The Mechanism of Polyribosome Disaggregation in Brain Tissue by Phenylalanine

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The injection of neonatal mice with phenylalanine resulted in a rapid decrease in brain polyribosomes and a concomitant increase in monomeric ribosomes. Animals of 1–16 days of age were equally affected by phenylalanine, although the brain polyribosomes of 60-day-old mice were relatively resistant to the effects of phenylalanine. The population of free polyribosomes appeared to be more sensitive to phenylalanine treatment than bound polyribosomes, which were somewhat more resistant to disruption by high concentrations of the amino acid. The effects of phenylalanine were more pronounced with polyribosomes in the cerebral cortex than with those in the cerebellar tissue. The mechanism of polyribosome disruption was shown to be independent of hydrolysis mediated by ribonuclease. Virtually all of the monomeric ribosomes that resulted from phenylalanine treatment were shown to be inactive with regard to endogenous protein synthesis and were present in the cell cytoplasm as vacant couples. These ribosomes were readily dissociated by treatment with 0.5M-KCl and subsequent ultracentrifugation. These results are discussed in the light of the possibility that high concentrations of phenylalanine disrupt brain protein synthesis by a molecular mechanism that is associated with initiation events.

A chronic excess of phenylalanine in the blood circulatory system has been shown to have marked effects on the developing brain of animals and humans (Waisman *et al.*, 1960; Schlesinger *et al.*, 1969). Acute administration of phenylalanine also has serious effects on brain protein metabolism. Brain amino acid concentrations are altered by high concentrations of phenylalanine both *in vivo* (McKean *et al.*, 1968; Swaiman *et al.*, 1968) and *in vitro* (Appel, 1966). Others have demonstrated that the administration of phenylalanine causes a 'disaggregation' of brain cell polyribosomes (Aoki & Siegel, 1970; Siegel *et al.*, 1971; MacInnes & Schlesinger, 1971). However, the mechanism of the phenylalanine-induced disruption of neural protein synthesis is presently unknown.

Several theories have been proposed to explain these previous findings. Appel (1966) and McKean *et al.* (1968) have suggested that excess of one amino acid (i.e. phenylalanine) could decrease the concentrations of other essential amino acids by competition for common transport mechanisms. Appel (1966) has suggested that some amino acids may also compete or interfere with the enzymic activation and subsequent acylation of amino acids to tRNA molecules. Roscoe *et al.* (1968) have found that high concentrations of phenylalanine also inhibited the incorporation of leucine in Krebs 2 ascites cells and cell-free systems. Although it is not difficult to understand how altered precursor concentrations (aminoacyl-tRNA) may cause a decreased measured rate of protein synthesis, it does not easily explain the

relationship between amino acid concentrations and the observed polyribosome 'disaggregation' in brain cells. Under some circumstances the presence of a rate-limiting supply of amino acids has been shown apparently to stabilize cell polyribosomes and decrease the transition of ribosomes from the polyribosomes to the monomeric ribosomes (Baliga *et al.*, 1968). A deficiency of only tryptophan, and not of other amino acids, evidently leads to a 'disaggregation' of polyribosomes in rabbit reticulocytes (Hori *et al.*, 1967).

Mammalian brain polyribosomes have been reported to be more unstable than those of other tissues (Zomzely *et al.*, 1966, 1968). This lability may be of some importance with regard to the polyribosome alterations that are evoked by high concentrations of phenylalanine, since the brain tissue, particularly that of neonatal animals, appears to be most susceptible (Aoki & Siegel, 1970). The present paper is primarily directed towards an elucidation of the mechanism by which phenylalanine disrupts brain polyribosomes and the effects of aging on polyribosome stability in the presence of high concentrations of amino acid.

