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**DEVELOPMENTAL ASSESSMENT OF INFANT MACAQUES RECEIVING
DIETARY ASPARTAME OR PHENYLALANINE**

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RECEIVING DIETARY ASPARTAME OR PHENYLALANINE**

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INTRODUCTION

Extraordinary sweetness or the ability to be substituted for sucrose is a desirable component of the diet for many people. Aspartame (APM), a nutritive artificial sweetening agent, thus holds promise because it consists of aspartic acid and phenylalanine, amino acids which are present in many dietary proteins. Aspartame is a dipeptide, and is split into amino acids by peptidases in the digestive tract. It is approximately 200 times as sweet as sucrose. The increasing incidence of juvenile onset diabetes and obesity in the population of the United States makes a nutritive artificial sweetener very appealing both to the public and to the health professional. The relationship between sucrose and dental caries provides another reason for utilization of artificial sweeteners in lieu of sucrose during childhood.

When administered in very high dosages, aspartic acid alone or Aspartame have been shown to cause damage to certain hypothalamic neurons in newborn mice (1, 2). It is noteworthy, however, that administering similar high dosages (2 mg/gm) of Aspartame to infant monkeys could not elicit hypothalamic damage (3).

The phenylalanine moiety of the molecule is of interest because of the hereditary disease, phenylketonuria. It has been demonstrated experimentally that elevating phenylalanine levels in the blood by administering large amounts of phenylalanine in the diet can produce abnormalities in the weanling rat (4) or the infant monkey (5). Perhaps of greatest concern was the finding that the infant monkey raised on a diet containing 3 gm/kg of body weight per day exhibited

grand-mal convulsions as well as persistent intellectual deficits (5). Subsequently, Waisman (6) began a study evaluating the effects of chronic APM ingestion upon growth and development in the infant macaque which was prematurely terminated by Dr. Waisman's death. The study was further flawed by lack of access to adequate numbers of infant rhesus monkeys at the time, the inclusion of one infant monkey with severe birth defects, and the presence of a Shigella sp. infection in two of the treatment groups essentially throughout the duration of the diet administration.

Since the approval of APM for dietary use would make it extensively available and even desirable in the diets of toddlers and children, exhaustive testing for safety in developing animal systems is mandatory. The most appropriate and available test animal prior to clinical studies is the Old World monkey. With respect to endocrine regulation, metabolic parameters, brain maturation, and nearly any other indices that have been investigated, the Old World macaque provides an excellent model for studies involving human growth and development. Dietary requirements and amino acid metabolism in the macaque have been well defined (7). This study provides for the intake of Aspartame and phenylalanine by a relatively large number of infant monkeys to assess the safety of APM as a dietary component during infancy. A special feeding protocol was designed to avoid an inherent problem in the prior Waisman studies (4, 6) involving administering special diets to infant monkeys. The infant monkeys were hand-held and fed from a nursing bottle approximately four times during a typical technician working-day. Thus, the desired dosage of amino acid being administered on a kg/day basis was in fact administered

over an 8-hour period. This meant that the infant monkeys were exposed to extraordinarily high amino acid intake levels during 8 hours, and were not exposed to the diet at all during the remaining 16 hours of each day.

MATERIAL AND METHODS

Infant Macaca arctoides were selected for the study. All infants were born in the closed M. arctoides colony in the Primate Facility of the Biologic Resources Laboratory, University of Illinois at the Medical Center. M. arctoides is closely related to M. mulatta, the rhesus monkey, and interbreeds with this species in the wild.

The doses of APM chosen were 1.0, 2.0 and 3.0 gm/kg per day, which are massive intakes. Phenylalanine was given at a level of 1.65 gm/kg per day, equimolar to the phenylalanine level in a 3 gm/kg APM load. Four infants were placed in each of these four treatment groups and four infants were assigned to a control group (Table 1).

Following birth, each infant was allowed to remain with its mother for a minimum of one week to insure stability and good sucking ability. Each infant was then weaned by being placed in an incubator and taught to nurse itself from a plastic bottle with a rubber nipple (Fig. 1). At the beginning of the experimental diet period, each infant was assigned at random to a diet of plain infant formula (Similac with iron) or one of formula plus an amino acid addiye. Water was available at all times to the infant monkeys. When infant monkeys reached the point that incubators no longer provided comfortable accomodations, they were moved into individual large activity cages. In these cages water and formula were initially available in the plastic bottles with rubber nipples used in the incubators. Usually around three months of age, large glass bottles were suspended on the outside cage walls with metal drinking spigots projecting into the cages.

Each week the prior week's intake of formula, with or without the added amino acid, was assessed in order to estimate the next week's daily intake. APM or phenylalanine was added to sufficient quantity during formula preparation each day so as to achieve the desired dose. Each morning, after cage cleaning, fresh formula and water were substituted for the previous day's allotment. Careful records were kept on a daily basis with respect to milk and water intake. This procedure provides a more normal feeding situation over a 24-hour period than did the protocol of Waisman and colleagues where the infants were given the APM or phenylalanine loads only over the 8-hour work day. At four months of age when weaning normally occurs, infant monkeys are usually started on solid monkey biscuits. Because this study mandated being on a liquid diet for 270 days, only the customary daily allotment of one piece of fruit (apples, oranges or bananas) and a weekly vitamin sandwich were initiated at 3 months of age. The infant monkeys were not started on monkey biscuits until the end of the 270 day feeding period on the liquid diet. At this time, the infant monkeys were moved from the individual cages and placed into large gang cages and given the routine monkey chow diet.

Blood Analyses: Blood samples were obtained from the saphenous vein from all of the infant monkeys at 2, 4, 6, 8 and 9 months of treatment. On days that blood samples were to be drawn, the infants were not allowed access to formula from 6 until 10 AM when the blood samples were drawn. Since the infants were on a liquid diet, this would essentially provide a fasted sample. Bloods were then analyzed for serum electrolytes, serum osmolality, CBC, and fasting glucose levels. For amino acid analyses, blood samples were immediately centrifuged to remove erythrocytes and the plasma prepared as described by Stegink et al. (8).

Amino acid analyses were carried out on automated amino acid analyzers (Beckman 121 M, Beckman Instruments, Palo Alto, California).

Urine: Urine was checked at intervals by labstix for pH, occult blood protein, glucose, ketones, and bilirubin and by Phenistix for phenylketones.

Growth: Each infant was weighed on a weekly basis. Once a month, each infant was measured with respect to crown-rump and crown-heel length, head circumference, and examined for various developmental milestones. These included extent of teething, ability to vocalize, alertness, tractibility, and general behavior. Two research assistants were involved in this study from its beginning to its end and the infant monkeys socialized with them.

Electroencephalograms: Electroencephalograms were administered to the infant monkeys prior to or immediately after going on the diet. Electroencephalograms were also performed at 4 and 9 months of treatment. After the monkeys were removed from the experimental diets, eight animals were continued on the infant formula without additive for a month and then received EEG's. Some infants received EEG's at quarterly intervals following the termination of the experimental diet. All EEG's were performed in the Department of Neurology by the same EEG technician and were analyzed blindly by faculty in the Department of Neurology, University of Illinois at the Medical Center. The EEG's were performed utilizing techniques similar to those for newborn infants. Because of the rapid motor development of the infants,

it was necessary to wrap them securely in towels before lightly sedating with 10 % chloral hydrate as a prelude to attaching EEG leads.

The exact dates for embarking on each experimental diet, the times of EEG administration and blood sampling can be found in Appendix 1.

RESULTS

Dietary intakes: The feeding regimen chosen in order to provide each infant with constant access to formula or formula plus amino acid and yet to insure consumption of the desired amount of APM or phenylalanine on a per kg body weight basis was successful (Fig. 2). Average intakes closely approached the desired level of dosing, especially for those animals receiving phenylalanine or APM at the 1 and 2 gm/kg dose level. The animals in group 5, who were to consume 3 gm/kg per day of APM or typically consumed on the order of 2.5 - 2.7 gm/kg per day. It should be noted that in this context that APM has a very low solubility and the 3.0 gm/kg load was technically difficult to achieve. When placed in the suspended bottles, the APM tended to come out of solution upon standing and also at times clogged the feeding spigots. Various methods were attempted to remedy this technical difficulty, including agitation of milk within the bottles to little avail. Nevertheless, as depicted in Figure 2, the series of animals receiving APM did experience markedly different dose levels, and intake levels within each group were uniform throughout the study. (The standard errors were less than 1% of the mean for all the monthly values.)

