either is responsible for very rare species of RNA or is never transcribed. In regions where no polyribosomes are attached, numerous small granules the size of the putative RNA polymerase molecules are associated with the chromosome.

The kinetics of ribosomal RNA (rRNA) synthesis in *E. coli* indicate that the 16*S* and 23*S* cistrons are transcribed simultaneously by a relatively large number of RNA polymerase molecules as compared to the transcription of structural genes (*13*). In addition, ribosomal proteins, rather than ribosomes, become associated with the rRNA's as they are synthesized (*14*). Chromosomal segments with 60 to 70 attached fibrils have been observed (Fig. 4). Since the length of such segments is close to the length of DNA necessary to code for one 16*S* and one 23*S* rRNA (*15*), we suggest that these segments are rRNA genes. Studies with *Bacillus subtilis* (*16*) and *Proteus mirabilis* (*17*) have shown that the 16*S* and 23*S* cistrons are contiguous in those species.

Experiments on RNA-DNA hybridization (*18*) have shown that approximately 0.4 percent of the *E. coli* chromosome contains cistrons coding for rRNA. Taking into consideration the length of the chromosome and the amount of DNA necessary to code for the 16*S* and 23*S* rRNA molecules, this value indicates that the *E. coli* chromosome contains no more than six segments with tandem 16*S* and 23*S* cistrons (*19*). Our observations suggest that these sites are quite widely spaced on the chromosome. This conclusion is supported by biochemical data in another study using *E. coli* (*20*).

We believe that refinement of the techniques used in this study should provide a powerful tool for direct observation of specific active genetic loci in microbial systems, and that the procedures will prove generally useful for cytogenetic analysis at the molecular level.

O. L. MILLER, JR.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee
BARBARA A. HAMKALO*
C. A. THOMAS, JR.
Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

References and Notes

1. O. L. Miller, Jr., and B. R. Beatty, *Science* **164**, 955 (1969); *J. Cell. Physiol.* **74** (Suppl. 1), 225 (1969).
2. Strain N181 of the sucrose dependent mutant *sud* 24 was supplied by Dr. David Schlessinger, Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri. Procedures for handling this fragile mutant are given in G. Mangiarotti and D. Schlessinger, *J. Mol. Biol.* **20**, 123 (1966). Briefly, the cells are osmotically stable and grow exponentially in a medium containing 20 percent sucrose. In mediums with low or no sucrose, the cells become fragile and lyse spontaneously. If, however, the sucrose is replaced with Carbowax 4000 (Dow), the cells become fragile without lysing. The strain sometimes is fragile in cultures in the log phase of growth in high sucrose medium, and this condition is evidenced by a slight rounding of the normally rod-shaped cells. Our best preparations so far have been obtained with the latter type of culture.
3. See table 2 in W. E. Hill, J. D. Thompson, J. W. Anderegg, *J. Mol. Biol.* **44**, 89 (1969).
4. The diameter of double-helix DNA determined by electron microscopy of shadow-cast molecules [C. E. Hall, *J. Biophys. Biochem. Cytol.* **2**, 625 (1956)] and uranyl acetate–stained molecules [W. Stoeckenius, *ibid.* **11**, 297 (1961); M. Beer and C. R. Zobel, *J. Mol. Biol.* **3**, 717 (1961)] is approximately 20 Å.
5. G. Zubay and M. R. Watson, *J. Biophys. Biochem. Cytol.* **5**, 51 (1959).
6. G. Mangiarotti and D. Schlessinger, *J. Mol. Biol.* **29**, 395 (1967).
7. G. Stent, *Science* **114**, 816 (1964); R. Byrne, J. C. Levin, H. A. Bladen, M. Nirenberg, *Proc. Nat. Acad. Sci. U.S.* **52**, 140 (1964).
8. M. Lubin, *J. Mol. Biol.* **39**, 219 (1969).
9. R. F. Baker and C. Yanofsky, *Proc. Nat. Acad. Sci. U.S.* **60**, 313 (1968); F. Imamoto, *ibid.*, p. 305.
10. F. Jacob, A. Ullman, J. Monod, *Compt. Rend.* **258**, 3125 (1964).
11. The five-gene tryptophan operon in *E. coli* is ~2.3 μm of double-helix DNA [F. Imamoto and C. Yanofsky, *J. Mol. Biol.* **28** 1 (1967)]; the nine-gene histidine operon in *Salmonella typhimurium* is ~3.75 μm of double-helix DNA [R. Benzinger and P. E. Hartman, *Virology* **18**, 614 (1962)].
12. D. Kennel, *J. Mol. Biol.* **34**, 85 (1968).
13. H. Bremer and D. Yuan, *ibid.* **38**, 163 (1968); H. Manor, D. Goodman, G. Stent, *ibid.* **39**, 1 (1969).
14. G. Mangiarotti, D. Apirion, D. Schlessinger, L. Silengo, *Biochemistry* **7**, 456 (1968).
15. The molecular weights for 23*S* and 16*S* rRNA are 1.1 and 0.56×10^6, respectively [C. G. Kurland, *J. Mol. Biol.* **2**, 83 (1960)]. Approximately 1.66 μm of double-helix DNA is required to code for one each of these molecules [A. R. Peacocke and R. B. Drysdale, *The Molecular Basis of Heredity* (Butterworths, Washington, D.C., 1965) p. 34)].
16. W. Colli and M. Oishi, *Proc. Nat. Acad. Sci. U.S.* **64**, 642 (1969).
17. I. Purdom, J. O. Bishop, M. L. Birnstiel, *Nature*, in press.
18. S. A. Yankofsky and S. Spiegelman, *Proc. Nat. Acad. Sci. U.S.* **48**, 1466 (1962); D. Kennel, *J. Mol. Biol.* **34**, 85 (1968).
19. The *E. coli* chromosome has been estimated to be about 1100 μm in length [J. Cairns, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 43 (1963)]. Approximately 1.66 μm of double-helix DNA is required to code for the rRNA's (*15*); 0.4 percent hybridization (*18*) indicates that 0.8 percent of double-helix DNA codes for the rRNA genes; 0.8 percent of 1100 μm is 8.8 μm; a length which accommodates no more than six cistrons of each of the rRNA's.
20. R. G. Cutler and J. E. Evans, *J. Mol. Biol.* **26**, 91 (1967).
21. Sponsored by AEC under contract with the Union Carbide Corporation (O.L.M.), NIH postdoctoral fellowship 5-FO2-CA41226-02 (B.A.H), and NSF grant GB-8611 and NIH grant 2-ROI-AI-08186-02 (C.A.T.).
* Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