Materials and Methods

Materials

SCH:ARS (ICR), non-inbred Swiss albino mice were purchased from ARS/Sprague-Dawley, Madison, Wis., U.S.A. RNAase*-free sucrose was pur-

* Abbreviation: RNAase, ribonuclease.

chased from Schwarz/Mann, Orangeburg, N.Y., U.S.A. L-Phenylalanine was purchased from Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.

Methods

Administration of phenylalanine. L-Phenylalanine was dissolved in 0.15M-NaCl to a final concentration of 30mg/ml. The resulting amino acid solution was pre-warmed to 37°C before injection and administered by intraperitoneal injection at a dose of 1 or 2mg of phenylalanine/g body wt. (30 μ l or 60 μ l/g). Matched control animals were injected with a comparable amount of saline (0.9% NaCl) alone. Animals less than 12 days of age were maintained at 37°C after their injection with either saline or phenylalanine.

Isolation of brain polyribosomes. Brain polyribosomes were isolated by a modification of the technique of Lerner *et al.* (1971). Whole brains or specific areas, as described in the text, were quickly removed and homogenized in an ice-cold TKM buffer solution (50mM-Tris-HCl, pH 7.2, 25mM-KCl, 5mM-MgCl₂) which contained 1mg of bentonite/ml (Lerner & Johnson, 1970). In some experiments, which are indicated in the text, bentonite was omitted from the homogenization medium. The tissue was homogenized with a motor-driven glass-Teflon homogenizer and the resulting homogenate was centrifuged at 8400g for 15 min at 4°C to remove mitochondria, synaptosomes and cellular debris. After this preliminary centrifugation the supernatant containing the free polyribosomes was removed, and sodium deoxycholate and Tween-40 were added to a final concentration of 1.0%. The polyribosomes were then purified by gel filtration through a Sephadex G-200 column (1.1cm $\times$ 10cm) that had been previously equilibrated with TKM buffer at 4°C. Both the polyribosomes and monomeric ribosomes were eluted from the column in the void volume and subjected to centrifugation analysis.

Isolation of membrane-bound brain polyribosomes. Membrane-bound polyribosomes were isolated from the 8400g pellet that was obtained from the preliminary centrifugation step described above. The pellet was resuspended in TKM buffer at 4°C, re-centrifuged at 8400g and the resulting pellet resuspended in TKM buffer. Sodium deoxycholate and Tween-40 were then added to final concentrations of 0.5% and 1.0% respectively (Lerner *et al.*, 1971). The suspension was incubated at 4°C for 10min, centrifuged at 8400g for 15min and the freed polyribosomes in the resulting supernatant were removed. Without the addition of detergents, extensive washing and rehomogenization of the washed 8400g pellet did not release a significant amount of ribosomal material. Therefore the bound polyribosomes that were liberated by sodium deoxy-

cholate and Tween-40 did not appear to be a result of insufficient cell breakage during the homogenization step or the adventitious entrapment of ribosomal material by cellular debris during centrifugation.

Sucrose-gradient analysis of brain polyribosomes. The brain polyribosome preparations (approx. 3–5 E_{254} units) were layered on 3.8ml linear (0.7–1.5M) sucrose gradients in TKM buffer. The samples were centrifuged at 280000g_{av.} for 72min in an International model SB405 rotor at 4°C. Duplicate gradients were always run for each experimental or control determination. After centrifugation the sucrose was removed by a peristaltic pump from the top through a Buchler Densi-Flow apparatus and the E_{254} monitored with an Isco model UA-2 u.v. analyser. Areas under specific peaks were determined with the aid of a compensating polar planimeter.

Gradient analysis of ribosomal subunits. The quantity of native ribosomal subunits was measured by analysis in 0.7–1.5M-sucrose gradient in TKM buffer essentially as described above except that the time of centrifugation at 280000g_{av.} was extended to 156min. After centrifugation the E_{254} was continually monitored and the areas under the monoribosome and subunit peaks were determined by planimetry.