Milk and water intakes: As would be expected, the daily milk (Table 2) and water intake (Table 3) increased markedly as the infants grew over the nine months of diet administration. Both analysis of variance and the Student-Newman-Kuels test were performed on these data and no significant differences were found between any of the group with

respect to milk or water intake during the 270 days each group was involved in the study. It should be noted that group 2, receiving 1.65 gm/kg of phenylalanine per day, exhibited the lowest milk intake throughout the study (Table 2). Group 5, receiving 3 gm/kg of APM per day exhibited the most extensive water intake throughout these studies (Table 3). Total daily intakes of milk plus water are depicted in Figure 3. Again, no significant differences between any of the groups were noted when milk and water intakes were added together to indicate the entire amount ingested during the study. When a ratio was developed of mean milk intake/mean water intake and graphed (Fig. 4) some interesting relationships emerged. Group 3, receiving 1 gm/kg APM, exhibited the largest formula consumption in relation to water consumed. This difference was significant at 5 months from the other groups ($p < .05$). The two groups exhibiting the largest water intake in relationship to formula intake were those animals receiving the most amino acid added to the diet, 3 gm/kg of APM or 1.65 gm/kg of phenylalanine. Thus, animals receiving the greatest concentration of amino acid simply consumed more water in relation to their formula intake.

Growth rates: Whether analyzed on an individual basis (Fig. 5) or as groups (Fig. 6), incremental weight gain was remarkably uniform. The regression coefficients (Fig. 6) were also very similar. Group 2, receiving 1.65 gm/kg of phenylalanine, exhibited a slightly lower growth rate than did the other four groups, whose rates were essentially identical. The crown-heel growth rate was also uniform for all five groups (Fig. 7) and no differences were present between the control

animals of Group 1, and any of the four experimental groups.

Blood indices: Hematocrit, hemoglobin levels, WBC and RBC were normal for all groups throughout the study. Blood glucose levels ranged from 33 to 100 mg% which is well within the normal range for the infant macaque during the administration of the diet. Sodium, chloride and potassium levels, and serum osmolality were all within normal ranges for the duration of the study.

Urine indices: A normal pH and no significant levels of protein, glucose or ketones were observed in the urine specimens tested at intervals from each animal throughout the study.

EEG determinations: Fortuitously, an infant rhesus monkey not involved in any experimental study was diagnosed as having grand mal seizures coincidentally with the present study. An extensive series of electroencephalograms were performed on this infant monkey who exhibited the typical spike and wave discharges seen in brain activity characteristic of individuals with phenylketonuria or epilepsy. In contrast, seizure patterns were never observed in any of the 20 infant macaques. The occasional abnormalities in EEG's indicated in Table 4 are typical of EEG recordings from growing human infants. It can be noted that no infant persisted in exhibiting spikes or transient waves throughout the period of the study. It should be noted that the last three categories or the so-called "follow-up" EEG's were obtained on animals in the study at intervals simply because they were waiting to be sent to another state for behavioral testing.

Blood amino acid analyses: The data in Table 5 show mean (\pm S.D.) fasting plasma phenylalanine, tyrosine, glutamate and aspartate levels in the 5 groups of animals calculated for the entire feeding period.

The data show no significant differences in plasma aspartate and glutamate levels between animal groups. These data indicate a rapid metabolism of the aspartate portion of aspartame when it is administered with the diet. These data are consistent with data for glutamate, the other major dicarboxylic amino acid. These data indicate a very rapid metabolism and low toxicity when glutamate is administered as part of a meal (2-4).

Fasting plasma phenylalanine levels were increased in all animal groups ingesting the diet with added phenylalanine or aspartame. Fasting plasma phenylalanine levels in animals ingesting aspartame at 1 gm/kg body weight were only slightly higher (8.88 ± 5.15 umoles/dl) than values noted in control animals receiving no aspartame or phenylalanine (5.49 ± 1.49 umoles/dl). Similarly, plasma tyrosine levels were only slightly higher (9.38 ± 5.38 umoles/dl) than values in control animals (7.61 ± 2.49 umoles/dl). This undoubtedly reflects the rapid metabolism of this level of aspartame by the monkey when ingested during the course of the entire day and the rapid metabolism and clearance during the fasting period.

Fasting plasma phenylalanine levels in animals ingesting aspartame at 2 gm/kg body weight were also higher (28.7 ± 48 umoles/dl) than values in control animals. The elevated fasting levels indicate that plasma phenylalanine levels were significantly higher during the postprandial hours. Plasma phenylalanine levels were measured in one animal postprandially, and a value of 227 umoles/dl was noted. This indicated that postprandial plasma phenylalanine levels were considerably higher than levels noted during the fasting period. Fasting plasma tyrosine

levels in these animals are also elevated (13.1 ± 8.58 umoles/dl) above levels noted in control animals (7.61 ± 2.49 umoles/dl). The elevated tyrosine levels undoubtedly reflect the conversion of phenylalanine to tyrosine.

Fasting plasma phenylalanine levels in animals ingesting aspartame at 3 gm/kg body weight were significantly higher (66.2 ± 83.3 umoles/dl) than levels in control animals. Considerable variation was noted in the fasting phenylalanine levels in animals ingesting aspartame, undoubtedly reflecting the variable length of time since the monkeys had last eaten. Fasting plasma tyrosine levels were also elevated (23.3 ± 11.4 umoles/dl), indicating considerable conversion of phenylalanine to tyrosine.

The diet with phenylalanine added at 1.65 gm/kg provides an equivalent quantity of phenylalanine as the diet providing 3 gm/kg body weight aspartame. As expected, fasting plasma phenylalanine and tyrosine levels were similar on these two diets. Mean fasting plasma phenylalanine levels were 54.4 ± 65 umoles/dl in animals ingesting the diet with added phenylalanine, while fasting plasma tyrosine levels were 19.3 ± 6.37 umoles/dl.

The data in Table 6 compare fasting plasma phenylalanine and tyrosine levels in each group of animals with the length of time the animals were fed the diet.

General Observations: Because of the early initiation to a liquid diet immediately after weaning there was no problem with respect to acceptance of liquid diet by the infant monkeys. Beginning at 2-3 months of age, all of the infants were troubled with chronic diarrhea.

The first five animals involved in the study did acquire a Salmonilla sp. infection which responded quickly to Keflex (Eli Lilly & Co.). Subsequently, repeated stool samples were obtained, cultured, and no common enteric bacteria that cause diarrhea could be identified. After the study was nearly complete, it was learned that Old World monkeys nearly universally tend to become lactose intolerant at about 3 months of age. Thus, the animals were experiencing chronic diarrhea because of a normal diminishing in their capacity to produce lactase. The good weight gain achieved by each and every animal in the study indicates that the lactose intolerance did not interfere with nutrient absorption or utilization.

Extensive observations were made on the time of tooth eruption, changes in pelage, and comprehensive anecdotal behavioral records were maintained. With few exceptions, each of the monkeys eagerly ate the daily fruit allotment. However, the animals in Group 2 showed an almost greedy interest in fruit.

DISCUSSION

The growth and maturational data are essentially identical for all 20 infants regardless of APM or phenylalanine intake. Thus, even at so-called "abuse" levels, APM can be considered without measurable effect when present in the diet of the infant primate. Although all of the monkeys thrived on their respective diets, some subtle differences in milk and water intakes did emerge. The five infant monkeys receiving 1.65 gm/kg of phenylalanine were the most voracious eaters of the daily fruit supplement. It is interesting to speculate that this might be because of the somewhat bitter taste of the phenylalanine additive. Some of the control infants and those on the diets with APM added were rather indifferent to the fruit supplement, which is typical of diet preferences encountered in monkey populations. The obvious craving of the monkeys on the phenylalanine supplemented diet could reflect a desire for a sweet taste.

It is noteworthy that the infant on the diet containing phenylalanine consistently drank less formula than the other groups (Table 2). The groups receiving 3 gm/kg APM and 1.65 gm/kg phenylalanine drank more water in relation to formula consumed (Fig. 4) than the other groups. The diet of infants on 3 gm/kg APM contained greater levels of amino acid than were present in the other four groups. If renal solute loads are calculated (Table 7), it can be seen that the available water per millimole was essentially the same for the control animals, those ingesting phenylalanine or those receiving the highest APM load. The fact that serum osmolality and electrolytes were comparable for all groups indicated that there was plenty of free water available and homeostasis was readily achieved by each infant monkey.

