Heterogeneity of Template RNA in Adrenal Glands

Abstract. *Template RNA in adrenal glands appears to be heterogeneous in stability. The RNA that regulates synthesis of a large fraction of adrenal protein has a turnover time of 4 hours or less. The remainder of adrenal-protein synthesis, including synthesis of protein that mediates the rapid steroidogenic response to ACTH, depends on RNA with considerably greater stability.*

The increase in adrenal-corticosteroid biosynthesis that occurs within minutes of administration of ACTH can be blocked in vivo by inhibition of protein synthesis with puromycin or cycloheximide (*1*). These observations have led to the hypothesis that synthesis of specific protein (steroid-regulating protein) is necessary for the rapid stimulation of steroidogenesis by ACTH. Turnover of the steroid-regulating protein appears to be very rapid, since corticosterone secretion in the rat decreases from maximum to baseline values within 20 minutes after synthesis of new protein is inhibited by cycloheximide.

However, actinomycin D does not acutely inhibit the steroidogenic response by the rat adrenal in vivo (*1*) or in vitro (*2, 3*), although this drug appears to inhibit steroidogenesis by bovine adrenal slices in vitro (*3*). It has been inferred that stimulation of steroid-regulating protein, in the rat adrenal at least, is not caused by an increase in RNA synthesis but rather by a modification in the utilization of preformed template RNA. It has been suggested that the inhibitory effect of actinomycin D in the bovine adrenal may result from instability of template RNA; continuous replacement of RNA may therefore be required for protein synthesis and steroidogenesis to continue (*3*).

We have examined the stabilities of the RNA involved in the steroidogenic process and of the RNA regulating overall synthesis of adrenal protein in the rat. We have blocked new RNA synthesis with actinomycin D and then estimated the turnover rate of the preformed RNA that regulates protein synthesis; the rate was estimated by measuring the rate of decrease in the incorporation of C^{14}-labeled amino acids into adrenal protein (which we defined as overall synthesis of adrenal protein) and by measuring the rate of decrease in the steroidogenic response to ACTH (thus assaying the decrease in the postulated steroid-regulating protein).

Table 1. Incorporation of C^{14}-uridine into adrenal RNA. Holtzmann rats (200 g) were either untreated, hypophysectomized, or hypophysectomized and immediately given actinomysin D intraperitoneally. At the time indicated after treatment, each animal was given 10 μc of C^{14}-uridine (30 mc/mmole) in the femoral vein, and the adrenal was removed 20 minutes later for measurement of C^{14} incorporation in RNA (*15*). The adrenals were homogenized in 2 ml of tris buffer, *p*H 7.4, 0.01*M* Mg^{++}, and incubated for 5 minutes at 37°C with 5μg of deoxyribonuclease per milliliter. Two milliliters of 20-percent trichloroacetic acid was then added, the supernatant was aspirated, and the precipitate was washed successively with 3 ml of 5-percent trichloroacetic acid (three washes), 3 ml of ether : ethanol (3 : 1), and 3 ml of ether. The final washed precipitate was dissolved in hyamine hydroxide. Radioactivity was measured with a liquid-scintillation spectrometer. After counting, C^{14} standard was added to each tube to correct for quenching. Results are means ± S.E. of each mean; number of animals in each group is in parentheses. Abbreviations: H, hypophysectomy; A, Actinomycin D.

Treatment		Incorporation in adrenal	
Type	Injection (mg)	Time (min)	Amount (dpm/100 mg)
None			543 ± 157 (5)
H		480	514 ± 78 (7)
A	1	30	128 ± 19 (9)
A	1	480	57 ± 4 (9)

A dose of 1 mg of actinomycin D was initially selected for administration to rats, since it was shown that this dose abolishes synthesis of all hepatic RNA with the exception of small quantities of transfer RNA (*4*). The rats were hypophysectomized in order to prevent variations in endogenous secretion of ACTH. RNA synthesis was determined by measuring incorporation of intravenously injected C^{14}-uridine (*5*) into adrenal RNA (Table 1). Hypophysectomy itself did not cause decrease in RNA synthesis over the experimental period. Actinomycin D, 1 mg, markedly inhibited RNA synthesis within 30 minutes. The incorporation of C^{14}-uridine into adrenal RNA was almost completely inhibited within 2 hours (*1*); in our experiments the incorporation into adrenal RNA was similarly reduced within 8 hours of the administration of actinomycin D (Table 1).