The relative proportion of inactive ribosomes was determined by their dissociation with KCl and subsequent sedimentation profiles. Brain polyribosome preparations were adjusted with 3M-KCl to a final concentration of 0.5M-KCl, placed on linear 0.7–1.5M-sucrose gradients in TKM buffer–0.5M-KCl and centrifuged at 280000g_{av.} for 156min as described above.

Results

To measure the effect of phenylalanine on the sedimentation properties of mouse brain polyribosomes, animals were given a single intraperitoneal injection of phenylalanine at a dose of 2mg/g body wt. Then 30min later the brains were removed and the polyribosomes of saline-injected control animals and phenylalanine-treated animals were resolved on linear sucrose gradients as described in the Materials and Methods section. The introduction of this dose of phenylalanine resulted in a decrease in the proportion of ribosomes that were associated with the polyribosome fractions and a concomitant increase in the proportion of single monomeric (80S) ribosomes (Fig. 1). The decrease in polyribosome material was readily apparent on inspection of the gradient profiles and could be accurately quantified by measuring the quantity of absorbing material (E_{254}) in the monoribosome and polyribosome regions by planimetry. This

Fig. 1. Sedimentation profiles of brain polyribosomes isolated from saline- and phenylalanine-injected mice

At 30 min after injection of 2-day-old mice with either saline (a) or 2 mg of phenylalanine/g body wt. (b) brains were removed and samples for centrifugation were prepared as described in the Materials and Methods section. A portion (3.0 E_{260} units) of each sample was placed on a linear (0.7–1.5 M) sucrose gradient in TKM buffer which was centrifuged for 72 min and analysed as described. The direction of sedimentation was from right to left.

allowed an assessment, quantitatively, of the percentage of ribosomal material associated with each region of the gradient. This method also circumvented any experimental error that may have been introduced by the addition of variable amounts of material absorbing at 254 nm to individual sucrose gradients. Additional experiments (not shown) clearly illustrated that the injection of 0.9% NaCl alone did not lead to changes in brain polyribosome patterns.

The kinetics of the loss of polyribosome material, as a result of a single injection of phenylalanine, was studied over a 90 min period. The shift of ribosomes from the polyribosome regions of the gradients of the monoribosome (80S) fractions occurred rapidly and most of the effects of phenylalanine were expressed by 30 min (Table 1). A dose of 2 mg of phenylalanine/g body wt. did not result in any measurable recovery within the 90 min incubation

Table 1. Increase in brain monomeric ribosomes as a result of phenylalanine injection

Mice (5 days old) were injected with 2 mg of phenylalanine/g body wt. and the free polyribosomes were isolated and analysed by linear (0.7–1.5 M) sucrose-gradient centrifugation as described in the Materials and Methods section. The relative proportion of 254 nm-absorbing material of the monoribosome region to that of the total ribosomal material was measured with a compensating polar planimeter. Duplicate gradients were used in each experiment and the number of independent experiments for each time-interval is indicated in parentheses. No change in polyribosome profiles was observed in saline-injected control animals for any time-period indicated below.

Time after phenylalanine injection (min)	E_{254} of monoribosomes/total E_{254}	Increase in monoribosomes (%)
0	0.225 (9)	—
5	0.193 (1)	0
15	0.273 (3)	21
30	0.332 (7)	48
40	0.318 (6)	41
45	0.323 (1)	44
90	0.338 (2)	50

Table 2. Effect of age on brain polyribosome sensitivity to phenylalanine

Animals were injected with either saline or 2 mg of phenylalanine/g body wt., and 30 min after injection the percentage of monoribosomes was determined by sucrose-gradient centrifugation as described in the Materials and Methods section. Duplicate gradients were used in each experiment and the number of independent experiments for each age group is indicated in parentheses.

