momycin A_3) in the presence of natural DNA is best rationalized as an expression of the degree of binding of these antibiotics. The degree of binding is a reflection of the G+C percentage of the DNA under observation. This mechanism is different from the mode of binding of aminoacridines (quinacrine) to DNA, where the binding to DNA is not sequence specific but the variable quantum efficiency of fluorescence is a function of the base composition at the binding site. The production of R bands by the G-C–specific fluorochromes supports the suggestion that nucleotide sequence arrangement along the chromosome plays an important role in determining the display of fluorescent bands (*3, 4, 6*).

Further qualitative examination of chromosome banding with these and other fluorescent probes should provide additional information on the mechanism of chromosome banding, and also may lead to a better understanding of the structure and function of chromosomes.

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References and Notes

1. T. Caspersson, S. Farber, G. E. Foley, J. Kudynowski, E. J. Modest, E. Simonsson, U. Wagh, L. Zech, *Exp. Cell Res.* **49**, 219 (1968).
2. I. Hilwig and A. Gropp, *ibid.* **75**, 122 (1972).
3. C. C. Lin and J. H. van de Sande, *Science* **190**, 61 (1975).
4. B. Weisblum and P. L. de Haseth, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 629 (1972).
5. R. K. Tubbs, W. E. Ditmars, O. Van Winkle, *J. Med. Biol.* **9**, 545 (1964); L. M. Chan and J. A. McCarter, *Biochim. Biophys. Acta* **204**, 252 (1970); U. Pachmann and R. Rigler, *Exp. Cell Res.* **72**, 602 (1972).
6. D. E. Comings and M. E. Drets, *Chromsoma*, in press.
7. Olivomycin and chromomycin A_3 were obtained from Schwartz/Mann. Mithramycin was kindly provided by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.
8. W. Kersten, *Gann Monogr.* **6**, 65 (1968).
9. ——— and H. Kersten, *Biochem. Z.* **341**, 174 (1965).
10. W. Behr and G. Hartmann, *ibid.* **343**, 519 (1965).
11. D. Ward, E. Reich, I. H. Goldberg, *Science* **149**, 1259 (1965).
12. W. Kersten and H. Kersten, *Molecular Associations in Biology*, B. Pullman, Ed. (Academic Press, New York, 1968), p. 289.
13. W. Behr, K. Honikel, G. Hartmann, *Eur. J. Biochem.* **9**, 82 (1969).
14. W. Kersten, H. Kersten, W. Szybalski, *Biochemistry* **5**, 236 (1966); M. Waring, *J. Mol. Biol.* **54**, 249 (1970).
15. National Foundation, *Birth Defects Orig. Art. Ser.* **8** (No. 7) (1972).
16. M. Bobrow, H. E. A. C. Collacott, K. Madan, *Lancet* **1972-II**, 1311 (1972).
17. W. Schnedl and R. Czaker, *Cytogenet. Cell Genet.* **13**, 246 (1974); C. C. Lin, D. R. Newton, W. K. Smink, R. B. Church, *Can. J. Anim. Sci.* **56**, 27 (1976).
18. K. W. Jones, *Nature (London)* **225**, 912 (1970); M. L. Pardue and J. G. Gall, *Science* **168**, 1356 (1970).
19. This work was supported by Medical Research Council of Canada grants MA 4655 and MA 4885.

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Hyperphenylalanemia: Effect on Brain Polyribosomes Can Be Partially Reversed by Other Amino Acids

Abstract. *The effect of a single injection of phenylalanine (2 mg/g of body weight) on brain polyribosomes, which increases the number of inactive monoribosomes, persists for 2 to 3 hours. A single injection of seven large neutral amino acids after phenylalanine administration results in a reversal of the effect on brain polyribosomes with a resultant decrease in monoribosomes to near normal levels. The other common amino acids are apparently not limiting during hyperphenylalanemia, because an injection of these did not increase recovery.*

A single injection of phenylalanine leads to a shift in polyribosome profiles of mammalian brain tissue and increases the number of monoribosomes (*1–3*). The monoribosomes are apparently inactive and accumulate as a result of a decreased rate of the initiation of protein synthesis (*3*). In addition to the effects on protein metabolism, a state of hyperphenylalanemia produces a rapid and extensive decrease in the intracellular concentration of several amino acids in brain cells both in vivo and in vitro (*4*). The amino acids which are most affected are the large neutral amino acids which are cotransported with phenylalanine. Alterations in the intracellular concentrations of these amino acids have been suggested as the mechanism by which phenylalanine decreases protein synthesis in neural tissue (*4, 5*). If this amino acid imbalance is directly responsible for the inhibition of neural protein synthesis, then the administration of the proper amino acids to hyperphenylalanemic animals might restore the normal balance and reverse the inhibitory effect on neural protein synthesis.