The results of this study present some interesting questions with respect to the study of Waisman involving APM dosing in monkeys which was terminated prematurely (6) as well as his earlier work on inducing experimental phenylketonuria in infant monkeys by feeding them a diet high in phenylalanine (5). It should be noted that when phenylketonuria is induced in rats by feeding excess quantities of phenylalanine after weaning, behavioral deficits do appear. They are totally eliminated upon cessation of phenylalanine loading. Thus, the behavioral deficit is reversible in contrast to the infant monkeys receiving large amounts of dietary phenylalanine who exhibited apparently nonreversible disturbances in brain electrical activity. (5).

The methods of administering phenylalanine were markedly different in the two studies. As noted earlier, Waisman and colleagues always gave a given dosage over an 8-hour period when technicians were available to dose the infant monkeys. This technique meant that infant monkeys were receiving a very concentrated dose of a given amino acid in a relatively short period of time. One intended dosage of APM (6) was 4-6 gm/kg, but the infants would not consume that load, presumably due to the intense sweetness of the compound. Thus, the real dose was approximately 3.6 gm/kg. Although it cannot be determined from any of the publications or reports, in order to induce infant monkeys to swallow adequate amounts of formula for administering the desired amino acid loads, it is doubtful that water was provided during the 8-hour period dosing was going on. Thus, serious questions can be raised with respect to the blood osmolality levels and general metabolic state of the animals under abnormal conditions of food intake.

Convulsions, seioures and alterations in brain electrical activity are common sequelae to high blood solute loads (22. 23). The infant monkeys in the Waisman study (6) were observed to convulse or seize primarily when being handled, which would have been at the time of feeding. Thus, the question arises as to whether instead of the experimental phenylketonuria, those infant monkeys might not have been experiencing hyperosmolality or general hyperaminoacidemia. In the present study close attention was paid to providing free access to water as well as monitoring water intake in relation to formula intake. This ration was increased (Fig. 4), in the animals receiving 3 gm/kg of APM. Thus, simple access to water may be the key element in the contradictory findings between this study, and the prior work of Waisman both in inducing experimental fetal phenylketonuria in infant monkeys and in testing APM. Ausman (9) has recently observed a high mortality rate, probably from hyperosmolality in infant Cebus and squirrel monkeys raised on infant formula without free access to water.

The fasting plasma phenylalanine and tyrosine data indicate a rapid metabolism of aspartame and phenylalanine by the infant primate. Ingestion of aspartame at 1 gm/kg body weight resulted in only a minimal elevation in fasting plasma phenylalanine levels despite the large quantity fed. Fasting plasma phenylalanine levels do show a positive dose-response curve to increasing aspartame or phenylalanine load. The ingestion of phenylalanine as part of aspartame rather than as the pure amino acid did not seem to affect these values, since fasting plasma phenylalanine levels were similar in animals given 1.65 gm/kg phenylalanine and those given an equivalent amount of phenylalanine as aspartame (3 gm/kg body weight).

A considerable variation in fasting plasma phenylalanine levels was noted in animals ingesting diets with added aspartame and phenylalanine. This probably reflects the variable periods of fasting in each animal. Food was removed from the animals at 0600 hours, and blood samples were obtained at 1000 hours. However, no data are available when the animals had last ingested food. In some cases, no food remained at 0600 hours, while in other cases food was still available to the animals at 0600 hours. Thus, the length of fasting prior to blood sampling was at least 4 hours, but undoubtedly varied from animal to animal. Animals with a longer fasting period would be expected to show lower plasma phenylalanine levels.

It is difficult to predict postprandial plasma phenylalanine and tyrosine levels in these animals. It is clear, however, that postprandial values must be considerably higher than levels noted in fasting samples. A postprandial sample was obtained in one animal ingesting aspartame at 2 gm/kg body weight during the first two months of the study. In that animal, plasma phenylalanine was 227 umoles/dl.

The data from the present study also allow us to evaluate the hypothesis that the plasma phenylalanine/tyrosine ration plays a major role in controlling energy intake.

The control of food intake and the correlated regulation of body energy balance has been studied for many years. Recently, a considerable interest has evolved in the relationships among diet, plasma and brain amino acid levels, and brain neurotransmitter levels (14, 15). The importance of amino acids in neurotransmitter synthesis in the CNS has been realized for some time. However, only recently has it been shown that physiological changes in plasma amino acids can alter neurotransmitter synthesis in the brain, indicating that peripheral metabolism

may have a major influence on central metabolism.

In recent investigations (16), the concentration of tyrosine, hence catecholamines, in the brain has been shown to depend upon relative concentrations of tyrosine and the other large neutral amino acids (tryptophan, phenylalanine, valine, isoleucine, leucine).

Recently Anderson and colleagues (14, 15, 17, 18) have postulated that the plasma ratio of tyrosine to phenylalanine may be a major factor in energy intake in the rat. In their experiments, weanling rats were allowed to self select from two diets that differed only in protein content. After food selection patterns were established, the rats were allowed to eat overnight, and the blood was collected between 9 to 11 AM the following morning. Changes in plasma amino acid patterns were correlated with protein and energy intake by the various groups. In these studies, Anderson and colleagues (17, 18) reported that the plasma tyrosine to phenylalanine ratio, but not the tyrosine to large neutral amino acid ratio, correlated consistently with energy intake. The correlation between plasma tyrosine/phenylalanine ratio suggested that "changes in plasma phenylalanine/tyrosine ratio reflect, or stimulate, at least in part, a mechanism operating via the CNS to control energy intake (14)".

The dietary treatment in the present study caused a significant changes in the plasma tyrosine/phenylalanine ratio. These changes allow us to evaluate the tyrosine/phenylalanine ratio hypothesis in the primate. This is particularly important, since most feeding studies have been carried out in the rat, and it is not certain the primate will respond similarly.

The data in Table 8 correlates the overall mean fasting plasma tyrosine/phenylalanine ratio in the monkeys with the total milk intake over the entire 298 day study period. Tyrosine/phenylalanine ratios varied from 1.38 in control animals to 0.35 in animals ingesting either 3 gm/kg aspartame or 1.65 gm/kg phenylalanine as part of the diet. Evaluation of the self-selected milk intake in these animals reveals no significant differences between groups, and no correlation of energy intake with the tyrosine/phenylalanine ratio. Thus, our data either suggest that the tyrosine/phenylalanine ratio hypothesis is not correct, or that the hypothesis is appropriate only to the rat and not to the primate.

It must be pointed out that the imbalance in plasma tyrosine/phenylalanine ratios as measured in the fasting sample is likely to be less than values noted in postprandial plasma samples. The data in Tables 9 and 10 show plasma phenylalanine and tyrosine levels in infant monkeys given 2 gm/kg body weight loads of aspartame (Table 9) and in adult humans given 200 mg/kg aspartame loads (Table 10). These data indicate that plasma phenylalanine levels increase more rapidly than plasma tyrosine levels during the postprandial period. Thus, the postprandial tyrosine/phenylalanine ratio in animals fed aspartame or phenylalanine must be even lower than that indicated by the fasting plasma levels.

SUMMARY

Newborn monkeys (Macaca arctoides) were reared for nine months on infant formula diets to which the nutritive artificial sweetener, Aspartame (APM) had been added at levels of 1, 2 or 3 gm/kg of body weight per day. Control monkeys received only infant formula and one group received formula plus phenylalanine at a level of 1.65 gm/kg body weight per day, which is equivalent to the phenylalanine moiety of a 3 gm/kg APM addition to the diet. The feeding protocol allowed self-feeding to attain the desired dose level plus free access to water at all times.

The infant monkeys were monitored for formula and water intake, weight, and crown-rump length increases. Blood samples were taken at intervals and analyzed for electrolytes, osmolality, differential cell count, hematocrit, hemoglobin, glucose and amino acid levels. Urine samples were checked for protein, glucose, and ketones. Each animal was handled often and evaluated carefully for feeding habits and behavioral milestones. Electroencephalograms were performed at regular intervals.

The growth rates for all five groups of animals were indistinguishable, both with respect to weight gain and increments in crown-heel length. Blood chemistries and urine analyses were normal in all five groups throughout the dietary period and did not differ between groups. The water intake in relation to formula consumed was elevated for the groups receiving the 3.0 gm/kg APM or 1.65 gm/kg phenylalanine additives. The group consuming 3.0 gm/kg APM consumed more water in absolute numbers than the other groups; the group consuming 1.65 gm/kg phenylalanine became the most avid fruit eaters. The bitter taste imparted

by phenylalanine and the excessive sweetness of high levels of APM in addition to elevated amino acid intakes may have been responsible for these observations.

The electroencephalogram findings did not vary from group to group and resembled those characteristic of newborn human infants. No infant monkeys in any group exhibited convulsions, seizures, shudders or any sort of abnormal behavior during the diet administration or in the year following the change to solid food.