Age (days)	Percentage of monomeric ribosomes		Difference (a–b)	Increase (%)
	+Phenylalanine (a)	Saline (b)		
0–1	37.4 (2)	26.8 (2)	11.6	45
6–8	32.1 (5)	22.3 (4)	9.7	44
10–16	31.9 (7)	21.5 (4)	10.4	48
60–70	28.0 (3)	25.0 (3)	2.8	11

period. Similar experiments with a dose of 1 mg of phenylalanine/g body wt. showed a comparable increase in brain monomeric ribosomes, although complete recovery occurred within 120 min.

When animals of various ages were injected with phenylalanine as described in Fig. 1, a distinct maturation-dependent change in sensitivity to high concentrations of the amino acid was measured. During the first 2 weeks of postnatal life brain polyribosomes were sensitive to the presence of phenylalanine (Table 2). However, by the time the

Fig. 2. Maturation-dependent changes in the sensitivity of brain polyribosomes to an intraperitoneal injection of phenylalanine

Animals of various ages were injected with either saline (●) or 2mg of phenylalanine/g body wt. (○). Each point represents the mean of at least two independent experiments, each of which included duplicate gradients.

Table 3. Sensitivity of mouse brain free and membrane-bound polyribosomes to phenylalanine

Mice (7 days old) were injected with phenylalanine or saline as described in Table 1, and 30min after injection the free and bound polyribosomes were isolated as described in the Materials and Methods section. Each determination represents duplicate gradients; three independent experiments were performed.

Poly-ribosomes	Percentage of monomeric ribosomes		Difference (a-b)	Increase (%)
	+Phenylalanine (a)	Saline (b)		
Free	26.5	18.0	8.5	47.2
Bound	28.7	22.5	6.2	27.6

animals were 60 days old there was a marked decrease in polyribosome 'disaggregation' when phenylalanine was injected. There was a slight change in the percentage of total ribosomes that were found as native monoribosomes during postnatal development (Fig. 2). Despite this slight, but measurable, maturation-dependent alteration, it was obvious that the resistance of more mature brain polyribosomes to phenylalanine was not the result of a major change in the monoribosome/polyribosome ratios that could be attributed to aging alone (Fig. 2).

All of the above experiments were carried out with brain polyribosome and ribosome preparations which

Table 4. Sensitivity of cerebral-cortex and cerebellum polyribosomes to phenylalanine

Mice (7 days old) were injected with saline or phenylalanine as described in Table 1, and 30min after injection polyribosomes were isolated from the cerebral cortex and cerebellum. Each determination represents duplicate gradients; four independent experiments were performed.

Brain area	Percentage of monomeric ribosomes		Difference (a-b)	Increase (%)
	+Phenylalanine (a)	Saline (b)		
Cerebral cortex	33.9	20.2	13.7	68
Cerebellum	32.3	24.2	8.1	33

were primarily free and not bound to membranous structures (Lerner *et al.*, 1971). Therefore a series of experiments was designed to test the relative sensitivity of free and bound brain polyribosomes to phenylalanine injection. The results clearly illustrated that both populations of brain polyribosomes were affected by high concentrations of phenylalanine. The free polyribosomes, as measured by the absolute change in material absorbing at 254nm or the percentage increase in monomeric ribosomes, showed a response of greater magnitude than that measured with bound polyribosomes (Table 3). In the light of these observations, further experimentation was limited to effects of phenylalanine on free polyribosome preparations.

To measure possible differences in polyribosome sensitivity between major brain areas, animals were injected with 2mg of phenylalanine/g body wt., and 30min later the polyribosomes of the cerebral cortex and the cerebellum were analysed by sucrose-gradient centrifugation. Before homogenization the cerebellum and tissue slices from the cerebral cortex of phenylalanine- and saline-injected animals were quickly prepared by hand dissection. Cerebellar and cerebral tissue from five brains were pooled before homogenization, to obtain sufficient material for duplicate sucrose gradients. The polyribosome profiles from these animals indicated that the effects of phenylalanine were greater in the cerebral cortex than in the cerebellum (Table 4). However, we consider that some degree of caution should be taken in this interpretation, since the proportion of monomeric ribosomes in the saline-injected control animals showed that slightly more of the material absorbing at 254nm was initially in polyribosomes in samples of the cerebral cortex than in samples of the cerebellum. Therefore the difference in response to phenylalanine, at least in part, may be the result of the degree of polyribosome formation in these two areas before phenylalanine treatment.