The injection of neonatal mice with L-phenylalanine (2 mg per gram of body

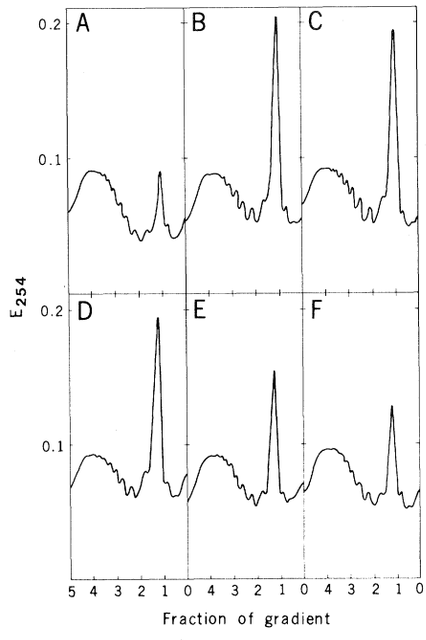

Fig. 1. Sedimentation profiles of brain polyribosomes isolated after injection of various amino acids. Noninbred Swiss albino mice (*6*) received intraperitoneal injections of phenylalanine as described (*3*) at a dose of 2 mg/g of body weight (65 μl/g) from a stock solution containing 30 mg of L-phenylalanine per milliliter of saline; control animals received a comparable amount of saline alone. (A) Saline was injected 60 minutes before the animals were killed (the same profile is found if saline is injected 90 to 180 minutes before killing). (B) Phenylalanine was injected 60 minutes before the animals were killed. (C) Phenylalanine was injected 120 minutes before the animals were killed (similar polyribosome profiles were observed for at least 180 minutes after injection with phenylalanine). When two injections were used, the second injection was given 60 minutes after the administration of phenylalanine. The second injection consisted of a mixture of the seven large neutral amino acids (*7*) (3.75 mg of each amino acid per milliliter of saline, injected at a dose of 0.075 mg of each amino acid per gram of body weight, or 20 μl/g). (D) Phenylalanine was injected 60 minutes before a saline injection (20 μl/g); animals were killed either 30 or 60 minutes later. (E) Phenylalanine was injected 60 minutes before the seven amino acids were injected; the animals were killed 30 minutes later. (F) Phenylalanine was injected 60 minutes before the seven amino acids were injected; animals were killed 60 minutes later. Brain polyribosomes were isolated as described (*3*) by homogenizing brains with a motor-driven glass-Teflon homogenizer in TKM buffer solution (50 m*M* tris-HCl, *p*H 7.2; 25 m*M* KCl; 5 m*M* $MgCl_2$) containing 1 mg/ml of bentonite. The resulting homogenate was centrifuged at 8400*g* for 15 minutes at 4°C. The supernatant fluid containing the polyribosomes was removed and sodium deoxycholate and Tween-40 were added to a final concentration of each of 1 percent. The polyribosomes were then purified by gel filtration through a Sephadex G-200 column (1.1 cm by 10 cm) at 4°C. The brain polyribosome preparations were then layered (approximately 2.0 E_{254} units) on a 5.0-ml linear (0.7 to 1.5*M*) sucrose gradient in TKM buffer and centrifuged at 190,000*g* for 105 minutes in a Beckman SW 50.1 rotor at 4°C. Duplicate gradients were run for each condition in each experiment. After centrifugation the sucrose was removed from the top of the gradient by a peristaltic pump, the optical absorption at 254 nm (E_{254}) was continuously monitored with an ISCO model UA-2 ultraviolet analyzer, and the area under the monoribosome and polyribosome region was determined (*3*).

weight) (*6*) results in an increase in the proportion of ribosomes present as monoribosomes in neural tissue (Fig. 1). The effect on neural polyribosomes reaches a maximum at 30 minutes and persists for at least 180 minutes without any significant recovery. The effects of phenylalanine on brain polyribosomes are shown in Fig. 1, A to C. The lack of recovery for up to 3 hours after injection allowed us time in which to attempt to reverse the effect of phenylalanine on brain polyribosomes.