Fasting plasma phenylalanine levels showed a positive dose-response curve to the quantity of APM and/or phenylalanine ingested. Phenylalanine levels were similar in monkeys ingesting APM (3 gm/kg) in those ingesting the dose equivalency of phenylalanine (1.65 gm/kg). Considerable variation in the values attained may reflect the length of the fast prior to obtaining blood samples.

It was impossible to confirm earlier work suggesting that experimental phenylketonuria and accompanying neurological deficits can be induced in infant monkeys by adding phenylalanine to the diet. Free access to water and self-feeding of formula characterized this study. Possibly, hyperosmolality and/or a general hyperaminoacidemia induced by forced feeding was responsible for nonspecific neurological abnormalities reported previously. Thus, large intakes of APM on a body weight basis as a part of the diet have no effect upon numerous developmental parameters in the infant macaque.

Table 1

Dose Levels

	Dose (gm/kg/day)	Number of Infants
1. Control		4
2. Phenylalanine	1.65*	4
3. APM	1.0	4
4. APM	2.0	4
5. APM	3.0	4

* Equimolar to phenylalanine in 3gm/kg APM load

Table 2 AVERAGE GROUP DAILY MILK INTAKE

($\bar{X} \pm \text{SEM}$)

MONTH	1	2	3	4	5	6	7	8	9
GROUP 1 (CONTROL)	277.7 \pm 9.5	305.9 \pm 8.2	353.6 \pm 8.2	382.0 \pm 9.7	392.9 \pm 11.1	449.1 \pm 16.2	501.4 \pm 19.8	552.5 \pm 15.7	584.4 \pm
GROUP 2 (1.65 gm PHE/Kg/day)	235.8 \pm 10.7	264.6 \pm 11.3	303.5 \pm 11.5	313.4 \pm 13.3	339.2 \pm 16.3	415.1 \pm 18.2	455.8 \pm 22.0	464.7 \pm 20.5	499.0 \pm
GROUP 3 (1 gm APH/Kg/day)	265.7 \pm 9.5	284.3 \pm 7.9	325.8 \pm 10.3	368.8 \pm 9.1	396.9 \pm 13.4	485.8 \pm 12.3	530.3 \pm 13.1	593.2 \pm 11.5	604.5 \pm
GROUP 4 (2 gm APH/Kg/day)	259.2 \pm 8.4	298.2 \pm 8.5	370.2 \pm 10.3	445.7 \pm 8.7	467.1 \pm 16.2	563.6 \pm 14.8	625.1 \pm 16.4	604.7 \pm 16.6	614.5 \pm
GROUP 5 (3 gm APH/Kg/day)	220.6 \pm 7.6	218.6 \pm 9.3	318.4 \pm 8.1	359.2 \pm 36.7	410.6 \pm 11.9	406.3 \pm 15.2	500.4 \pm 16.4	521.9 \pm 15.9	544.4 \pm

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GROUP 2 (1.65 gm PHE/kg/day)	235.8 ± 10.7	264.6 ± 11.3	303.5 ± 11.5	313.4 ± 13.3	339.2 ± 16.3	415.1 ± 18.2	455.8 ± 22.0	464.7 ± 20.5	499.0 ± 21.6
GROUP 3 (1 gm APN/kg/day)	265.7 ± 9.5	284.3 ± 7.9	325.8 ± 10.3	368.8 ± 9.1	396.9 ± 13.4	485.8 ± 12.3	530.3 ± 13.1	593.2 ± 11.5	604.5 ± 14.0
GROUP 4 (2 gm APN/kg/day)	259.2 ± 8.4	298.2 ± 8.5	370.2 ± 10.3	445.7 ± 8.7	467.1 ± 16.2	563.6 ± 14.8	625.1 ± 16.4	604.7 ± 16.6	614.5 ± 19.7
GROUP 5 (3 gm APN/kg/day)	220.6 ± 7.6	218.6 ± 9.3	318.4 ± 8.1	359.2 ± 36.7	410.6 ± 11.9	406.3 ± 15.2	500.4 ± 16.4	521.9 ± 15.9	544.4 ± 19.3

Table 3 AVERAGE GROUP DAILY H₂O INTAKE

($\bar{X} \pm \text{SEM}$)

MONTH	1	2	3	4	5	6	7	8	9
GROUP 1 (CONTROL)	52.4±5.8	46.4±5.2	48.6±5.0	48.4±5.1	56.1±7.4	78.8±10.1	122.5±11.4	124.5±11.4	150.4±14.
GROUP 2 (1.65 gm PHE/Kg/day)	62.8±5.0	77.8±7.0	65.7±6.3	70.8±6.8	99.0±9.7	124.9± 9.2	140.3±12.4	143.3±11.0	163.8±16.
GROUP 3 (1 gm APN/Kg/day)	57.6±5.4	45.1±6.1	40.7±5.1	46.1±6.6	40.7±5.7	71.6± 8.6	114.2±10.4	150.0±13.1	157.6±20.
GROUP 4 (2 gm APN/Kg/day)	66.8±5.0	43.9±5.7	43.7±5.7	53.8±5.7	66.8±7.1	93.9±10.0	143.3±10.9	144.9±10.4	180.9±18.
GROUP 5 (3 gm APN/Kg/day)	91.2±6.5	83.7±6.9	76.2±7.5	81.7±5.8	94.7±7.8	125.8± 9.5	172.7±14.9	210.1±15.6	271.9±24.

Table 3 AVERAGE GROUP DAILY H₂O INTAKE
($\bar{X} \pm \text{SEM}$)

MONTH	1	2	3	4	5	6	7	8	9
GROUP 1 (CONTROL)	52.4±5.8	46.4±5.2	48.6±5.0	48.4±5.1	56.1±7.4	78.8±10.1	122.5±11.4	124.5±11.4	150.4±14.1
GROUP 2 (1.65 gm PINE/Kg/day)	62.8±5.0	77.8±7.0	65.7±6.3	70.8±6.8	99.0±9.7	124.9± 9.2	140.3±12.4	143.3±11.0	163.8±16.1
GROUP 3 (1 gm APN/Kg/day)	57.6±5.4	45.1±6.1	40.7±5.1	46.1±6.6	40.7±5.7	71.6± 8.6	114.2±10.4	150.0±13.1	157.6±20.1
GROUP 4 (2 gm APN/Kg/day)	66.8±5.0	43.9±5.7	43.7±5.7	53.8±5.7	66.8±7.1	93.9±10.0	143.3±10.9	144.9±10.4	180.9±18.1
GROUP 5 (3 gm APN/Kg/day)	91.2±6.5	83.7±6.9	76.2±7.5	81.7±5.8	94.7±7.8	125.8± 9.5	172.7±14.9	210.1±15.6	271.9±24.1

Table 4 EEG: INFANT MACAQUES

DAYS (0=START OF STUDY)	PRE-STUDY (-7 - 0)	(12 - 71)	STUDY (102 - 155)	(259 - 288)	1 MONTH POST STUDY SIMILAC DIET (300 - 306)	POST STUDY FOLLOW-UP (306 - 450) (455 - 518)
CONTROL						
3344		WNL	WNL	WNL	WNL	WNL
3507		WNL	WNL	WNL	WNL	WNL
3585		WNL	WNL	one episode sharp act.	WNL	WNL
3750	WNL		WNL	WNL	WNL	WNL
APM (1 gm/Kg/day)						bi-par ind. spikes
3348		WNL	WNL	WNL	WNL	WNL
3493		WNL	WNL	WNL	WNL	WNL
3697	WNL	WNL	WNL	WNL	WNL	WNL
3799	WNL		sharp transients	WNL	WNL	WNL
APM (2 gm/Kg/day)						
3349		spikes	spikes	WNL	WNL	WNL
3476		WNL	ques spikes	WNL	WNL	WNL
3711	WNL		WNL	WNL	WNL	fast BKG
3789	WNL		WNL	WNL	WNL	WNL
APM (3 gm/Kg/day)						
3351		sugg. spikes	WNL	WNL	ques. spikes	WNL
3453		WNL	WNL	fast BKG	WNL	WNL
3713	WNL		WNL	WNL	WNL	WNL
3794	WNL		WNL	WNL	WNL	WNL
PHE (1.63 gm/Kg/day)						
3346		WNL	WNL	WNL	bi-occ. spikes	WNL
3494	WNL	WNL	WNL	WNL	WNL	WNL
4	WNL	WNL	WNL	WNL	WNL	WNL