Fig. 3. Sedimentation profiles of brain ribosomes and native ribosomal subunits from saline- and phenylalanine-injected mice

At 30min after injection of either saline (a) or phenylalanine (b) as described in Fig. 1, samples were prepared, centrifuged and analysed as described, except that centrifugation was carried out for 156min (see the Materials and Methods section). The direction of sedimentation was from right to left.

Fig. 4. Effects of phenylalanine on the relative proportion of ribosomes in distinct polyribosome fractions

Mice (5 days old) were injected with saline (●) or 2mg of phenylalanine/g body wt. (○) and the polyribosomes subjected to sucrose-gradient analysis as described in the Materials and Methods section. The areas under each polyribosome peak (two to seven ribosomes per polyribosome) and the area under the monoribosome region were compared with the total material absorbing at 254nm on each gradient. Each point represents the mean of two independent experiments and duplicate gradients.

We reasoned that the mechanism of the phenylalanine effects on brain protein synthesis might be approached by a characterization of the products that result from polyribosome 'disaggregation'. For instance, it was possible that phenylalanine caused an increase in ribosomal subunits that was comparable with the measured increase in monoribosomes. Previous centrifugation conditions were not adequate to resolve this possibility. Therefore samples from phenylalanine-treated and saline-injected animals were placed on sucrose gradients and centrifuged at $180000g_{av}$ for 156min. To avoid any loss of native ribosomal subunits during preparation, the gel-filtration step was not carried out before centrifugation analysis. These methods allowed the measurement of free 60S ribosomal subunits in both experimental and control animals. However, 40S ribosomal subunits, if present at all, were not of sufficient quantity to be detected and were most probably masked by soluble protein and nucleic acid material near the top of the sucrose gradients.

Even the 60S ribosomal subunits were found in small quantities in brain tissue, and, despite the marked increase in monomeric ribosomes in phenylalanine-treated animals, there was not a significant alteration in free ribosomal subunits (Fig. 3).

We considered the possibility that high concentrations of phenylalanine might cause an activation of RNAase which could lead to the hydrolysis of mRNA and an increase in the relative proportion of brain monoribosomes. During the course of these experiments several lines of evidence suggested that the disruption of brain polyribosomes was not mediated by the action of RNAase. We found that the $8400g$ supernatants could be stored at $0^{\circ}C$ for as long as 24h without a detectable increase in the percentage of monomeric ribosomes. In addition, polyribosome profiles were identical in freshly prepared and stored brain preparations whether or not bentonite was used in the original isolation procedure. However, these data primarily ruled out the possibility that RNAase action might have influenced the gradient profiles after the removal of the brain tissue and the preparation of the tissue

Fig. 5. Effect of phenylalanine on KCl dissociation of brain monomeric ribosomes obtained from saline- and phenylalanine-injected mice

Mice (5 days old) were injected with saline (a), phenylalanine (1mg/g body wt.) (b), or phenylalanine (2mg/g body wt.) (c), and 30min after injection brains were removed and samples for centrifugation were prepared. Before centrifugation the samples were made 0.5M with respect to KCl and centrifuged on linear (0.7–1.5M) sucrose gradients in TKM–0.5M-KCl buffer as described in the Materials and Methods section. The direction of sedimentation was from right to left.

