buffer, and combined, and the double-labeled tRNA was chromatographed as described below.

Column Chromatography. Reversed-phase Freon columns (Kelmers *et al.*, 1965) were prepared as previously described (Nishimura and Weinstein, 1969). Details on the operation of these columns and the BD-cellulose columns (Gillam *et al.*, 1967) are given in the figure captions.

Determination of Radioactivity. After the addition of 500 μg of carrier DNA the double-labeled samples from benzoylated DEAE-cellulose or reversed-phase Freon columns were precipitated with 5% trichloroacetic acid, deposited on 24-mm membrane filters (0.45 μ pore size), washed with cold 5% trichloroacetic acid followed by 60% ethanol, and

[1] Abbreviation used is: BD-cellulose; benzoylated DEAE-cellulose.

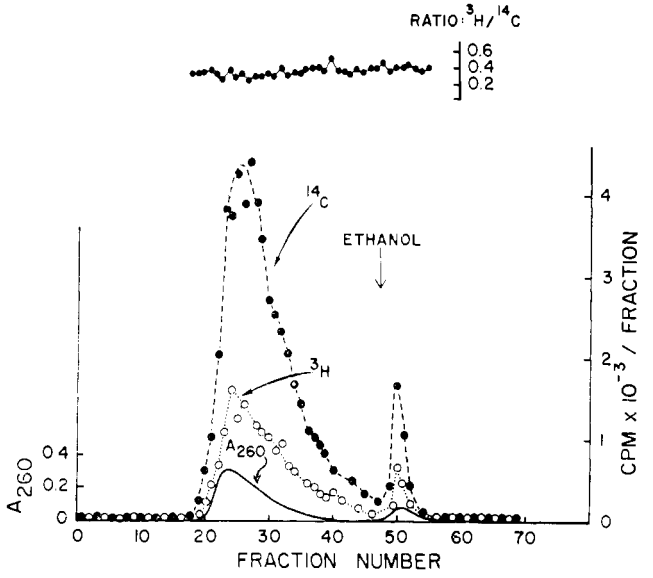

FIGURE 1: Cochromatography of tRNA obtained from control and cortisone-treated rats, labeled *in vivo* with [^{14}C]- and [^{3}H]orotic acid, respectively. A benzoylated DEAE-cellulose column (0.8 × 12 cm) was equilibrated with 0.01 M sodium acetate buffer (pH 4.5), 0.01 M $MgSO_4$, and 0.3 M NaCl. tRNA (4 mg) in 2 ml of water, prepared as described in the text, was applied onto the column. A linear gradient elution was carried out using 100 ml of 0.3 M and 100 ml of 1.3 M NaCl, both buffered as described above. The column was then washed with a linear 5–30% ethanol gradient, in 1.5 M NaCl, 0.01 M sodium acetate (pH 4.5), and 0.01 M $MgSO_4$. Fractions of 4 ml each were collected at a flow rate of 30 ml/hr and processed for determination of radioactivity as described under Materials and Methods.

counted in 10 ml of Bray's solution (Baliga *et al.*, 1969). All radioactivity measurements were made with a Packard TriCarb liquid scintillation counter equipped with automatic external standardization. All activities depicted are corrected for background radioactivity, quenching, and spilling of ^{14}C to ^{3}H window. In the sucrose gradient analysis of binding of corticosteroids to tRNA, aliquots of fractions were either precipitated and collected as described above or added directly to Bray's solution. In the assay of amino acid acceptor activity of tRNA, 0.08-ml aliquots (from 0.1-ml reaction mixtures) were placed on 24-mm Whatman No. 3MM filter papers, washed three times with cold 5% trichloroacetic acid containing 1% Casamino Acids, once with 1:1 (v/v) cold ethanol–ether, and finally with cold ether. These were counted in 10 ml of toluene solution containing 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2(phenyloxozalyl)]benzene.