Since it seemed reasonable that most or all of the large neutral amino acids might be limiting during hyperphenylalanemia (*4*), were employed various mixtures of all these amino acids in attempts to reverse the effects of phenylalanine. The injection of a mixture of seven large neutral amino acids (*7*), at a dose of 0.075 mg of each amino acid per gram of body weight, resulted in a decrease in the number of ribosomes present as monoribosomes and a concomitant increase in polyribosomes (Fig. 1, D to F). The maximum effect occurred about 60 minutes after injection and resulted in a partial, though never complete, reversal of the phenylalanine effects. The effect on polyribosomes could be quantitated by determining both the areas under the monoribosome peak and the total ribosomal material, and expressing the monoribosomes as a proportion of the total ribosomes (Table 1). The 50 to 60 percent increase in the number of monoribosomes that occurred after the administration of phenylalanine decreased to 17 to 20 percent (Table 1) after the injection of the seven large neutral amino acids (*7*). The dose of the mixture of the seven amino acids was apparently very important, since neither smaller (0.05 mg/g) nor larger (0.10 mg/g) concentrations could achieve the same amount of recovery as was obtained with 0.075 mg/g. The larger doses may not have been effective since high concentrations of most of these amino acids have inhibitory effects on brain polyribosomes (*2, 8*).

These seven amino acids appear to be those primarily related to the effects of phenylalanine on brain protein synthesis. An injection of the 12 other common amino acids (*9*) was neither able to reverse the effects of phenylalanine nor to enhance the reversal occurring with the seven large neutral amino acids alone (see Table 1). Several concentrations of the 12 common amino acids were tried (0.01 to 0.03 mg of each amino acid per gram of body weight) with the seven large neutral amino acids, but there was no enhancement of recovery.

Table 1. The effects of various amino acids on brain polyribosomes. Some mice received a single injection of phenylalanine (or saline, control) before they were killed, while others received a second injection (of the seven large neutral amino acids, or the 12 amino acids, or both, or saline) (see legend, Fig. 1). The area under the monoribosome peak as well as the area for the total ribosomal material was determined from the polyribosome profiles after sucrose density gradient centrifugation as described in Fig. 1. Results are expressed as concentrations of monoribosomes as proportions of the total ribosomal material ± 1 standard deviation. The numbers of independent experiments, each carried out with duplicate gradients, are indicated in parentheses. The increase in monoribosomes is reported as the difference between the mean experimental values and the saline control values, expressed as a percentage of the control; for example, 60 minutes after the injection of phenylalanine, [(0.277 − 0.171)/0.171] × 100 = 62.0 percent.

Time between first injection and second injection or killing (minutes)	Second injection	Time between second injection and killing (minutes)	Ratio of monoribosomes to total ribosomes†	Increase in monoribosomes (%)
60 (control)*			0.171 ± .014 (6)	
60			0.277 ± .005 (2)	62.0
90			0.257 (1)	50.2
120			0.253 ± .010 (2)	48.0
60	Saline	30	0.277 ± .004 (2)	62.0
60	Saline	60	0.281 ± .025 (6)	64.3
60	7 amino acids	30	0.236 ± .017 (2)	38.0
60	7 amino acids	60	0.201 ± .012 (3)	17.5
60	7 amino acids	90	0.207 ± .007 (2)	20.7
60	12 amino acids	60	0.275 ± .005 (2)	60.8
60	7 + 12 amino acids	60	0.214 ± .006 (3)	25.1

*First injection was saline; all other animals received phenylalanine as first injection. †The ratio of the optical absorption of each at 254 nm.

It appears that a balanced amino acid mixture, containing all of the amino acids cotransported with phenylalanine, may be necessary to restore brain polyribosomes to near normal levels. When single amino acids of the large neutral amino acid class (methionine, tryptophan, and leucine) were injected, we found no measurable reversal of the effects of phenylalanine. The necessity for a balanced amount of all the large neutral amino acids, and the narrow range of concentrations that was required for even a partial recovery, are probably related to the inhibitory effects that several of the large neutral amino acids have on brain polyribosomes and protein synthesis (*2, 8*). We do not know why all of the effects of phenylalanine on brain polyribosomes cannot be reversed. Perhaps there is an optimum concentration of each large neutral amino acid in the mixture that would allow full recovery.