homogenates. The possible role of RNAase action *in vivo* was assessed by a direct analysis of the brain polyribosome profiles in phenylalanine- and saline-injected animals. A random hydrolysis of mRNA by RNAase would be expected to decrease the material absorbing at 254nm in the larger polyribosome regions of the gradients and increase the proportions of this material in the areas of smaller polyribosomes. The resolution that we obtained between various-sized polyribosomes by sucrose-gradient centrifugation allowed a quantitative measurement of the amounts of ribosomal material from monoribosome areas to polyribosome areas that were composed of seven ribosomes (Fig. 1). This type of analysis showed that phenylalanine did not cause an increase in smaller polyribosomes. In fact, when polyribosomes of control and phenylalanine-treated mice were compared, there was either a decrease in the percentage of material absorbing at 254nm in the polyribosome region (four to seven ribosomes per polyribosome) or no detectable change (two or three ribosomes per polyribosome) as a result of phenylalanine treatment (Fig. 4). Although the resolution of individual polyribosome species greater than seven ribosomes per chain was not sufficient to permit accurate measurement of the

areas under specific peaks, it was possible to observe that phenylalanine caused a decrease in the proportion of material absorbing at 254nm in these larger polyribosomes. This ruled out the possibility that larger polyribosomes may have accumulated as a result of high concentrations of phenylalanine. Therefore it is doubtful that the primary effect of hyperphenylalaninaemia on protein synthesis is related to a decreased rate of translocation or termination.

A change in the rate of the initiation of protein synthesis was compatible with the measured decrease in brain polyribosomes and the increase in monomeric ribosomes. Since mRNA and peptidyl-tRNA prevent the dissociation of active ribosomes by KCl (Belitsina & Spirin, 1970; Hamburger *et al.*, 1973; Mathews & Wettstein, 1974), we were able to discriminate between active monoribosomes and vacant couples (Martin & Hartwell, 1970) and characterize the 80S ribosomes that resulted from the 'disaggregation' of polyribosomes after an injection with phenylalanine. Polyribosome preparations from saline-injected control animals and mice injected with either 1 mg or 2 mg of phenylalanine/body wt. were subjected to KCl dissociation. The results clearly showed that the number of active monoribosomes (80S) were not appreciably altered by the

presence of high concentrations of phenylalanine (Fig. 5). In addition, the degree of aggregation of brain polyribosomes were not affected by the addition of KCl to a final concentration of 0.5M. However, there was a marked increase in ribosomal subunits (60S and 40S) in both groups of phenylalanine-treated animals in comparison with the saline-injected control group (Fig. 5). These results suggest that the presence of phenylalanine leads to the normal run-off of ribosomes from the mRNA with a decreased rate of initiation. Therefore it is most likely that brain translational events continue at normal, or near-normal, rates in the presence of high concentrations of phenylalanine with normal termination and release of ribosomes from the template RNA molecules. The primary molecular event of protein synthesis that is disrupted during hyperphenylalaninaemia is apparently associated with the inability of the cells to reinitiate polypeptide synthesis. These data also support our previous conclusion that the increase in monomeric ribosomes is not mediated by RNAase, since enzymic hydrolysis of mRNA would result in monoribosomes with mRNA and peptidyl-tRNA still attached, and these ribonucleoprotein particles would be resistant to KCl dissociation (Martin & Hartwell, 1970).

Discussion

The injection of 1–2mg of phenylalanine/g body wt. resulted in a marked and rapid disruption of mouse brain polyribosomes. In general, our results agree with those previously reported by other investigators (Aoki & Siegel, 1970; Siegel *et al.*, 1971; MacInnes & Schlesinger, 1971). In addition, we confirm their reports that suggested that neural maturation leads to an increased resistance of brain polyribosomes to high concentrations of phenylalanine. It is difficult to compare directly the quantitative aspects of our data with theirs, particularly the analyses of specific polyribosome species, since some authors have not included representative polyribosome profiles and others were not able to obtain a comparable resolution of discrete polyribosome peaks. We were not able to observe any significant increase in ribosomal dimers as was reported by Siegel *et al.* (1971). This difference may be related to the ability of ribosomes from rats to form mRNA-independent dimers more readily (Reader & Stanners, 1967) or the action of RNAase during their isolation procedure.