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L-Dihydroxyphenylalanine: Effect on S-Adenosylmethionine in Brain

Abstract. *Forty-five minutes after intraperitoneal injection of a single dose (100 milligrams per kilogram) of* L-*dihydroxyphenylalanine, the concentration of* S-*adenosylmethionine in rat brain was lowered by 76 percent. As little as 10 milligrams of* L-*dihydroxyphenylalanine per kilogram decreased content of* S-*adenosylmethionine in the adrenal medulla by 51 percent, whereas 100 milligrams per kilogram did not significantly depress concentration of* S-*adenosylmethionine in the liver in this time interval. Concentration of* S-*adenosylmethionine in the brain varied diurnally;* L-*dihydroxyphenylalanine lowered this concentration whether administered at the daily peak or at the nadir.*

The efficacy of L-dihydoxyphenylalanine (L-dopa) in the treatment of Parkinson's disease has been correlated with the fact that this catechol amino acid is the physiological precursor for brain dopamine. Its administration to experimental animals causes an increase in content of dopamine in the brain (*1*). Moreover, brains of parkinsonian patients often contain subnormal amounts of dopamine (*2*), and the concentration of its chief metabolite, homovanillic acid (HVA), in their cerebrospinal fluid is depressed (*3*). Finally, the administration of L-dopa to human subjects elevates the HVA content of the urine and cerebrospinal fluid (*3, 4*), which indicates that significant quantities of the exogenous amino acid are converted to dopamine, as occurs with endogenous dopa.

After intraperitoneal administration, L-dopa is largely methylated to 3-*O*-methyl-dopa, which is then decarboxylated and converted to HVA. A surprisingly high percentage (more than half) of a dose of L-dopa is *O*-methylated within the first 20 minutes after administration (*5*). Conversion to central catecholamines is actually a very minor metabolic route of exogenous L-dopa. Since *S*-adenosylmethionine (SAMe) is the methyl donor in the *O*-methylation of L-dopa and dopamine (*6*), it seemed likely that large amounts of SAMe must be utilized in

Table 1. Relation between dose of L-dopa and extent of depletion of SAMe content in tissue. Groups of five rats received L-dopa intraperitoneally and were killed 45 minutes later. Data are presented as mean concentration of SAMe (micrograms per gram of wet tissue) ± standard error of the mean.