Table 4 EEG INFANT MACAQUES

DAYS (0=START OF STUDY)	PRE-STUDY (-7 - 0)	1 MONTH POST STUDY SIMILAC DIET			POST STUDY FOLLOW-UP		
		(12 - 71)	STUDY (102 - 155)	(259 - 288)	(300 - 306)	(306 - 450)	(455 - 518)
CONTROL							
3344		WNL	WNL	WNL	WNL	WNL	WNL
3507		WNL	WNL	WNL	WNL	WNL	WNL
3585		WNL	WNL	one episode sharp act.	WNL	WNL	WNL
3750	WNL		WNL				bi-par ind. spikes
APH (1 gm/kg/day)							
3348		WNL	WNL	WNL	WNL	WNL	WNL
3493		WNL	WNL	WNL	WNL	WNL	WNL
3557	WNL	WNL	sharp transients	WNL	WNL	WNL	WNL
3799	WNL						
APH (2 gm/kg/day)							
3349		spikes	spikes	WNL	WNL	WNL	WNL
3476		WNL	ques spikes	WNL	WNL	WNL	WNL
3711	WNL		WNL	WNL	WNL	fast BKG WNL	WNL
3789	WNL		WNL	WNL			
APH (3 gm/kg/day)							
3351		sugg. spikes	WNL	WNL	ques. spikes	WNL	WNL
3453		WNL	WNL	fast BKG	WNL	WNL	WNL
3713	WNL		WNL	WNL	WNL	WNL	WNL
3794	WNL		WNL	WNL			
PHE (1.63 gm/kg/day)							
3346		WNL	WNL	WNL	bi-occ. spikes	WNL	WNL
3494		WNL	WNL	WNL	WNL	WNL	WNL
3584	WNL	WNL	WNL	WNL	WNL	WNL	WNL
3753	WNL	WNL	WNL	WNL	WNL	WNL	WNL

* within normal limits

Table 5: Mean (\pm S.D.) plasma phenylalanine, tyrosine, aspartate and glutamate levels (umoles/dl) in infant monkeys fed diets supplemented with aspartame or phenylalanine

<u>Animal Group</u>	<u>Plasma Levels (umoles/dl) while on diet</u>			
	<u>Phenylalanine</u>	<u>Tyrosine</u>	<u>Aspartate</u>	<u>Glutamate</u>
Control	5.49 \pm 1.94	7.61 \pm 2.49	1.08 \pm 0.66	6.06 \pm 2.31
+ Aspartame (1 gm/kg)	8.88 \pm 5.15	9.38 \pm 5.33	1.23 \pm 0.90	4.48 \pm 1.31
+ Aspartame (2 gm/kg)	26.7 \pm 48.8	13.1 \pm 8.58	1.61 \pm 1.21	5.33 \pm 1.59
+ Aspartame (3 gm/kg)	66.2 \pm 83.3	23.2 \pm 11.4	1.78 \pm 1.43	5.90 \pm 2.41
+ Phenylalanine (1.65 gm/kg)	54.4 \pm 64.9	19.3 \pm 6.37	1.08 \pm 0.54	4.96 \pm 1.35

Table 5: Mean (\pm S.D.) plasma phenylalanine, tyrosine, aspartate and glutamate levels (μ moles/dl) in infant monkeys fed diets supplemented with aspartame or phenylalanine

<u>Animal Group</u>	<u>Plasma Levels (μmoles/dl) while on diet</u>			
	<u>Phenylalanine</u>	<u>Tyrosine</u>	<u>Aspartate</u>	<u>Glutamate</u>
Control	5.49 \pm 1.94	7.61 \pm 2.49	1.08 \pm 0.66	6.06 \pm 2.3
+ Aspartame (1 gm/kg)	8.88 \pm 5.15	9.38 \pm 5.33	1.23 \pm 0.90	4.48 \pm 1.3
+ Aspartame (2 gm/kg)	26.7 \pm 48.8	13.1 \pm 8.58	1.61 \pm 1.21	5.33 \pm 1.5
+ Aspartame (3 gm/kg)	66.2 \pm 83.3	23.2 \pm 11.4	1.78 \pm 1.43	5.90 \pm 2.4
+ Phenylalanine (1.65 gm/kg)	54.4 \pm 64.9	19.3 \pm 6.37	1.08 \pm 0.54	4.96 \pm 1.3

Table 6.

MEAN (\pm S.D.) PLASMA PHENYLALANINE AND TYROSINE LEVELS (μ moles/dl) WITH TIME ON DIET

ANIMAL GROUP	First Sample	Second Sample	Third Sample	Fourth Sample	Fifth Sample
Phenylalanine Levels (μ moles/dl)					
Control	4.74 \pm 0.45	4.82 \pm 0.79	7.98 \pm 4.22	6.24 \pm 1.75	5.33 \pm 1.32
+Aspartame (1 gm/kg)	7.98 \pm 5.41	13.4 \pm 6.02	8.68 \pm 2.13	5.67 \pm 0.57	9.68 \pm 7.78
+Aspartame (2 gm/kg)	62.5 \pm 110	25.7 \pm 29.4	16.8 \pm 16.0	11.0 \pm 2.85	17.7 \pm 10.7
+Aspartame (3 gm/kg)	95.3 \pm 89.9	107 \pm 135	59.9 \pm 102	66.2 \pm 63.9	17.9 \pm 19.5
+Phenylalanine (1.65 gm/kg)	27.4 \pm 24.2	63.0 \pm 98.1	59.1 \pm 82.1	31.7 \pm 40.7	81.6 \pm 75.9
Tyrosine Levels (μ moles/dl)					
Control	8.24 \pm 3.77	7.57 \pm 1.84	8.49 \pm 2.04	8.34 \pm 3.02	6.98 \pm 2.40
+Aspartame (1 gm/kg)	8.73 \pm 4.84	14.5 \pm 8.94	9.55 \pm 2.20	7.65 \pm 2.36	6.36 \pm 3.41
+Aspartame (2 gm/kg)	14.0 \pm 7.69	13.7 \pm 7.45	10.7 \pm 3.01	12.5 \pm 7.50	14.8 \pm 17.2
+Aspartame (3 gm/kg)	24.6 \pm 14.7	24.8 \pm 5.89	19.2 \pm 15.2	28.9 \pm 12.3	22.4 \pm 11.1
+Phenylalanine (1.65 gm/kg)	18.5 \pm 11.6	18.0 \pm 2.94	23.1 \pm 3.03	16.4 \pm 7.93	19.4 \pm 3.35

Table 7

Calculated Daily Intake of Renal Solute Load*

Group	Solute (mosmols) Formula	APM or PHE	Total Volume Free Water	Available Water per mosmol
1 (CONTROL)	43	0	451	10.5
2 (1.65 gm PHE/ kg/day)	37	5	427	10.0
5 (3.0 gm APM/ 41 kg/day)	41	10	485	9.5

*Renal Solute = (Na + K + Cl + Urea)
Na, K, Cl contribute one mosmole per mEq
Each gram protein yield 4 mosmole urea

Similac provides 102 mosmoles/liter.

To calculate potential renal solute, it was assumed each
160 mg N yields 4 mosmoles urea.

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Each gram protein yields 4 mosmole urea

Similac provides 102 mosmoles/liter.

To calculate potential renal solute, it was assumed each 160 mg N yields 4 mosmoles urea.

Table 8

Correlation of fasting plasma tyrosine/phenylalanine ratio with milk intake in infant monkeys fed diets supplemented with aspartame or phynylalanine

Animal Group	Tyr/PHE	Average Daily Milk Intake (ml)
Control	1.38	422
+ Aspartame (1 gm/kg)	1.05	428
+ Aspartame (2 gm/kg)	0.49	472
+ Aspartame (3 gm/kg)	0.35	389
+ Phenylalanine (1.65 gm/kg)	0.35	366

Table 9: Mean (\pm S.D.) plasma phenylalanine and tyrosine levels in neonatal monkeys administered aspartame at 2 gm/kg body weight (n = 9).

