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.

JOHN A. RESKO
Department of Reproductive Physiology and Behavior, Oregon Regional Primate Research Center, Beaverton 97005

4 APRIL 1969

References and Notes

1. L. T. Samuels and T. Uchikawa, in *The Adrenal Cortex*, A. B. Eisenstein, Ed. (Little, Brown, Boston, 1967), p. 61.
2. W. E. Balfour, R. S. Combine, R. V. Short, *Nature* **180**, 1480 (1957).
3. R. V. Short, *Biochem. Soc. Symp.* **18**, 59 (1960).
4. E. Endröczi, *Acta Physiol. Acad. Sci. Hung.* **21**, 195 (1962).
5. H. H. Feder, J. A. Resko, R. W. Goy, *J. Endocrinol.* **41**, 563 (1968).
6. M. X. Zarrow, J. M. Yochim, J. L. McCarthy, *Experimental Endocrinology, A Sourcebook of Basic Techniques* (Academic Press, New York, 1964), pp. 194–196.
7. H. J. van der Molen and D. Groen, *J. Clin. Endocrinol. Metab.* **25**, 1625 (1965).
8. K. Diem, Ed., *Documenta Geigy* (Geigy Pharmaceuticals, Division of Geigy Chemical Corp., Ardsley, New York, ed. 6, 1962), pp. 314–315.
9. R. N. Dexter, L. M. Fishman, R. L. Ney, G. W. Liddle, *J. Clin. Endocrinol. Metab.* **27**, 473 (1967).
10. L. L. Hibbitt, W. R. Starnes, S. R. Hill, *ibid.* **18**, 1315 (1958).
11. K. Yoshinaga, S. A. Grieves, R. V. Short, *J. Endocrinol.* **38**, 423 (1967).
12. I. Hashimoto, D. M. Henricks, L. L. Anderson, R. M. Melampy, *Endocrinology* **82**, 333 (1968).
13. F. D. Dean, P. M. Cole, I. C. Jones, *J. Endocrinol.* **18**, 3 (1959); V. Critchlow, R. A. Liebelt, M. Bar-Sela, W. Mountcastle, H. S. Lipscomb, *Amer. J. Physiol.* **205**, 807 (1963).
14. M. Holzbauer and H. M. Newport, *J. Physiol.* **193**, 131 (1967).
15. I thank Ciba Pharmaceutical Co. and Merck Sharp and Dohme for aminoglutethimide and dexamethasone, respectively. I thank C. Paris, who followed the rat cycles; J. Ploem and H. Stadelman for technical assistance; and Drs. R. Goy and D. Bullock for reading the manuscript. Publication No. 378 of the Oregon Regional Primate Research Center; supported by NIH grants MH-08634 and FR-00163.

12 November 1968

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		

Table 2. The effect of fusidic acid on the guanosine triphosphatase activity of ribosomes, factor T_2 alone, and combined. The assay mix contained, in a total volume of 0.125 ml: tris-HCl (*p*H 7.4), 6.25 μmole; KCl, 10 μmole; $MgCl_2$, 1.25 μmole; DTT, 2.0 μmole; GTP-γ-P^{32} (31,000 count/min per nanomole), 2.0 nmole; and ribosomes, T_2 factor, and fusidic acid, as indicated. Incubation was for 15 minutes at 37°C. The liberated P_i^{32} was determined as by Felicetti and Lipmann (*2*).

Ribosomes (μg)	T_2 (μg)	Fusidic acid (m*M*)	P_i^{32} released (count/min)	Inhibition (%)
119			4,801	
119		1.6	1,172	75
	7.5		3,003	
	7.5	1.6	136	95
119	7.5		24,176	
119	7.5	1.6	3,177	86
119	7.5	0.16	10,950	53
119	7.5	0.016	15,760	32

is desirable to strengthen the argument for general analogy (*2*).

A new opportunity for comparison seemed to be offered by the study of Tanaka *et al.* (*8*) on inhibition of bacterial protein synthesis by the steroid antibiotic fusidic acid, which has a lanostane-like skeleton (*9*). They report it to inhibit specifically the bacterial elongation factor G. They found an inhibition of amino acid polymerization parallel with that of G-linked guanosine triphosphatase (*4*, *8*). Haenni and Lucas-Lenard (*10*) have confirmed the inhibition specificity of G by showing that after transpeptidation fusidic acid prevents a translocation of newly formed peptidyl-tRNA.

We report here on inhibition of reticulocyte protein synthesis by fusidic acid, which we find is specific for T_2, the mammalian analog of G. In an assay with a suspension of intact rabbit reticulocytes (*11*), 0.8 m*M* fusidic acid causes a 96 percent inhibition of the incorporation of C^{14}-valine into hemoglobin, which shows permeability of the cell membrane to fusidic acid. The cell-free system responds similarly, with the use of poly U–dependent phenylalanine polymerization (Table 1). Judging from its activity at a concentration of 0.016 m*M*, which causes 37 percent inhibition, fusidic acid seems to be slightly more active with the reticulocytes than with the preparation of *Escherichia coli* (*8*).