Tissue	L-Dopa dose (mg/kg)			
	0	10	30	100
Brain	16.8 ± 0.6	16.0 ± 1.1	10.7 ± 0.3*	5.5 ± 0.4*
Adrenal	39.4 ± 1.8	19.4 ± 3.9†	15.1 ± 4.9†	14.1 ± 3.6*
Liver	56.8 ± 3.5	65.9 ± 2.0	71.2 ± 5.3	61.4 ± 5.4

* $P < .001$ differs from control group. † $P < .01$ differs from control group.

the process. We now present evidence that the administration of L-dopa to rats in a single dose equivalent to that generally used in treating Parkinson's disease causes a marked reduction of SAMe content in the brain.

All experiments utilized adult male Sprague-Dawley rats housed in individual cages under alternating 12-hour periods of light (Vita-Lite, 40 to 60 $\mu w/cm^2$) and darkness; the animals had free access to Purina chow and to water. The L-dopa (Nutritional Biochemicals) was dissolved in 0.05*M* HCl (10 mg/ml) and administered intraperitoneally; control animals received only the diluent. Concentrations of SAMe in tissue were assayed by the double label, isotope dilution, isotope derivative method of Baldessarini and Kopin (*7*).

The concentrations of norepinephrine and dopamine in several regions of rat brain exhibit significant diurnal fluctuations (*8*). Since these catecholamines are methyl acceptors (*6, 9*), a study was performed to determine whether concentrations of SAMe in the brain also varied diurnally. Such variations, if present, could influence the amount of the cofactor utilized after a given dose of L-dopa. Concentrations of SAMe in the brain were lowest at the middle of the daily light period and rose by 50 percent to a maximum in the middle of the dark period 12 hours later; the concentrations at the ends of both the light and the dark periods were similar, 25 percent above those present at the nadir. The SAMe content in the adrenal medulla also varied diurnally ($P < .01$); however, the amplitude of the daily rise was slightly less than that seen in the brain (31 percent), and the nadir and peak occurred at the ends of the light and dark periods, respectively.

To examine the effects of exogenous L-dopa on concentration of SAMe in the brain, groups of five rats received 0, 10, 30, or 100 mg of the catechol amino acid per kilogram in the middle of the daily dark period and were killed 45 minutes later. Treatment with 30 mg of L-dopa per kilogram was associated with a 36 percent reduction ($P < .001$) of SAMe in the brain (Table 1); treatment with 100 mg/kg caused a 67 percent reduction ($P < .001$). As little as 10 mg of L-dopa per kilogram caused a 51 percent decrease in concentration of SAMe in the adrenal medulla ($P < .01$); in contrast, the content of SAMe in the liver was not significantly altered 45 minutes after the injection of as much as 100 mg of L-dopa per kilogram. In another experiment, we observed that the decrease in SAMe concentration in brains of rats receiving 100 mg of L-dopa per kilogram was at least as pronounced in the middle of the daily light period (that is, when these contents were normally lowest) as at their daily peak in the middle of the dark period. The decrease in concentration of SAMe in the brain after administration of L-dopa was observed in four separate experiments.

The time course of the effect of L-dopa on SAMe in the brain was next examined among groups of four rats that received 100 mg of L-dopa per kilogram and were killed at various intervals thereafter. Untreated rats were also killed with each experimental group to control for changes due to the daily rhythm in SAMe concentration in the brain. Forty-five minutes after administration of L-dopa, there was a 76 percent reduction in concentration of SAMe in the brain (Table 2). By the 6th hour after treatment with L-dopa, SAMe concentration was no longer depressed in the brain.

Table 2. Time course of effect of L-dopa on concentration of SAMe in the brain. Groups of four rats received L-dopa (100 mg/kg) intraperitoneally. Data are presented as mean ± standard error of the mean.

Time after injection	Control ($\mu g/g$ brain)	L-Dopa ($\mu g/g$ brain)
45 minutes	17.5 ± 1.7	4.2 ± 0.3*
6 hours	18.2 ± 1.1	21.9 ± 1.5
24 hours	18.4 ± 1.7	24.6 ± 4.4

* $P < .01$ differs from control group.