Time (Minutes)	Plasma Levels (umoles/dl)		TYROSINE PHENYLALANINE
	Phenylalanine	Tyrosine	
0	6.40 \pm 2.32	6.63 \pm 1.79	1.04
20	57.1 \pm 68.8	12.8 \pm 4.02	0.22
40	70.4 \pm 65.2	17.1 \pm 7.94	0.24
60	83.8 \pm 46.9	19.2 \pm 10.2	0.23
90	97.7 \pm 68.1	23.8 \pm 12.3	0.24
120	104 \pm 95.9	28.4 \pm 12.9	0.27
180	84.6 \pm 98.0	29.8 \pm 10.8	0.35
240	44.0 \pm 18.0	31.7 \pm 8.76	0.72

Table 10 Mean (\pm S.D.) plasma phenylalanine and tyrosine levels in adult humans administered aspartame at 0.2 gm/kg body weight (n = 6)

Time (minutes)	Plasma Levels (umoles/dl)		Tyrosine Phenylalanine
	Phenylalanine	Tyrosine	
0	5.26 \pm 0.67	5.69 \pm 0.78	1.08
15	17.2 \pm 7.16	6.78 \pm 1.31	0.39
30	32.8 \pm 7.31	7.93 \pm 1.39	0.24
45	37.7 \pm 8.08	9.14 \pm 1.75	0.24
60	42.3 \pm 14.6	10.7 \pm 2.48	0.25
90	48.7 \pm 15.5	12.0 \pm 2.58	0.25
120	48.4 \pm 15.3	13.5 \pm 3.46	0.28
180	31.7 \pm 7.98	13.7 \pm 4.01	0.43
240	22.8 \pm 5.14	12.8 \pm 3.62	0.56
300	16.3 \pm 3.78	11.8 \pm 2.74	0.72
360	13.2 \pm 2.80	10.3 \pm 1.89	0.78
420	10.8 \pm 2.13	8.93 \pm 1.64	0.83
480	8.67 \pm 1.42	7.74 \pm 1.59	0.89

Figure 1.

Infant M. arctoides were housed in incubators. Water and formula or formula plus amino acids were freely available on a 24-hour basis in plastic bottles with rubber nipples.

Figure 1

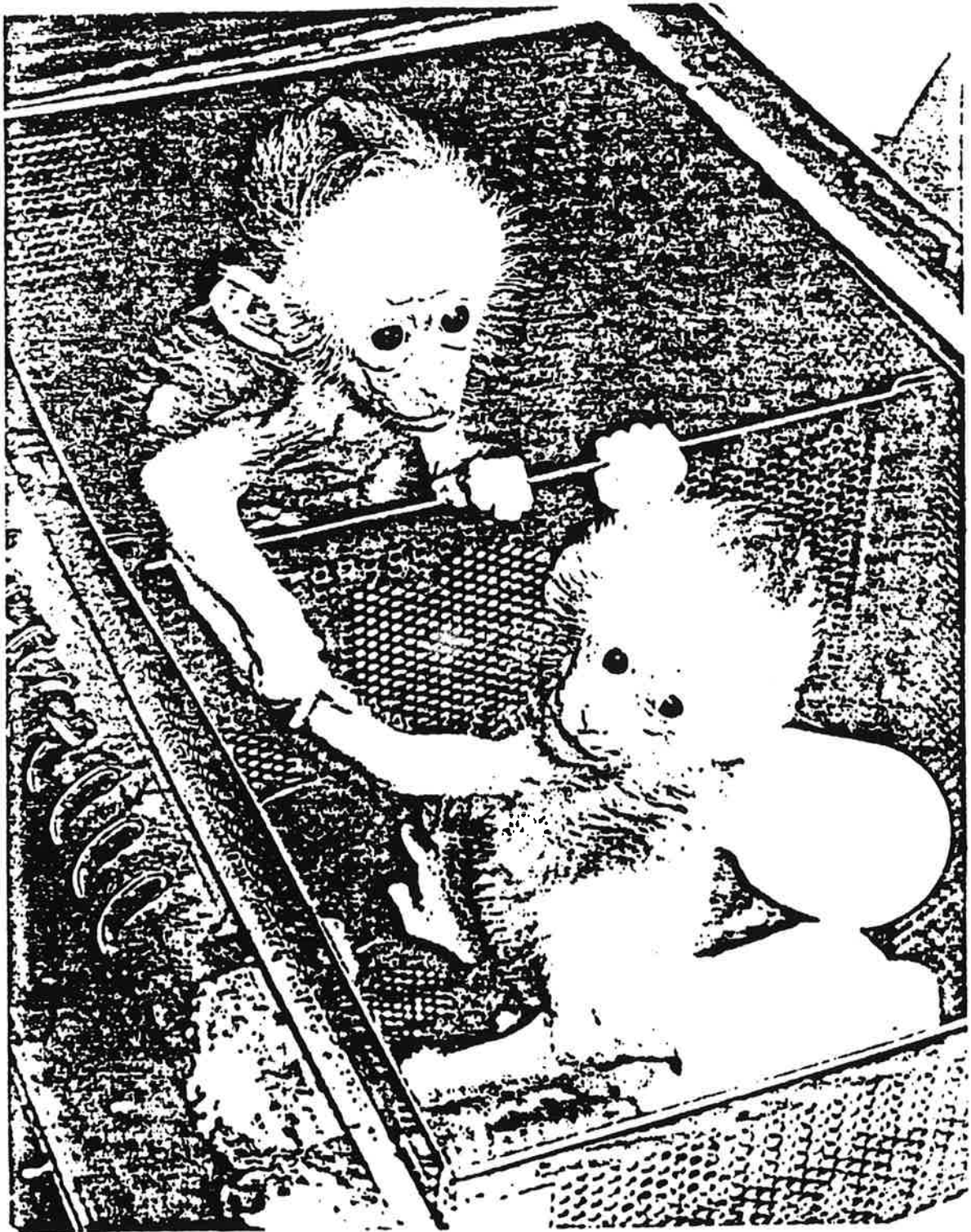


Figure 2.

Target doses for Groups 2 through 4 were essentially attained by the feeding regimen employed in this study. It was technically difficult to provide infants of Group 5 access to a 3 gm/kg load because of the relatively low solubility of APM. The graph demonstrates the dose levels achieved while maintaining uniformity within each group throughout the study.

Figure 2

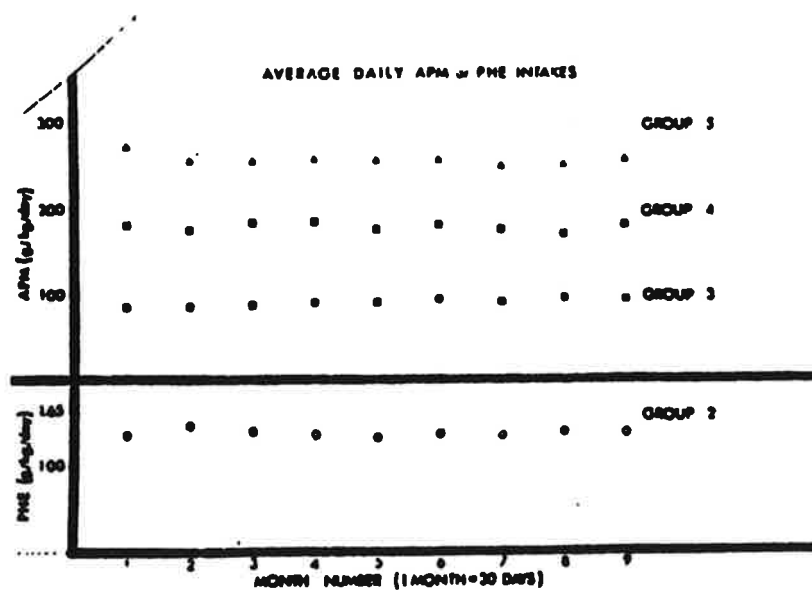


Figure 3.

**The mean total daily liquid intake for each group increased over time.
No significant differences between groups were observed.**

Figure 3

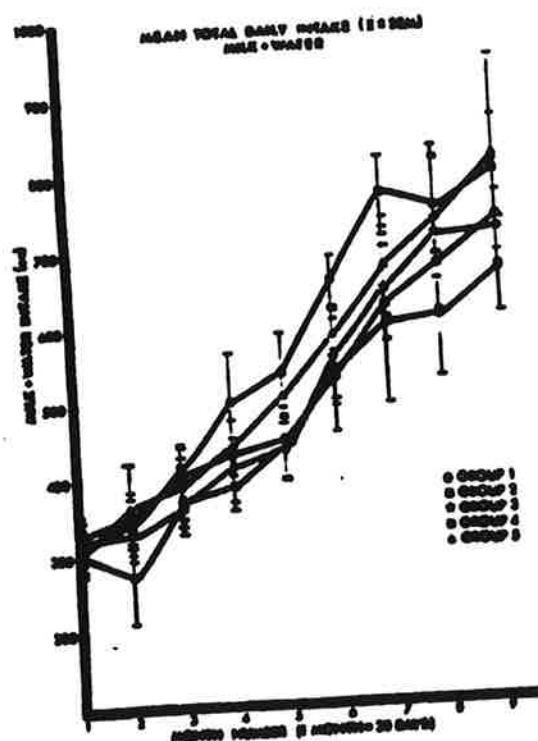


Figure 4.