Results

Table I indicates that as early as 30 min after cortisone administration there was a 28% increase in labeling of tRNA from [^{3}H]orotic acid, when compared were the corresponding control. The increased labeling was also apparent at 3 and 5 hr after cortisone administration. These results are in agreement with earlier studies (Feigelson *et al.*, 1962; Wicks *et al.*, 1965) and it has been previously established that this increased labeling is not due just to labeling of the CCA terminus of tRNA (Greenman *et al.*, 1965). Data in this table also demonstrate that this stimulation was only

TABLE I: Effect of Cortisone on [^{3}H]Orotic Acid Incorporation into Acid-Soluble Pool and tRNA of Rat Liver.[a]

	Incorporation Time		
	30 min	3 hr	5 hr
tRNA			
Control	81	347	413
Cortisone treated	119	474	500
% change	46	36	21
Acid-soluble pool			
Control	8,464	10,488	8,131
Cortisone treated	10,046	10,342	7,219
% change	+18	0	−11

[a] Male, Holtzman rats were injected concurrently with [^{3}H]orotic acid (20 μCi/100 g of body weight) and either cortisone acetate (5 mg/100 g body weight) or saline. They were sacrificed at the indicated times thereafter. The specific activities of tRNA and acid-soluble pool were calculated according to Yu and Feigelson (1969b). Each value is the mean of separate determinations for two to three animals in each group. Units in cpm/A_{260} units.

partially dependent on the increase in the specific activity of the precursors, as judged by the acid-soluble pool. Thus, there is a true augmentation in synthesis of tRNA. The results to be described below were obtained in order to determine whether this stimulation reflects a general increase in all tRNAs, or the preferential synthesis of only certain types of tRNA.

A double-isotope-labeling technique was initially employed to detect any specific alterations in liver tRNA synthesis following cortisone administration. For this purpose, rats injected with either saline or 5 mg of cortisone acetate/100 g body weight 2 hr previously were given [^{14}C]orotic acid (50 μCi/100 g body weight) or [^{3}H]orotic acid (100 μCi/100 g body weight), respectively. Following a 2-hr isotope incorporation, equal weights of liver from animals of each group were extracted with phenol and the tRNA was isolated and purified on a sucrose gradient, as described under Methods. The ^{14}C- and ^{3}H-labeled preparations representing the control and the cortisone-treated animals, respectively, were then combined and cochromatographed on a benzoylated DEAE-cellulose column and fractions were processed for determination of ^{3}H:^{14}C ratio. As is evident by the data in Figure 1 the ^{3}H- and ^{14}C-labeled tRNAs had very similar elution profiles and the ^{3}H:^{14}C ratios were the same in all fractions. The small scatter in this ratio possibly reflects variations between animals since any slight differences noted in Figure 1 were not reproduced when the entire procedure was repeated, but with the isotopes reversed. There was also no significant variation in the ^{3}H:^{14}C ratio when the isotope was given concurrently with cortisone and the animals sacrificed 2 hr later. A similar double-isotope-labeling procedure has been previously employed with *Escherichia coli* tRNA to exclude the possibility of minor variations in the tRNA population associated with induction of β-galacto-

TABLE II: Amino Acid Acceptance Activity of Liver tRNA from Normal and Cortisone-Treated Rats.[a]

Amino Acid	μμmoles of [^{14}C]Amino Acid/mg of tRNA		
	Control	Cortisone Treated	Cortisone: Control
Phenylalanine	160	186	1.16
Tyrosine	181	189	1.04
Proline	312	295	0.94
Alanine	323	347	1.07
Threonine	812	767	0.94
Leucine	124	149	1.20
Serine	445	453	1.01
Aspartic acid	504	531	1.05
Valine	163	185	1.13
Histidine	195	195	1.00
Tryptophan	56	68	1.21
Glycine	874	767	0.87
Arginine	263	231	0.87
Glutamic acid	335	322	0.96
Lysine	177	166	0.93

[a] Three hours after the injection of either saline or cortisone (5 mg/100 g body weight), the tRNA was isolated in the usual manner from the 105,000*g* supernatant fraction, stripped of endogenous amino acids by incubation for 60 min at 37° in 0.5 M Tris-HCl (pH 8.8), and reprecipitated with ethanol. The tRNAs from control and cortisone-treated rats represent pools obtained from five rats in each group. Amino acid acceptance assays were performed on replicate 50- and 100-μg amounts of tRNA from both sources. Within this range the response was approximately linear. Reaction mixtures were incubated at 37° for 10 min, by which time the aminoacylation of tRNA had reached a plateau. Additional details of the assay are described under Materials and Methods. Each value is the mean of the determination done at the two tRNA concentrations, expressed as micromicromoles of [^{14}C]amino acid per milligram of RNA.