Doses of L-dopa proportional on the basis of milligrams per kilogram of body weight to those administered to parkinsonian patients (*10*) produce a marked and rapid decline in SAMe concentration in the brain. The decrease after a single dose is of relatively short duration. Moreover, no significant decrease is observed in the liver, an organ that contains large amounts of the methionine-activating enzyme and can synthesize SAMe rapidly (*7*). Thus, it seems most likely that this effect of L-dopa reflects the transient inability of SAMe synthesis to keep pace with the amounts of the compound needed for *O*-methylation after administration of L-dopa. There are now a considerable number of patients who have been receiving as much as 40 to 50 mmole (8 to 10 g) of L-dopa per day for many months (*10*). Inasmuch as dietary methionine, the main source of methyl groups available to humans, is generally consumed in much smaller daily quantities [approximately 10 to 15 mmole (*11*)], it seems possible that long-term administration of L-dopa might cause a relative depletion of methionine in the body. Unless this depletion were corrected (for example, by increasing the consumption of the methyl donor choline or of proteins rich in methionine, or by decreasing the fraction of ingested L-dopa that is metabolized by *O*-methylation), a variety of metabolic consequences might follow. One such consequence might be decreased concentration of SAMe in tissues and decreased availability of this methyl donor for transmethylation reactions.

A significant fraction of endogenously synthesized dopamine and norepinephrine in the brain is normally metabolized by *O*-methylation (*9*). If the reduction in concentration of SAMe in the brain that follows administration of L-dopa is sufficient to limit the rate of *O*-methylation of catecholamines, it seems possible that one additional mechanism by which L-dopa might produce its neurological effects would be to potentiate the actions of endogenous dopamine and norepinephrine, that is, by slowing the rate at which they are metabolized. This mechanism would allow administered L-dopa to influence central noradrenergic transmission in spite of the fact that very little of the amino acid is converted to norepinephrine (*5*). It is also possible that 3-*O*-methyl-

dopamine, formed by the decarboxylation of 3-*O*-methyl-dopa, may play a role in the actions of L-dopa. For example, *O*-methylated metabolites including 3-*O*-methyl-dopamine, enhance the uptake of norepinephrine into adrenergic nerve endings (*12*).

Methods of potentiating the effect of a dose of L-dopa have been under consideration for some time. Thus, inhibitors of the peripheral decarboxylation of L-dopa enhance the conversion of the precursor to catecholamines in the brain (*13*). Methyl-group acceptors or inhibitors of methyl transfer might similarly be of use in potentiating the clinical efficacy of L-dopa.

R. J. WURTMAN
C. M. ROSE
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139
S. MATTHYSSE, J. STEPHENSON
R. BALDESSARINI
Laboratory of Neuropsychopharmacology, Massachusetts General Hospital, Boston

References and Notes

1. A. Carlsson, M. Lindqvist, T. Magnusson, B. Waldeck, *Science* **127**, 471 (1958); G. F. Murphy and T. L. Sourkes, *Arch. Biochem. Biophys.* **93**, 338 (1961); R. J. Wurtman, *Catecholamines* (Little, Brown, Boston, 1966).
2. H. Ehringer and O. Hornykiewicz, *Klin. Wochenschr.* **38**, 1236 (1960); O. Hornykiewicz, *Pharmacol. Rev.* **18**, 925 (1966).
3. W. Wiener, W. Harrison, H. Klawans, *Life Sci.* **8**, 971 (1969).
4. D. B. Calne, F. Karoum, C. R. J. Ruthven, M. Sandler, *Brit. J. Pharmacol.* **37**, 57 (1969).
5. C. M. Rose, C. Chou, R. J. Wurtman, *Fed. Proc.* **29**, 511 (1970).
6. J. Axelrod and R. Tomchick, *J. Biol. Chem.* **233**, 702 (1958).
7. R. J. Baldessarini and I. J. Kopin, *J. Neurochem.* **13**, 769 (1966).
8. D. Reis and R. J. Wurtman, *Life Sci.* **7**, 91 (1968); R. W. Piepho and A. H. Friedman, *Pharmacologist* **11**, 255 (1969); M. J. Zigmond and R. J. Wurtman, *J. Pharmacol. Exp. Ther.* **172**, 416 (1970).
9. J. Glowinski, I. J. Kopin, J. Axelrod, *J. Neurochem.* **12**, 25 (1965); J. Glowinski and R. Baldessarini, *Pharmacol. Rev.* **18**, 1201 (1966).
10. G. C. Cotzias, P. S. Papavasiliou, R. Gellene, *N. Engl. J. Med.* **280**, 337 (1969).
11. W. C. Rose, M. J. Coon, H. B. Lockhart, G. F. Lambert, *J. Biol. Chem.* **215**, 101 (1955).
12. L. Iversen, J. Fisher, J. Axelrod, *J. Pharmacol. Exp. Ther.* **154**, 56 (1966).
13. G. Bartholini and A. Pletscher, *ibid.* **161**, 14 (1968).
14. Supported in part by grants from NASA (NGR-22-009-272), NIH (MH-16674-02), and the Scottish Rite Program of Research in Schizophrenia. We thank C. Chou for technical assistance.