A ratio of mean daily milk to water intake uncovered interesting inter-group variations. In Group 3, 1gm APM/kg/day, the greatest preference for milk was observed. Groups 2 and 5 received the greatest amount of amino acid additive and consumed the most water in relation to formula.

Figure 4

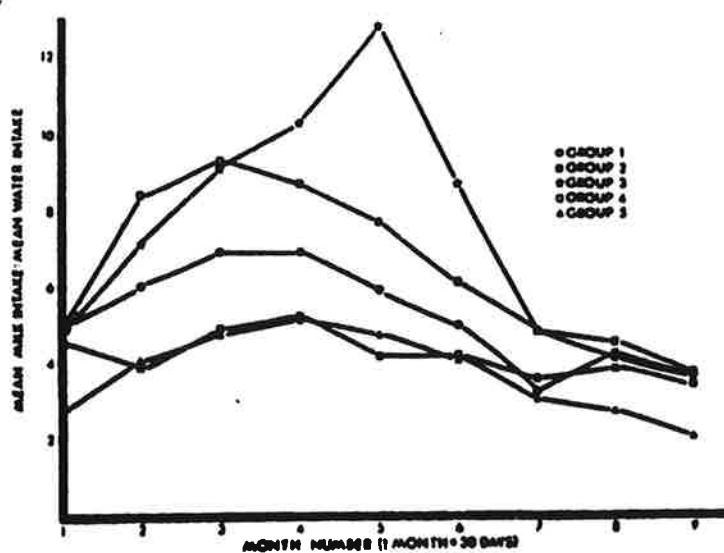


Figure 5.

Weight increase over time for individual animals within a group, as shown by Group 3, was uniform.

Figure 5

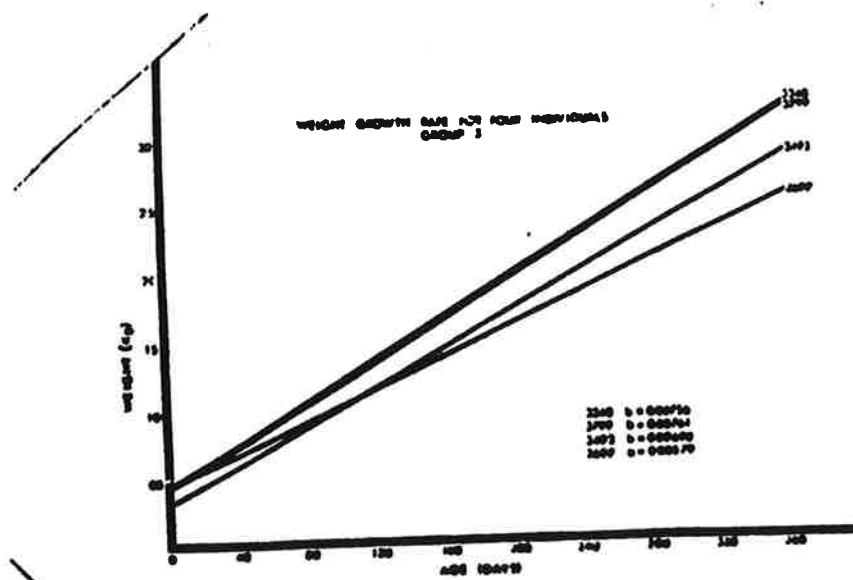


Figure 6.

Growth over the study period, as measured by weight gain, was essentially identical for all five groups. Group 2, 1.65 gm PHE/kg/day, exhibited a slightly more sluggish weight increment in keeping with its lower fluid intake values.

Figure 6

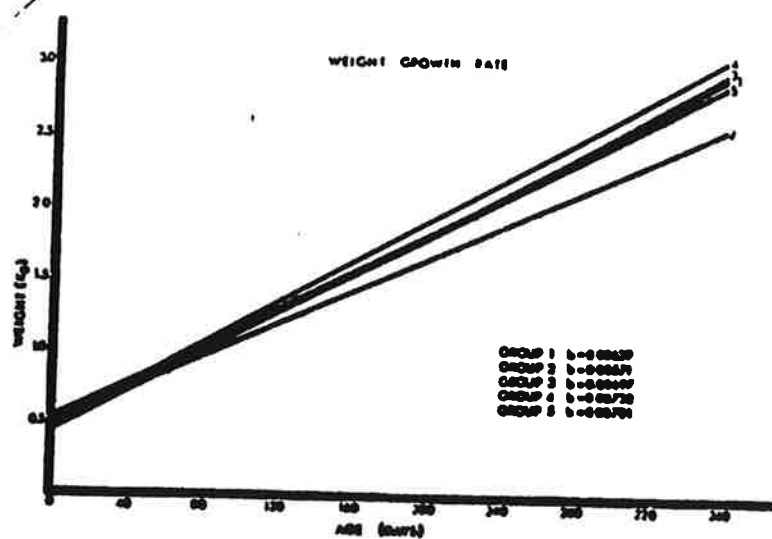
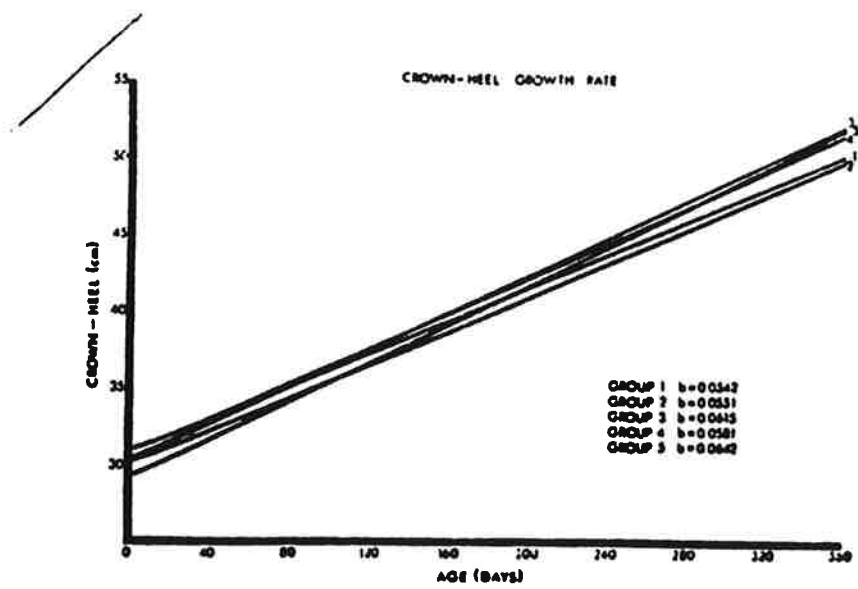


Figure 7.

Crown-heel growth rates, monitored monthly, again demonstrate the uniformity among groups. The experimental monkeys showed a length increase not significantly different from the control monkey.

Figure 7



REFERENCES

1. Olney, J. W., O. L. Ho, and V. Rhee. Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. *Exp. Brain Res.* 14: 61-76 (1971).
2. Reynolds, W. A., V. Bulter, and N. Lemkey-Johnston. Hypothalamic morphology following ingestion of aspartame or MSG in the neonatal rodent and primate: A preliminary report. *Journal of Toxicology Environ. Health* 2: 471-480 (1971).
3. Reynolds, W. A., L. D. Stegink, L. J. Filer, Jr., and E. Renn. Aspartame administration to the infant monkey: Hypothalamic morphology and blood amino acid levels. Manuscript in preparation, (1979).
4. Kerr, G. R. and H. A. Waisman. Dietary induction of hyperphenylalanine in the rat. *J. Nutrition* 92: 10-18 (1967).
5. Waisman, H. A. and H. P. Harlow. Experimental phenylketonuria in infant monkeys. *Science* 147: 3659 (1965).
6. Rao, K. S., R. G. McConnell, and H. A. Waisman. SC-18862: 52 week oral toxicity study in the infant monkey. Department of Biological Research, Searle Laboratories. October 10, 1972.
7. Nutrient Requirements of Nonhuman Primates, No. 14. Nutrient Requirements of Domestic Animals, The National Research Council, National Academy of Sciences, Washington, D.C. (1978).
8. Stegink, L. D., L. J. Filer, Jr., and G. L. Baker. Effect of aspartame and aspartate upon plasma and erythrocyte free amino acid levels in normal adult volunteers. *J. Nutr.* 107: 1837-1845 (1977).
9. Ausman, L. M., personal communication (1979).