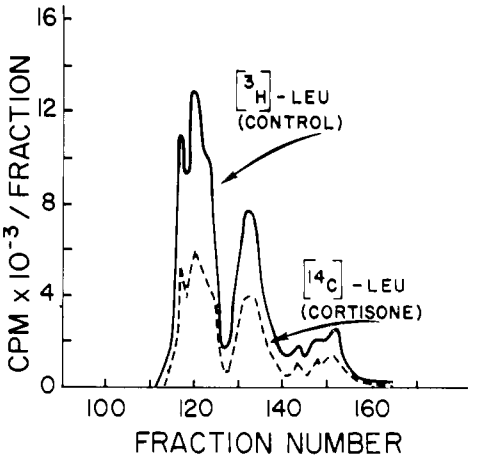

FIGURE 2: Cochromatography of leucyl-tRNA from control and cortisone-treated animals on a reversed-phase freon column. The column (0.5 × 130 cm) was equilibrated with 0.2 M NaCl, 0.01 M $MgCl_2$, 0.001 M EDTA, and 0.01 M sodium acetate (pH 4.5). Equal amounts of tRNA from control and cortisone-treated animals, previously stripped of endogenous amino acids, were charged with [^{3}H]- and [^{14}C]leucine, respectively, and mixed. The mixture (2 mg) was applied to the column and a linear gradient elution was carried out using 500 ml each of 0.2 and 0.6 M NaCl, buffered as described above. Fractions of 6 ml were collected at a flow rate of 20–25 ml/hr and absorbance at 260 mμ was determined. The conditions for aminoacylation and determination of radioactivity are described under Materials and Methods.

sidase (Smith, 1968). As described in Table I, the stimulation of total tRNA synthesis following cortisone administration amounted to 27% at 3 hr. If this increase was confined to one or a few types of tRNA, this probably would have been detected by this procedure, since each type of tRNA is eluted as a rather sharp peak at a characteristic position in the eluate from benzoylated DEAE-cellulose columns (Gillam *et al.*, 1967; Fink *et al.*, 1968; Nishimura and Weinstein, 1969). On the other hand, since there is considerable overlap in the elution of individual tRNAs, particularly in the central region of the major A_{260} peak, this experiment does not exclude the possibility of minor variations in the relative abundance of one or two molecular species of tRNA.

To further examine possible qualitative changes in the tRNA population after cortisone administration, we tested the acceptance capacity for 15 amino acids of tRNA prepared from control rats and from animals sacrificed 3 hr after cortisone injection. The tRNAs were tested at a limiting concentration, *i.e.*, 50 and 100 μg per 0.1-ml assay system, and in the presence of excess enzyme. Samples were incubated for 10 min at 37°, by which time the reaction had reached a plateau. The data in Table II indicate that for 15 amino acids there was no significant difference in amino acid acceptance of the two tRNA preparations. The cortisone:control ratio of aminoacylation for the 15 amino acids had a mean of 1.02 with a range of 1.20–0.87. The small differences in this ratio obtained with leucine, glycine, arginine, tryptophan, and valine (Table II) were not reproduced in replicate experiments.