Table 2. Adrenal-vein corticosterone secretion in response to ACTH. Holtzmann rats (200 g) were either untreated, hypophysectomized, or hypophysectomized and immediately given actinomycin D or 5-fluorouracil intraperitoneally. At the time indicated after treatment, each animal was given 2 milliunits of ACTH in the femoral vein, and adrenal-vein blood was collected for a 3-minute period 7 to 10 minutes later. Methods for blood collection and for corticosterone measurement have been described (*7*). Results are means ± S.E. of each mean; number of animals in each group is in parentheses. Abbreviations: H, hypophysectomy; A, actinomycin D; F, 5-fluorouracil.

Treatment		Adrenal vein corticosterone	
Type	Injection (mg)	Time (hr)	Amount (μg/3 min)
None			1.54 ± 0.15 (8)
H		8	1.64 ± 0.23 (12)
A	1	8	1.51 ± 0.11 (14)
F	30	8	1.97 ± 0.25 (6)

Despite the marked inhibition of RNA synthesis by actinomycin D, the increase in adrenal vein corticosterone, in response to ACTH, remained normal for 8 hours (Table 2). These studies confirm previous results (*1–3*) in showing that the stimulation of steroid-regulating protein is not mediated by increased synthesis of RNA; furthermore they indicate that the RNA utilized in this process is stable for at least 8 hours.

Staehelin (*6*) has reported that, if 5-fluorouracil (30 mg) is given to rats and their adrenals are removed 1 hour later, the adrenal response in vitro to ACTH is inhibited. Since his result was difficult to reconcile with our experiments with actinomycin D, we tested the response in vivo to ACTH in rats given 5-fluorouracil (30 mg); the response was normal for 8 hours after drug administration (Table 2). Although we administered ACTH in vivo while Staehelin did so in vitro, in both studies the 5-fluorouracil was administered in vivo. Thus the reason for the discrepancy in the reported results is not apparent. Our results with actinomycin D and 5-fluorouracil are, however, consistent in indicating that the RNA involved in the synthesis of steroid-regulating protein is stable for at least 8 hours.

In order to determine whether administration of actinomycin D would eventually inhibit the steroidogenic response to ACTH, longer-term experiments with actinomycin D were performed. Since in these experiments hypophysectomy itself would have led to decreased adrenal response to ACTH (*7*), all animals were given depot ACTH: 2.5 units every 12 hours subcutaneously, beginning immediately after hypophysectomy. Five animals were also given 0.2 mg of actinomycin D after hypophysectomy; 24 hours later they were given 2 milliunits of ACTH intravenously and 7 to 10 minutes later their adrenal-vein corticosterone was found to be 0.76 ± 0.21 μg/3 minutes (3-minute bleeding) (mean ± S.E.). Five animals that received no actinomycin D but were otherwise treated identically had an adrenal-vein

Table 3. Incorporation of C^{14}–algal protein hydrolysate into adrenal protein. Holtzmann rats (200 g) were either untreated, hypophysectomized, or hypophysectomized and immediately given actinomycin D or 5-fluorouracil intraperitoneally. At the time indicated after treatment, each animal was given 4 μc of C^{14}–algal protein hydrolysate (1.8 mc/mg) in the femoral vein, and the adrenals were removed 10 minutes later for measurement of C^{14} incorporation in adrenal protein as described (*1*). Results are means ± S.E. of each mean; number of animals in each group is in parentheses. Abbreviations: H, hypophysectomy; A, actinomycin D; F, 5-fluorouracil.

Treatment		Incorporation in adrenal protein	
Type	Injection (mg)	Time (hr)	Amount (dpm/100 mg)
None			4644 ± 482 (17)
H		8	3987 ± 314 (6)
A	1	2	3194 ± 352 (7)
A	1	4	2197 ± 66 (8)
A	1	6	2512 ± 514 (8)
A	1	8	2370 ± 352 (4)
F	30	4	3693 ± 138 (4)
F	30	8	3373 ± 204 (4)

corticosterone of 3.02 ± 0.21 μg/3 minutes.