10. Stegink, L. D., L. J. Filer, Jr., G. L. Baker, S. M. Mueller, and M. Y-C Wu-Rideout. Factors affecting plasma glutamate levels in normal adult subjects. In: *Glutamic Acid: Advances in Biochemistry and Physiology*, (L. J. Filer, Jr., S. Garattini, M. R. Kare, W. A. Reynolds, and R. J. Wurtman, editors), Raven Press, New York, pp. 333-351 (1979).
11. Baker, G. L., L. J. Filer, Jr., and L. D. Stegink. Effect of carbohydrate on glutamate metabolism. *Fed. Proc.* 38: 610 (1979).
12. Takasaki, Y. Studies on brain lesions after administration of monosodium L-glutamate to mice: II. Absence of brain damage following administration of monosodium L-glutamate in the diet. *Toxicology* 9: 307-318 (1978).
13. Stegink, L. D., W. A. Reynolds, L. J. Filer, Jr., G. L. Baker, and T. T. Daabees. Comparative metabolism of glutamate in mouse, monkey and man. In: *Glutamic Acid: Advances in Biochemistry and Physiology* (L. J. Filer, Jr., A. Garattini, M. R. Kare, W. A. Reynolds, and R. J. Wurtman, editors), Raven Press, pp. 85-102 (1979).
14. Anderson, G. H. Regulation of protein intake by plasma amino acids. *Advances in Nutritional Research* 1: 145-166 (1977).
15. Anderson, G. H. Control of protein and energy intake: Role of plasma amino acids and brain neurotransmitters. *Cand. J. Physiol. and Pharmacol.*, in press (1979).
16. Fernstrom, J. D. The effect of nutritional factors on brain amino acid levels and monamine synthesis. *Fed. Proc.* 35: 1151 (1976).

17. Anderson, G. H. and D. V. M. Ashley. Correlation of the plasma tyryptophan and tyrosine to neutral amino acid ratios with protein and energy intakes in the self-selecting weanling rat. *Proc. Canad. Fed. Biol. Soc.* 19: 24 (1976).
18. Anderson, G. H. and D. V. M. Ashley. Correlation of the plasma tyrosine to phenylalanine ratio with energy intake in self-selecting weanling rats. *Life Science* 21: 1227-1234 (1977).
19. Reynolds, W. A., V. Butler, N. Lenkey-Johnston, and L. D. Stegink. Hypothalamic morphology and plasma amino acid levels in neonatal primates administered large doses of aspartame with and without monosodium glutamate. Report to G. D. Searle, July 26, 1979.
20. Stegink, L. D., L. J. Filer, Jr., G. L. Baker, M. C. Brummel, and T. R. Tephly. Aspartame metabolism in human subjects. In: *Health and Sugar Substitutes*, (B. Guggenheim, editor), Krager, Basel, pp. 160-165 (1978).
21. Polidora, V. J., R. F. Cunningham, and H. A. Waisman. Phenylketonuria in rats: Reversibility of behavioral deficit. *Science* 151: 219-21 (1966).
22. Gauthier, B., R. Freeman, and J. Beveridge. Accidental salt poisoning in a hospital nursery. *Aust. Paediat. J.* 5: 101-105 (1969).
23. Simmons, M. A., E. W. Adcock, H. Bard, and F. C. Battaglia. Hypermnatremia and intracranial hemorrhage in neonates. *New Engl. J. Med.* 291: 6-10 (1974).

Appendix 1

ANIMAL NAME	BIRTH DATE	MOTHER #	DATE STARTED	DATE LAST DOSE	DATE ON CRD	CIR						BLOND						
						1	2	3	4	5	6	7	1	2	3	4	5	6
3346 SOWIT	5-10-76	1865	6-21-76		6-14-77	7-31-76	11-13-76	3-7-77	4-23-77	9-10-77	11-14-77		6-13-76	10-8-76	12-3-76	1-31-77	3-23-77	4-21-77
3307 GROWER	12-1-76	505	1-4-77		11-8-77	3-10-77	6-23-77	6-8-77	11-5-77	2-2-78			6-20-76	10-15-76	12-10-76	9-2-77	10-2-77	11-8-77
3305 OLIVER	2-4-77	1276	2-23-77		11-22-77	3-10-77	6-20-77	11-18-77	3-10-78	5-24-78			4-29-77	5-17-77	6-25-77	10-20-77	11-22-77	
3350 CORALIE	7-12-77	2026	8-5-77		5-29-78	8-5-77	12-7-77	5-17-78					10-5-77	12-1-77	2-9-78	4-6-78	5-29-78	
3348 CLYDE	5-14-76	2004	6-21-76	3-22-77	5-13-77	7-31-76	11-13-76	3-1-77	4-23-77	9-10-77	10-14-77		6-13-76	10-8-76	12-3-76	1-31-77	3-23-77	4-21-77
3494 DORELY	11-2-76	1245	11-22-76	8-17-77	9-22-77	2-1-77	3-21-77	6-15-77	9-21-77	12-23-77	1-4-78		6-20-76	10-15-76	12-10-76	9-2-77	10-2-77	11-8-77
3384 ACE	1-31-77	379	3-10-77	12-7-77	1-5-78	3-3-77	6-20-77	12-3-77	1-4-78	4-13-78	6-29-78		1-11-77	4-6-77	5-20-77	7-22-77	8-10-77	9-22-77
3353 FANNON	7-16-77	1242	8-16-77	5-28-78	5-29-78	6-15-77	12-20-77	5-17-78					1-11-77	4-6-77	5-20-77	7-22-77	8-10-77	9-22-77
3348 ORLY	5-17-76	1247	6-21-76	3-22-78	6-13-77	7-31-76	11-13-76	3-1-77	4-23-77	9-10-77	11-14-77		6-13-76	10-8-76	12-3-76	1-31-77	3-23-77	4-21-77
3493 CRICALLY	10-20-76	1241	11-22-76	8-17-77	9-22-77	2-1-77	3-21-77	6-15-77	9-21-77	12-23-77			6-20-76	10-15-76	12-10-76	9-2-77	10-2-77	11-8-77
3497 BOONLES	5-5-77	1301	6-29-77	3-13-78	3-24-78	6-23-77	9-23-77	3-9-78	3-13-78	6-22-78			1-11-77	4-6-77	5-20-77	7-22-77	8-10-77	9-22-77
3379 BOCKT	10-23-77	3526	11-5-77	8-8-78	6-5-78	11-5-78	3-16-78	6-2-78					1-12-78	3-17-78	5-12-78	7-6-78	8-5-78	
3499 BOONER	5-19-76	1304	6-21-76	3-22-78	5-13-77	7-31-76	11-13-76	3-1-77	4-23-77	9-10-77	11-17-77		6-13-76	10-8-76	12-3-76	1-31-77	3-23-77	4-21-77
3476 MCGAR	10-3-76	1242	11-1-76	8-4-77	8-14-77	11-13-76	2-1-77	3-21-77	6-15-77	9-21-77	12-23-77		6-20-76	10-15-76	12-10-76	9-2-77	10-2-77	11-8-77
3311 BOCKT	5-11-77	2027	5-29-77	3-23-78	3-24-78	5-23-77	9-23-77	3-9-78	6-22-78				1-11-77	4-6-77	5-20-77	7-22-77	8-10-77	9-22-77
3379 MCGAR	10-3-77	1439	11-5-77	8-8-78	6-5-78	11-5-78	3-16-78	6-2-78					1-12-78	3-17-78	5-12-78	7-6-78	8-5-78	
3351 JAMION	5-24-76	2502	6-21-76	3-22-77	5-13-77	7-31-76	11-13-76	3-1-77	4-23-77	9-10-77	11-17-77		6-13-76	10-8-76	12-3-76	1-31-77	3-23-77	4-21-77
3453 BIRMO	9-2-76	2027	9-22-76	6-22-77	7-25-77	11-13-76	2-1-77	3-19-77	6-20-77	11-17-77	1-20-78		6-20-76	10-15-76	12-10-76	9-2-77	10-2-77	11-8-77
3313 STODOLIX	5-15-77	2445	6-15-77	3-16-78	3-24-78	6-8-77	11-9-77	3-13-78	6-22-78				1-12-78	3-17-78	5-12-78	7-6-78	8-5-78	
3374 FAIRCHILD	10-15-77	3537	11-5-77	8-8-78	6-9-78	11-5-78	3-16-78	6-2-78					1-12-78	3-17-78	5-12-78	7-6-78	8-5-78	

*ORLY SAMPLES TO CORRECT BLOND VALUE