To explore specificity of action, the fusidic acid effect was tested on the T_1-dependent binding of phenylalanyl-tRNA and on the ribosome-linked guanosine triphosphatase of T_2. The T_1-linked binding was not affected by the antibiotic (see column marked "Binding" in Table 1). However, T_2-linked guanosine triphosphatase (Table 2) was inhibited to an extent similar to that of polymerization. The ribosome and T_2-fraction alone caused less of a breakdown of GTP, which is also sensitive to fusidic acid. The ribosome-linked reaction, which exceeds about threefold that of the separate fractions, was strongly inhibited. In Table 3, hydrolysis of ATP and GTP are compared with the combined system. It shows that the preparation was contaminated with a nonspecific nucleotide triphosphatase, since ATP was hydrolyzed only slightly less than GTP. Hydrolysis of GTP, however, was more strongly inhibited by fusidic acid; ATP hydrolysis responded with slight inhibition, which may indicate a more general effect of fusidic acid on this type of reaction. It seems pertinent to add that there may be differences in the active centers of T_2 and of G; in contrast to fusidic acid, diphtheria toxin–diphosphopyridine nucleotide inhibits T_2 specifically (*12*), but does not inhibit G (*13*).

Table 3. The effect of fusidic acid on the breakdown of H^3-GTP and H^3-ATP. The reaction mixture was the same as in Table 2, with ribosomes and T_2 combined, except that H^3-GTP and H^3-ATP (0.25 μc) were used. After 15-minute incubation at 37°C, 20-μl samples were removed from the incubation mix and applied to strips of diethylaminoethyl-cellulose paper (Whatman DE-81) for ascending chromatography in 0.25*M* triethylamine in 16 percent acetic acid (adenine nucleotides), or in 0.5*M* triethylamine in 32 percent acetic acid (guanine nucleotides). After they had dried, the nucleotides were identified under ultraviolet light; the areas were cut out, heated for 10 minutes at 100°C, placed in vials containing 5 ml of Bray's solution (*15*), and counted in a Nuclear-Chicago scintillation counter. The percentage of breakdown of the nucleotide refers to the amount of radioactivity remaining as the triphosphate after incubation with and without fusidic acid.

Sample	Triphosphate	Breakdown (%)	Inhibition (%)
Control	GTP	65	
Fusidic acid (0.16 m*M*)	GTP	25	62
Control	ATP	55	
Fusidic acid (0.16 m*M*)	ATP	40	27

MARTIN MALKIN
FRITZ LIPMANN

Rockefeller University, New York 10021

References and Notes

1. L. Skogerson and K. Moldave, *Arch. Biochem. Biophys.* **125**, 497 (1968); *J. Biol. Chem.* **243**, 5354 and 5361 (1968).
2. L. Felicetti and F. Lipmann, *Arch. Biochem. Biophys.* **125**, 548 (1968).
3. J. Lucas-Lenard and F. Lipmann, *Proc. Nat. Acad. Sci. U.S.* **55**, 1562 (1966).
4. Y. Nishizuka and F. Lipmann, *Arch. Biochem. Biophys.* **116**, 344 (1966).
5. R. Arlinghaus, J. Shaeffer, J. Bishop, R. Schweet, *ibid.* **125**, 604 (1968).
6. Abbreviations: GTP, guanosine 5′-triphosphate; tRNA, transfer RNA; ATP, adenosine 5′-triphosphate; DTT, dithiothreitol; TCA, trichloroacetic acid; and poly U, polyuridylic acid.
7. B. Parisi, G. Milanesi, J. L. Van Etten, A. Perani, O. Ciferri, *J. Mol. Biol.* **28**, 295 (1967).
8. N. Tanaka, T. Kinoshita, H. Masukawa, *Biochem. Biophys. Res. Commun.* **30**, 278 (1968).
9. D. Arigoni, W. von Daehne, W. O. Godtfredsen, A. Melera, S. Vangedal, *Experientia* **20**, 344 (1964); W. O. Godtfredsen, W. von Daehne, S. Vangedal, D. Arigoni, A. Marquet, A. Melera, *Tetrahedron* **21**, 3505 (1965). We are indebted to Dr. Godtfredsen of Leo Pharmaceutical Products, Copenhagen, Denmark, for supplying us with fusidic acid.
10. A-L. Haenni and J. Lucas-Lenard, *Proc. Nat. Acad. Sci. U.S.* **61**, 1363 (1968).
11. B. Colombo, L. Felicetti, C. Baglioni, *Biochim. Biophys. Acta* **119**, 109 (1966).
12. T. Honjo, Y. Nishizuka, O. Hayaishi, *J. Biol. Chem.* **243**, 3553 (1968).
13. W. Johnson, R. J. Kuchler, M. Solotorovsky, *J. Bacteriol.* **96**, 1089 (1968).
14. M. Nirenberg and P. Leder, *Science* **145**, 1399 (1964).
15. G. A. Bray, *Anal. Biochem.* **1**, 279 (1960).
16. Supported by a grant from PHS (GM-13972).

15 November 1968

Cellulose: Refutation of a Folded-Chain Structure

Abstract. *Calculations of modulus of elasticity for extended- and folded-chain configurations have been compared with the experimental observations of mechanical properties of native cellulosic fibers. A recent folded-chain proposal is incompatible with the experimental evidence.*

The proposal of a folded-chain configuration for crystalline native cellulose (*1*) has led to a great deal of controversy concerning interpretation of evidence to favor such structure or the older extended-chain structure. We offer a simple calculation to show that the modulus of elasticity for the axial (microfibrillar) direction of the proposed folded chain would be several orders of magnitude lower than values obtained from tests on native cellulosic fibers.

Muggli has shown that determinations of molecular weight of carbanilated native (ramie) cellulose are consistent only with an extended-chain conformation (*2*). The molecular weights correspond closely with length