polyoma DNA is present in its entirety in transformed cells, our data suggest that nearly 60 percent of the DNA transcribed during lytic infection is repressed. Alternatively, if only a selected portion (for example, 40 percent) of the polyoma genome is integrated in these cells, the saturation values reported above may represent complete expression of a segment of virus DNA.

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Endocrine Control of Adrenal Progesterone Secretion in the Ovariectomized Rat

Abstract. *In the ovariectomized rat, the adrenal gland secretes progesterone. Adrenocorticotropin treatment elevates concentrations of this steroid in plasma, and inhibitors of adrenocorticotropin or progesterone biosynthesis lower the concentration. Adrenocorticotropin controls progesterone secretion by the adrenal but not by the ovary. Adrenal progesterone and its mode of control may have important influences on reproductive processes.*

Progesterone occupies a key position in the biosynthesis of the adrenal corticoids in vitro (*1*). In addition to its role as precursor, progesterone has been isolated in considerable quantities from adrenal venous blood of cattle, sheep, and pigs (*2*) and of humans (*3*) and dogs (*4*) treated with corticotropin (ACTH). Recently, progesterone has been found in systemic plasma of rats after ovariectomy. The disappearance of progesterone from systemic plasma 8 hours after removal of the ovary and the adrenal suggests an adrenal origin for this compound (*5*). The experiments described in this report were efforts to understand control of adrenal progesterone secretion in the rat.

Adult, cycling female rats were ovariectomized 1 week before treatment. Adrenalectomies were performed on intact animals (day 1 or 2 of the cycle) with two consecutive 4-day cycles prior to the operation (*6*). The only special postoperative care given the animals was an ample supply of 0.9 percent saline as drinking water. Blood was collected by cardiac puncture into heparinized glass syringes and centrifuged shortly after removal. Samples, of the plasma were stored at −16°C until steroid analyses were made. Within 3 weeks after collection, about 4 ml of plasma was used for progesterone determination. Gas-liquid chromatography with electron-capture detection was used for steroid analysis (*7*). This technique had been used in my laboratory for the estimation of progesterone in rat plasma (*5*).

Aminoglutethimide (AGT) (Ciba) [30 mg in 0.5 ml of acetate buffer, *p*H 4.0 (*8*)] was injected subcutaneously (*9*). Acetate buffer injection without AGT was used as a control. Three hours after injection animals were anesthetized with Nembutal (35 mg per kilogram of body weight) and the blood was removed by cardiac puncture. Dexamethasone (DM) (Merck Sharp and Dohme) (100 mg/kg) was injected subcutaneously in 0.5 ml of propylene glycol. The animals were anesthetized with Nembutal 3 hours after treatment, and blood was collected. Corticotropin (5 U.S.P. units, Armour "Acthar") was injected via the tail vein in 0.3 ml (total volume) of saline. Another group of animals was treated with luteinizing hormone (100 μg; Calbiochem equine). Animals treated with saline served as controls. Animals were anesthetized with Nembutal at the time of injection, and anesthesia persisted until blood was collected 1 hour later.

Table 1. The effects of ACTH, dexamethasone, and aminoglutethimide on progesterone concentrations in systemic plasma in the ovariectomized rat.

Treatment	*N*	Progesterone (ng/ml ± S.E.)
Buffer	10	5.0 ± 2.2
Aminoglutethimide	10	0.5 ± 0.2*
Dexamethasone	10	ND†
Saline	9	3.9 ± 2.6
ACTH	9	24.1 ± 2.9‡

* Aminoglutethimide-treated compared to buffer-treated rats, $P < .05$. † ND, not detectable. ‡ ACTH-treated compared to saline-treated, $P < .01$.

Analysis of variance was used to determine the general effects of treatment on the concentration of progesterone in systemic rat plasma. Two groups of analyses were made. The animals in group 1 were treated with saline (control), luteinizing hormone, and ACTH; those in group 2 were treated with buffer (control), AGT, and DM. In both cases a significant *F* value was obtained for the effect of treatment (group 1, $P < .01$; group 2, $P < .05$). A *t*-test was used to compare treatment means with control.

In the ovariectomized rat, progesterone remains in the systemic blood for as long as 25 days after ovariectomy, whereas combined adrenalectomy and ovariectomy reduce the concentrations of this hormone in plasma to undetectable amounts in 8 hours (*5*). It is shown here that adrenal progesterone secretion in the rat is under ACTH control.

The quantitative effects of ACTH, AGT, and DM are shown in Table 1. One hour after injection of a corticotropin preparation, significant increases (ACTH versus saline) in progesterone concentrations in systemic plasma were noted ($P < .01$). Animals treated with dexamethasone were without circulating progesterone in their systemic plasma. Aminoglutethimide, a biosynthetic inhibitor that blocks the conversion of cholesterol to pregnenolone (*9*), significantly reduced systemic quantities of progesterone compared with those in buffer-injected controls ($P < .05$).