We found that phenylalanine produced a comparable disruption of brain polyribosomes in mice of 1–16 days of age. However, similar doses of the amino acid to animals that were over 60 days of age had little effect on the stability of brain polyribosomes. Whether this maturation-dependent change in sensitivity primarily reflects the higher rate of protein synthesis in neonatal brain tissue (Johnson &

Luttges, 1966; Gilbert & Johnson, 1972, 1974) or a qualitative change in ribosomal or polyribosomal characteristics (Zomzely *et al.*, 1968; Lerner & Johnson, 1970) is presently unknown. It is also possible that phenylalanine is more rapidly catabolized in more mature animals. Although neonatal brain tissue is more sensitive to the effects of high concentrations of phenylalanine than is mature brain tissue, there was a distinct regional variation in the apparent response to a single injection with phenylalanine. The disruption of polyribosomes, which led to an increase in monomeric ribosomes, was more evident in the cerebral cortex than in the cerebellum (Table 3).

Bound polyribosomes were isolated from the brains of saline-injected control animals and phenylalanine-treated mice by the method of Lerner *et al.* (1971). We found that this population of polyribosomes was affected by phenylalanine, although the degree of disruption was less than that measured with free polyribosomes. The greater sensitivity of free brain polyribosomes is consistent with the observations that free polyribosomes of mammalian brain (Hemminki, 1972) and mammalian liver (Khawaja, 1972) are more sensitive to inhibitors of protein synthesis, e.g. cycloheximide and NaF. It should be noted that, since the mRNA and nascent polypeptide may be involved in the binding of polyribosomes to the endoplasmic reticulum (Lee *et al.*, 1971; Rosbash & Penman, 1971; Faierman *et al.*, 1973), it is possible that the increased monomeric ribosomes (80S) which resulted from phenylalanine treatment may have originated, at least in part, from the population of bound ribosomes. This is particularly notable, since we have shown that these 80S ribosomes are vacant couples, free of mRNA and peptidyl-tRNA (Fig. 5).

The effects of phenylalanine on brain polyribosome integrity is clearly independent of RNAase action. An analysis of the monoribosomes that resulted from phenylalanine treatment by KCl dissociation showed that virtually all of the monoribosomes that could be attributed to phenylalanine existed in an inactive form, i.e. not associated with mRNA or peptidyl-tRNA. Although active ribosomes and polyribosomes were not affected by the addition of KCl to a final concentration of 0.5M, the monoribosomes that resulted from phenylalanine treatment were completely dissociated to ribosomal subunits (Fig. 5). This suggests that the inhibition of brain protein synthesis may be primarily related to events associated with the initiation of polypeptide synthesis. In the light of these observations, we suggest that the term 'disaggregation' that has been frequently used to describe this phenomenon is misleading. The present report illustrates that phenylalanine does not mediate its influence directly on polyribosome stability, but rather that high concentrations of this

amino acid cause an imbalance between normal rates of termination and re-initiation of protein synthesis.

The amino acid imbalance that results from high concentrations of phenylalanine (McKean *et al.*, 1968; Swaiman *et al.*, 1968) may result in a shift in the availability of aminoacyl-tRNA. A decrease in the proportion of acylated tRNA has been shown in other systems to influence protein initiation (Vaughan & Hansen, 1973) and the percentage of ribosomes that are bound as polyribosomal complexes.

The relationship between the acute administration of phenylalanine, where approx. 10% of the brain polyribosomes appear to be lost, and the metabolic aminoacidopathies that are associated with impaired neural development is presently difficult to establish. However, it is possible that a chronic condition that results in a slight, but selective, decrease in neural translational events might have a severe effect on brain proteolipid synthesis and neural development (Menkes, 1968; Shah *et al.*, 1972).

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