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Height and Weight at Menarche and a Hypothesis of Critical Body Weights and Adolescent Events

Abstract. *Height and weight at menarche were found for each subject in three longitudinal growth studies. Early and late maturing girls have menarche at the same mean weight, but late maturers are taller at menarche. Two other major events of adolescence, initiation of the weight growth spurt and maximum rate of weight gain, also occur at an invariant mean weight. The hypothesis is proposed that a critical body weight may trigger each of these adolescent events. Such an interaction would explain the secular trend to an earlier menarche.*

We analyzed the adolescent growth spurt by determining the height and weight for each child at the age of initiation of the spurt in growth (*1*), and at the time of maximum rate of growth (*2*), using the data from three longitudinal (each child is measured at each successive age) growth studies (see *3*).

The mean weight of girls at the time of initiation of the spurt in weight growth, about 31 kg (68 lb), did not differ for early and late maturing girls (*4*), whereas the mean height at the time of the initiation of the spurt in height growth increased significantly (about 10 cm) with age of initiation (*1*). [The height and weight spurts begin practically simultaneously (*1*).] Similarly, at the time of maximum rate of weight gain, the mean weight of early and late maturing girls was the same, about 39 kg (86 lb), whereas the mean height increased significantly with age of maximum rate of height gain (*2*).

These results account for the many observations (*5*) that early maturers have more weight for height than late maturers before and throughout the adolescent growth spurt. What was unexpected, however, was that two of the major events of adolescence were related to an unchanging mean weight. We have now found that a third adolescent event, menarche, also occurs at an unchanging mean weight.

Menarche occurs after the adolescent maximum rates of growth in height and weight are attained (*2*). As far as we know there are no data on actual height or weight at menarche, although there are many general observations that girls at menarche are taller and heavier than those of the same age who have not achieved menarche (*5*).

We estimated weight and height at menarche for 181 girls of the three growth studies by interpolation of their height and growth data (*6*). The mean age of menarche of all girls is 12.9 ± .09 years; their mean weight at menarche is 47.8 ± .51 kg (106 lb); their mean height at menarche is 158.5 ± .50 cm. The mean height increases significantly ($P < .02$) from 156.4 ± .97 cm to 160.9 ± 1.4 cm as the mean age of menarche increases from 11.4 to 14.5 years. [If HSPH girls, who we know from (*2*) include unusually short latest maturers, are excluded, the means are 156.7 ± 1.2 cm and 162.1 ± 1.6 cm ($P < .01$).] The mean weight at menarche, 48 kg, does not change with increasing age of menarche (Table 1).

Early maturers grow more rapidly in height and weight than late maturers during the adolescent spurt (*7, 8*). We find they continue to do so during the year of menarche: the increments in height and weight in that year are 7.0 ± .24 cm and 6.8 ± .46 kg, respectively, for girls with a mean age of menarche of 11.4 years; they decrease gradually to 3.8 ± .21 cm and 4.2 ± .35 kg for girls with a mean age of menarche of 14.5 years (difference significant at $P < .01$).

We observed, as did Shuttleworth (*7*), that late maturers take longer to attain menarche after initiation of the growth spurt; as the mean age of menarche increased from 11.4 to 14.4 years, the time increased from 2.9 ± .19 to 3.6 ± .18 years (*1*). Late maturers also take longer to attain menarche after attainment of maximum rate of growth (*2, 7, 8*). It has been suggested that these time differences are possibly not real, but are statistical artifacts (*9*). Our data show that the observed longer intervals must be real; since late maturers attain all the critical events at the same weight as early maturers and grow more slowly, they necessarily take a longer time between events and from initiation to menarche.

At age 18, when growth in height has ceased for all girls (*2*), early and late maturers are the same height (Table 1) (*1, 2, 10*), but late maturers are lighter in weight (Table 1) (*1, 2, 7*), and remain so into adulthood (*2*; *5*, p. 97). These results at age 18 are expected from the findings previous to and at menarche: early and late maturers can achieve the same height at age 18 since the early maturers, though shorter,