It has been proposed that a loss of amino acids from the brain may be associated with the inhibition of neural protein synthesis and the effects on brain polyribosome profiles (*1, 4*). Because these seven amino acids can apparently reverse the effects of a single dose of phenylalanine on neural polyribosomes, it will be of interest to determine whether the large neutral amino acids are equally effective during the more chronic conditions of hyperphenylalanemia which can be experimentally induced through inhibitors of phenylalanine hydroxylase (*10*). Perhaps these amino acids will be able to restore some balance to the mammalian brain for protein synthesis and other metabolic reactions. Conceivably, a balanced diet of certain amino acids could have some application in avoiding the mental retardation that is associated with hyperphenylalanemia and phenylketonuria in humans.

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References and Notes

1. K. Aoki and F. L. Siegel, *Science* **168**, 129 (1970); F. L. Siegel, K. Aoki, R. E. Colwell, *J. Neurochem.* **18**, 537 (1971).
2. J. W. MacInnes and K. Schlesinger, *Brain Res.* **29**, 101 (1971).
3. F. Taub and T. C. Johnson, *Biochem. J.* **151**, 173 (1975).
4. C. M. McKean, D. E. Boggs, N. A. Peterson, *J. Neurochem.* **15**, 235 (1968); S. Roberts, *Prog. Brain Res.* **1967**, 335 (1967); J. V. Hughes and T. C. Johnson, *J. Neurochem.* **26**, 1105 (1976).
5. S. H. Appel, in *Protein Metabolism of the Nervous System*, A. Lajtha, Ed. (Plenum, New York, 1970), p. 621.
6. Only neonatal mice (2 to 7 days old) were used because it was reported previously that the brain polyribosomes of only young animals are significantly affected by phenylalanine (*1–3*). For each experimental condition the brains of five to six animals were pooled.
7. The seven large neutral amino acids include valine, leucine, isoleucine, methionine, tyrosine, tryptophan, and threonine, as outlined by

S. R. Cohen and A. Lajtha, in *Handbook of Neurochemistry*, A. Lajtha, Ed. (Plenum, New York, 1972), vol. 1, p. 543.
8. N. Tudball and R. Griffiths, *J. Neurochem.* **26**, 1149 (1976).
9. The other 12 amino acids included alanine, proline, glycine, serine, cysteine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine. These were injected at a dose of 0.01 mg of the L form of each amino acid per gram of body weight.
10. M. A. Lipton, R. Gordon, G. Guroff, S. Udenfriend, *Science* **156**, 248 (1967); K. N. Antonas, W. F. Coulson, J. B. Jepson, *Biochem. Soc. Trans.* **2**, 105 (1974); O. Greengard, M. S. Yoss, J. A. Del Valle, *Science* **192**, 1007 (1976).
11. Supported by grant 430-13 RD from the Illinois Department of Mental Health and Developmental Disabilities and grant BNS 76-04507 from the National Science Foundation.

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Congenital Transmission of a Papovavirus of the Stump-Tailed Macaque

Abstract. *Stump-tailed macaque virus, a newly recognized papovavirus of the SV40 polyoma subgroup, was demonstrated in kidney cultures from each of five stump-tailed macaque fetuses in the second half of gestation and from six adult stump-tailed macaques. Such regular presence of virus in the fetus is an unusual feature for a papovavirus.*

The presence of stump-tailed macaque virus (STMV) was first recognized by thin-section electron microscopy in each of 15 kidney cultures from normal stump-tailed macaques (*Macaca speciosa*) which spontaneously developed an intense cytoplasmic vacuolated cytopathic effect (CPE) upon a few in vitro passages of the cultures (*1*). The virus was identified as a new papovavirus of the SV40-polyoma subgroup on the basis of its morphology, intracellular distribution, antigenic relationships, and DNA size and structure (*1–3*). Because the virus was found in kidney cultures of all animals, young and old, as well as in kidney cultures of a near-term stump-tailed macaque fetus (*4*), we examined the possibility of consistent congenital transmission of the virus. Kidneys and other tissues of five fetuses of the stump-tailed macaques and kidneys of six adults were cultured and monitored for virus expression. In addition to finding the virus in all the adult kidneys, we isolated STMV from every fetal kidney. These findings indicate that STMV is consistently transmitted congenitally from one generation to the next. This feature of STMV biology is an unusual one for papovaviruses which, so far, have been known to be transmitted only as horizontal infections acquired in postnatal life (*5*).