Thus, preferential changes in the abundance or total acceptance capacity of specific tRNAs are not apparent after cortisone treatment. Although the total acceptance capacities remained unaltered, it is conceivable that changes in isoaccepting species of tRNA have, nevertheless, occurred, such as those reported in bacteria after T_2 phage infection (Sueoka *et al.*, 1966), in rat liver after ethionine feeding (Axel *et al.*, 1967), or in certain plasma cell tumors in mice (Yang and Novelli, 1968; Mushinski and Potter, 1969). To investigate this possibility, $tRNA^{Leu}$ was chosen because leucine is one of the most abundant amino acids in protein and because changes in isoaccepting species of $tRNA^{Leu}$ have been most frequently reported (Sueoka *et al.*, 1966; Axel *et al.*, 1967; Mushinski and Potter, 1969). The tRNAs from control rats, and from rats injected with cortisone 3 hr prior to sacrifice, were charged with [^{3}H]- and [^{14}C]leucine, respectively, and cochromatographed on a reversed-phase Freon column (Figure 2). The elution position and relative abundance of the multiple isoaccepting species were similar in both preparations and gave an essentially constant $^{3}H:^{14}C$ ratio in all fractions. The tRNAs from both sources contained three major leucine tRNA peaks and at least two minor components.

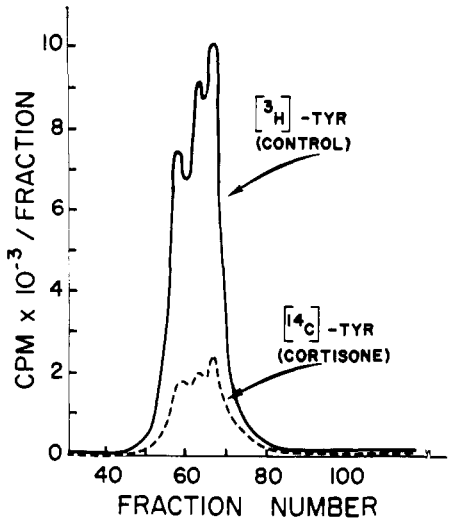

FIGURE 3: Cochromatography of tyrosyl-tRNA from control and cortisone-treated animals on a reversed-phase Freon column. The procedures for aminoacylation, chromatography, and determination of radioactivity were exactly the same as described in the legend for Figure 2 except that labeled tyrosine was used instead of labeled leucine and the elution was carried out with a linear gradient of 0.3–0.6 M NaCl.

The profiles are quite similar to those obtained for normal mouse liver by Mushinski and Potter (1969).

A similar study was carried out with $tRNA^{Tyr}$ because cortisone is known to induce hepatic tyrosine-α-ketoglutarate transaminase and to alter the pool size of liver tyrosine (Betheil *et al.*, 1965). As evident in Figure 3, there was a complete coelution of tyrosine tRNAs from control and cortisone-treated animals.

In the results described thus far aminoacylation of tRNA from either control or cortisone-treated animals was carried out with aminoacyl-tRNA synthetases from control animals. Qualitative variations in synthetases have been reported during sporulation in *Bortryodiplodia theobromae* (Van Etten and Bramble, 1968), during development of sea urchin eggs (Ceccarini *et al.*, 1967), after phage infection in *E. coli* (Chrispeels *et al.*, 1968), between different tissues in rabbits (Strehler *et al.*, 1967), and after hormone treatment in soy bean seedlings (Anderson and Cherry, 1969). In view of these reports, it was important to determine possible differences in aminoacylation when synthetases from cortisone-treated animals were employed instead of synthetases from control animals. These studies were carried out with an excess of tRNA (250 μg/0.1-ml assay system) and a limiting amount of enzyme (350 μg). These data, therefore, are not directly comparable with those in Table II. The results in Table III indicate that the charging of tRNA from cortisone-injected animals was identical for 15 amino acids, when enzymes from either control or cortisone-treated animals was used. It appears, therefore, that major changes in the activities of synthetases for the various amino acids do not ensue after cortisone administration. It also appears that the tRNAs from hormone-injected animals are recognized equally well *in vitro* by synthetases from either source.