Adrenocorticotropin does not seem to affect systemic progesterone concentrations when the adrenals are absent but the ovaries left intact. Animals treated with saline showed a progesterone concentration of 2.2 ± 0.9 (S.E.) ng per 100 ml of plasma ($N = 4$), and those treated with ACTH showed a concentration of 2.6 ± 1.1 (S.E.) ng per 100 ml of plasma ($N = 4$). These observations limit the action of ACTH on the biosynthesis of progesterone to

the adrenal, with no action on the ovary where progesterone is presumably synthesized by a similar biosynthetic pathway. They also exclude the possibility that gonadotropin contamination produced this effect in the corticotropin preparation. Specificity of action is limited to ACTH because human chorionic gonadotropin will not stimulate adrenal secretion in man (*10*). Luteinizing hormone does not stimulate progesterone secretion in ovariectomized rats. Animals treated with 100 μg of luteinizing hormone (Calbiochem) showed concentrations of progesterone of 3 ± 2.3 (S.E.) ng per 100 ml of plasma ($N = 9$), a value that did not differ from the saline controls. A similar treatment will increase progesterone in the intact rat (*11*).

The physiological importance of adrenal progesterone in the plasma of the rodent or any other species is not known. Recently, a preovulatory surge of progesterone in systemic plasma, preceding the display of behavioral estrus, was reported (*5*). A similar surge of progesterone, shown in ovarian venous plasma, seems to indicate that at least part of the preovulatory progesterone found in the rat is of ovarian origin (*12*). However, the quantities of adrenal corticoids in rat plasma vary with the estrous cycle, and corticoid concentrations are greater during proestrus than at any other time during the cycle. These facts suggest that corticotropin is released during proestrus (*13*). Likewise, since exogenous ACTH can influence systemic blood concentrations of progesterone, it is reasonable to conclude that the adrenal also contributes to the systemic progesterone pool.

Progesterone is found in systemic plasma of ovariectomized rats. Its concentrations in systemic plasma are influenced by ACTH, DM, and AGT. In addition, it has recently been shown that stress will elevate progesterone concentrations in the adrenal glands of rats (*14*) and that concentrations of the hormone increase with prolonged blood collection (*11*). These facts, together with the many regulatory and feedback effects of progesterone on brain structures, suggest an important role for adrenal progesterone in the reproductive process.

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Fusidic Acid: Inhibition of Factor T_2 in Reticulocyte Protein Synthesis

Abstract. *The steroid antibiotic fusidic acid inhibits reticulocyte protein synthesis. This inhibition appears to be due to interference with the activity of the T_2 supernatant fraction, and strengthens the proposition that T_2 is functionally analogous to the G-factor of bacterial protein synthesis, which is also specifically inhibited by this antibiotic.*

Recent work with mammalian systems of protein synthesis suggest that the mechanism in the phase of elongation of the polypeptide chain is functionally analogous to that in bacterial systems (*1, 2*). Two elongation or transfer factors have been isolated from particle-free supernatant fractions of each system—these are T [a complex of T_u and T_s (*3*)] and G in the bacterial system (*4*), and T_1 and T_2 in the mammalian system (*1, 5*). These factors complement washed ribosomes in the repeating process of the addition of amino acids to the growing polypeptide chain. It is indicated that bacterial T and mammalian T_1 activate the GTP-linked (*6*) binding of aminoacyl-tRNA to ribosomes, and that G and T_2 promote a GTP-linked translocation, on the ribosome, of freshly elongated peptidyl-tRNA and messenger RNA in preparation for the next addition. There is, however, good evidence against complete interchangeability of supernatant fractions (*7*) and, therefore, it

Table 1. The effect of fusidic acid on polymerization and binding of phenylalanyl-tRNA labeled with C^{14} to ribosomes. Ribosomes and supernatant factors, up to the stage of DEAE purification, were prepared according to Felicetti and Lipmann (*2*). The polymerization incubation mixture, in a final volume of 0.125 ml, contained: tris-HCl (*p*H 7.4), 6.25 μmole; KCl, 10 μmole; $MgCl_2$, 1.25 μmole; DTT, 2 μmole; GTP, 0.25 μmole; poly U, 25 μg; phenylalanyl-tRNA from *E. coli*, labeled with C^{14} (4 to 5 × 10^4 count/min), 44 μg; washed ribosomes, 83.5 μg; $T_1 + T_2$, 21.5 μg; and fusidic acid as indicated. Incubation was for 10 minutes at 37°C. The hot TCA-precipitate was counted as previously described (*2*). Binding was measured according to the procedure of Felicetti and Lipmann (*2*) in the absence of —SH compounds. For assay, the procedure of Nirenberg and Leder was used (*14*). The reaction mixture contained, in a final volume of 0.125 ml: tris-HCl (*p*H 7.4), 6.25 μmole; KCl, 5.0 μmole; $MgCl_2$, 1.0 μmole; GTP, 0.05 μmole; poly U, 25 μg; phenylalanyl-tRNA from *E. coli*, labeled with C^{14}, 44 μg; $T_1 + T_2$, 21.5 μg; washed ribosomes, 167 μg; and fusidic acid, as indicated. Incubation was for 10 minutes at 37°C.

Sample	Polymerization		Binding	
	Count/min	Inhibition (%)	Count/min	Inhibition (%)
Control	3746	0	1483	0
Fusidic acid (1.6 m*M*)	40	99	1552	0
Fusidic acid (0.4 m*M*)	93	97		
Fusidic acid (0.032 m*M*)	1157	69		
Fusidic acid (0.016 m*M*)	2375	37		