The acute increase in corticosterone secretion in response to ACTH was therefore inhibited 24 hours after the administration of actinomycin D. No animal mortality was observed during the experiment. One should note that there is no evidence that the adrenocortical response to given quantities of ACTH is inhibited by stress per se (*8*); thus it is unlikely that the inhibitory effect of actinomycin D reflected nonspecific toxicity of the drug. A decrease in adrenal-corticosterone content (*9*) and a small decrease in peripheral plasma corticosterone (*10*) in response to ACTH have also been noted 24 hours after the administration of actinomycin D. It is not clear whether single or multiple steps in corticosterone biosynthesis are inhibited by the antibiotic. It is therefore possible that the RNA involved in certain steps of biosynthesis of corticosterone are stable for more than 24 hours.

We next sought to determine whether overall protein synthesis is regulated by RNA of comparable stability. Rats were again hypophysectomized and immediately given actinomycin D (1 mg). Protein synthesis in vivo was determined by measurement of incorporation of intravenously injected C^{14}–algal protein hydrolysate into adrenal protein. Hypophysectomy itself resulted in only a slight decrease in protein synthesis over the 8-hour period (Table 3), although marked decreases have been observed over longer periods after hypophysectomy (*11*). Treatment with actinomycin D resulted in a rapid decrease in protein synthesis within 4 hours, with no further decrease between 4 and 8 hours after its administration.

Thus it appears that the RNA templates regulating overall synthesis of adrenal protein are heterogeneous in turnover rate. The RNA that regulates synthesis of a large fraction of adrenal protein has a turnover time of less than 4 hours, but the synthesis of the remaining adrenal protein is dependent on RNA that is stable for at least 8 hours. Fluorouracil only slightly decreased synthesis of adrenal protein (Table 3), suggesting that under the conditions of our experiments the drug is relatively ineffective in modifying the observed activity of template RNA.

Although our study does not entirely rule out the possibility that specific moieties of template RNA were not inhibited by actinomycin D, the large concentrations of the antibiotic used and the long period of observation after its administration mitigate against this explanation. Under certain experimental conditions actinomycin D inhibits protein synthesis by mechanisms other than its inhibition of RNA synthesis (*12*); thus there is the possibility that the turnover times of the RNA templates inferred from these experiments were even longer than the data indicate.

Template RNA in bacterial cells may be heterogeneous in stability (*13*). Heterogeneity in turnover of RNA that regulates synthesis of proteins in the rat thyroid has also been reported (*14*). Thus the template RNA for the major physiological function of the thyroid (thyroglobulin biosynthesis) and of the adrenal (steroidogenesis) appears to be relatively stable.

ROBERT L. NEY
Vanderbilt University School of Medicine, Nashville, Tennessee
WARREN W. DAVIS
National Institutes of Health, Bethesda, Maryland
LEONARD D. GARREN
Yale University School of Medicine, New Haven, Connecticut

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Retrograde Amnesia: Effects of Handling and Microwave Radiation

Abstract. *Rats that were irradiated with microwaves immediately after the training trial in a one-trial shock-avoidance learning task retained the conditioned avoidance response 24 hours later. However, rats that were handled a few minutes each day for 3 days before the experiment did not retain the response, although they were capable of learning in a later test.*

Electroconvulsive shock, anoxia, anesthesia, brain stimulation, and changes in body temperature can produce retrograde amnesia (*1*). Theoretically these treatments interfere with the perseverating neural activity required for consolidation of a memory trace. Microwave radiation should also interfere with recent memory. Heat production is the main effect of microwave radiation, although there are other effects (displacement and conduction currents, forces on dipoles, and intracellular orientation effects) which could alter neural activity (*2*). Heat produced a retrograde amnesia in goldfish, abolished the electroencephalogram (EEG) in rats, and desynchronized the EEG in rabbits (*3*). Microwave radiation abolished the action potential of a dog's sciatic nerve by direct heating to 50°C (*4*). I expected microwave radiation to interfere with the electrical activity of the nervous system and produce a retrograde amnesia for a conditioned avoidance response. I trained rats in one trial to avoid stepping off a small platform, by shocking them as they did so. Fifteen seconds after the training trial I irradiated the animals with 12-cm microwaves which quickly raised their body temperatures by 6°C. A test trial