Despite the lack of demonstrable qualitative changes in aminoacylation of tRNA, there remained the possibility that the hormone, or its metabolites, might be incorporated into, or interact directly with, liver tRNA, thereby altering other aspects of tRNA function. This was suggested by recent evidence that the plant hormone cytokinin (isopentenyladenosine) is present in specific tRNAs in yeast and mammalian cells (Helgelson, 1968). To explore this possibility, rat liver tRNA was incubated with [^{3}H]cortisol for 30 min at 37°. At the end of this time, the reaction mixture was fractionated on a 5–20% sucrose gradient. Data in Figure 4 reveal that the radioactivity does not bind to 4S tRNA. Furthermore, none of the radioactivity could be precipitated with trichloroacetic acid. To test for possible *in vivo* binding, liver cell sap was obtained from rats 20 min after injection of [^{3}H]cortisone. Liver uptake of cortisone is known to be maximal at this time (Yu and Feigelson, 1969b). The postribosomal supernatant fraction was dialyzed for 1 hr at 4° against 1000 volumes of 0.001 M Tris-HCl (pH 7.5), containing 0.005 M $MgCl_2$, to remove most of the free [^{3}H]cortisone, and applied on a sucrose gradient, as previously described. No evidence for binding to the 4S fraction was obtained

TABLE III: Amino Acid Acceptance Activity of tRNA from Cortisone-Treated Animals Tested with Aminoacyl-tRNA Synthetases from Either Control or Cortisone-Injected Rats.[a]

$\mu\mu$moles of [^{14}C]Amino Acid/mg of Enzyme Protein			
	Synthetase Source		
	Control	Cortisone Treated	Cortisone: Control
Phenylalanine	16	16	1.00
Tyrosine	20	16	0.84
Proline	132	137	1.04
Alanine	259	276	1.06
Threonine	600	619	1.03
Leucine	9	8	0.88
Serine	237	223	0.94
Aspartic acid	160	160	1.00
Valine	342	287	0.84
Histidine	226	247	1.09
Tryptophan	58	68	1.17
Glycine	682	693	1.01
Arginine	13	14	1.07
Glutamic acid	44	50	1.13
Lysine	435	398	0.91

[a] Three hours after the injection of either saline or cortisone (5 mg/100 g body weight), the synthetases were prepared from the 105,000*g* supernatant fraction as described under Methods. Amino acid acceptance assays were performed using replicate 350-μg amount of enzyme protein from either source. The tRNA obtained from cortisone-injected animals was used in 250-μg amounts in all cases. Reaction mixtures were incubated at 37° for 10 min. Under these conditions, the extent of aminoacylation was dependent upon the amount of enzyme added. Each value is the mean of replicate determinations expressed as micromicromoles of [^{14}C]amino acid per milligram of enzyme protein.

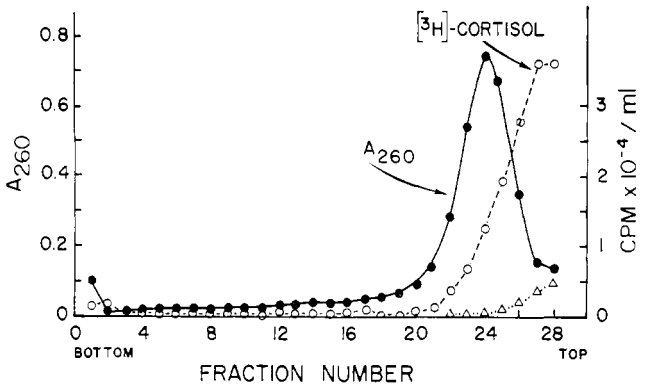

FIGURE 4: Absence of *in vitro* binding of [^{3}H]cortisol to rat liver tRNA. The tRNA (0.5 mg in 1 ml) was incubated with 0.5 μCi of [^{3}H]cortisol at 37° for 30 min, in the presence of 0.005 M $MgCl_2$–0.001 M Tris-HCl (pH 7.4), and 0.4 ml of the reaction mixture was layered on top of a 5–20% sucrose gradient and centrifuged at 39,000 rpm for 5 hr in the SW39 rotor of a Spinco ultracentrifuge, Model L2-65B. Fractions of 8 drops were collected and diluted with 1 ml of water. After determination of the absorbance at 260 mμ, aliquots of each sample were (a) counted directly in Bray's solution (○—○) and (b) precipitated with trichloroacetic acid on membrane filters (△------△).