Five stump-tailed macaque fetuses of gestational ages between 84 and 133 days were obtained from the Biologic Resources Laboratory, Chicago, Illinois. The gestation period for the stump-tailed macaque is 164 days. A total of 21 tissues—kidney, lung, and skin from each of five fetuses and placenta and brain from each of three fetuses—were examined. Cultures initiated by trypsinization of minced tissues were maintained, with and without serial passage, in Eagle's minimum essential medium (Earle's salts) supplemented with fetal calf serum (10 percent) and antibiotics. In order to ensure against accidental contamination, primary cultures from 13 of these tissues, derived from three fetuses, were prepared in duplicate sets. After the first week, one set of cultures was monitored in Baltimore, Maryland, and the other set was monitored in Covington, Louisiana. Cultures from kidney, lung, skin, and brain grew vigorously and were serially passed once every week or 15 days for the first few passages; those from placenta grew very slowly and subsequent passages were less frequent. Cultures were monitored weekly for CPE (*1*) and periodically for STMV-specific nuclear immunofluorescence (IF) (*2*), for up to 130 days. At the end of the study and when indicated by the occurrence of CPE, cultures were screened for virions of papovavirus morphology by electron microscopy (EM) after negative staining. At present, no cell line is available for an infectivity assay of STMV.

The details of observations and tests on the five fetal kidney cultures are sum-

Table 1. Development of STMV cytopathic effect and immunofluorescence (IF) in kidney cultures of fetal stump-tailed macaques.

Fetus		Passage	Cytopathic effect and viral IF at week*																	
No.	Age (days)		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1238	84	Pr	−	−/*	−	−	−	−	−												
		P-1		−	−	−/*	−	−	−	−	−	−	−	−	−	−	−	⊖			
		P-2			−	−	−/*	−	−	−	+	+									
		P-3				−	−	−	−/*	−	+	⊕									
		P-4						−	−	*/−	+										
		P-5								−	−	+/*									
		P-6										−	−								
424	97	Pr	−	−/*	−	−	−	−	−	*/−	−	−	−	−	−	−	−	−	−↘	−	−
		P-1		−	−	−/*	−	−	−	−	−	−	−	*/−							
		P-2			−	−	−/*	−	−	−	−	+	+		−	−	−	−	−		
		P-3					−	−	−	−/*	+	⊕	+	+	+	⊕					
		P-4								−	−/*	+	+	+							
		P-5									−	+	+	+							
		P-6																			
1281	118	Pr	−	−/*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*/−
		P-1		−	−	−/*	−	−	−	−	−	−	−	−	−	−	−	−	−/*	−↘	⊖
		P-2			−	−	−/*	−	−	−	−										
		P-3				−	−	−	−/*	−	−	+									
		P-4							−	−	−	−	⊕								
		P-5								−	−	+/*									
		P-6										⊕									
329	123	Pr	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		P-1		−	−/*	−	−	−	−	−	−	−	−	−	−	*/−					
		P-2			−	−	−/*	−	−	−	−	−	−	−	+	+	+				
		P-3					−	−/*	−	−	−	−	−	−	+						
		P-4						−	−	−/*	−	−	+	⊕							
		P-5								−	−	−	⊕	+	+						
		P-6											+	+	+						
588	133	Pr	−	−	−	−	−	−	−	−											
		P-1		−/*	−	−	−	+	+	+											
		P-2			−	−/*	−	−	−	⊕											
		P-3				−	−	−/*	−	+	+										
		P-4						−	−	+/*											
		P-5							−	+	+										
		P-6																			

*− and + refer to negative and positive CPE, respectively. Negative IF is indicated by an asterisk, and positive IF by a circle around the CPE symbol. Two passages in week 17 of observation are indicated by arrows. The STMV-immune serum used in IF tests was reactive with STMV antigens but not to antigens of SV40, BK virus, and polyoma virus (*3*).