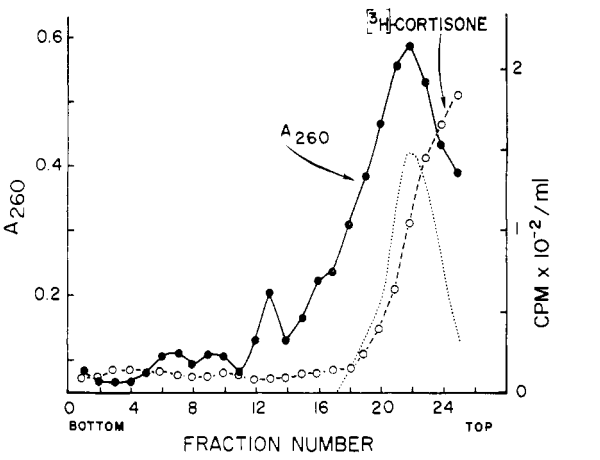

FIGURE 5: Absence of binding of [^{3}H]cortisone to rat liver tRNA *in vivo*. Rats starved overnight were injected with 8 μCi/100 g body weight of [^{3}H]cortisone and 5 mg of carrier cortisone. Twenty minutes later, the animals were sacrificed and an aliquot of the post-mitochondrial supernatant, prepared as described in the text, was layered onto a 5–20% sucrose gradient. Centrifugation and processing of samples were as described in the legend of Figure 4. The position of a marker tRNA was obtained in a parallel run and is shown in dotted lines (· · · · · ·).

(Figure 5). In other experiments, no detectable radioactivity was associated with the tRNA fraction 5 days after a pulse of 50 μCi/100 g body weight of [^{3}H]cortisone.

Discussion

The results described in this report confirm and extend earlier observations with sRNA (Feigelson *et al.*, 1962; Wicks *et al.*, 1965; Greenman *et al.*, 1965) that a definite stimulation of tRNA synthesis occurs following cortisone administration and that this is apparent as early as 30 min after injection of the hormone. All efforts to evaluate this as a general increase in all tRNAs *vs.* stimulation of selected tRNA species consistently favored the former thesis. We believe, therefore, that this general increase in liver tRNAs is likely to be a prelude to, and part of, the hepatomegaly which follows cortisone administration and is probably not causally related to the induction by cortisone of specific liver enzymes. On the other hand, it is conceivable that in addition to its general effect on tRNA synthesis, the hormone produces a specific change in certain minor species of tRNA, and that this escaped detection in the present studies. Resolution of the isoaccepting species of $tRNA^{Leu}$ and $tRNA^{Tyr}$ on reversed-phase Freon columns did not indicate that this was the case, but the isoaccepting species of tRNAs for each amino acid would have to be examined in detail to completely exclude this possibility. We were also unable to detect a significant increase in the activity of any of several aminoacyl-tRNA synthetases in extracts of liver obtained after cortisone administration nor could we find evidence for either *in vitro* or *in vivo* binding of labeled cortisone to liver tRNA. This was especially relevant since a variety of macromolecules have been studied as receptors for various hormones (Koide, 1969; Feigelson and Feigelson, 1965).

The corticosteroids do not, therefore, seem to modify translation by producing gross changes in the abundance of specific tRNAs or aminoacyl-tRNA synthetases. Our results do not rule out the possibility that cortisone may affect tRNA function *in vivo* by alterations in conformation *via* methylation or other secondary modifications. Nor do they argue against specific affects on tRNA function mediated *via* alterations in the intracellular concentration of Mg^{2+} or other ions (Adams *et al.*, 1967), levels of inhibitors or activators, or other factors known to modify the aminoacylation of tRNA (Yegian and Stent, 1969). Finally, it is conceivable that the known changes in amino acid pools following cortisone administration (Betheil *et al.*, 1965) alter *in vivo* the ratio of charged:uncharged tRNAs and in this way modulate translation. Such changes have recently been reported to occur after insulin administration (Davey and Manchester, 1969), and further studies are required to determine whether cortisone induces similar effects *in vivo*.

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