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Effects of Sodium Phenylpyruvate on Brain Amino Acids.* (27456)

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In analysis of the free amino acids of serum in phenylketonuric subjects it has been noted (1,2) that along with a high level of phenylalanine, there is a decrease in concentrations of most of the other amino acids. Glycine and histidine, however, are present in normal and excess amounts respectively. Similar data have been obtained in this laboratory(3). It would appear, then, that the high level of phenylalanine is associated with a depression in concentration of other amino acids.

Such studies, therefore, imply a generalized amino acid involvement in phenylketonuria. To examine this concept further, the effects of sodium phenylpyruvate (the characteristic excretory product of the phenylketonuric subject) on the free amino acids of rat brain were investigated.

Methods. Rats (Sprague-Dawley 150-200 g) were sacrificed by dislocation of the neck. After decapitation the brain was removed and separated into hemispheres. Each hemisphere was sliced as thinly as possible by hand in the cold room, weighed, and transferred to Warburg flasks. Incubation in air in the presence of glucose (0.01 mM) and Krebs-Ringer phosphate was carried out for one hour at 37°C. Sodium phenylpyruvate (0.0018 mM) dissolved in the buffer was added to the experimental flasks.

At the conclusion of the experimental period, the medium and tissue were separated

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TABLE I. Distribution of Amino Acids between Tissue and Medium after Incubation.

	A.A. in tissue (μmoles/g)		A.A. in medium (μmoles/ml)	
Amino acid	Control	Exp.	Control	Exp.
Taurine	2.4	2.0	.60	.56
Aspartic	2.1	1.8	.14	.13
Glutamine	2.8	2.3	.50	.40
Glutamic	6.7	6.2	.50	.50
Phenylalanine	.13	.58	.02	.27

System: glucose 0.01 mM; sodium phenylpyruvate 0.0018 mM; Krebs-Ringer phosphate buffer to volume of 3.0 ml. Approximately 400 mg of brain slices used in each experiment.

Concentrations of serine, threonine, glycine, alanine, cystine, cystathionine, methionine, isoleucine, leucine and tyrosine were not significantly changed in tissues and medium between experimental and controls, hence the values are not listed.

by centrifugation. The free amino acids were isolated from the tissue by the picric acid technic and chromatographed(4). The medium was also analyzed for its amino acids in a similar fashion. Sodium phenylpyruvate in amounts equal to those used in these experiments gave no detectable peak when chromatographed(5).

Results. The results of the amino acid analysis of both brain and medium after incubation in presence of glucose are indicated in Table I. When sodium phenylpyruvate is added to the medium, the brain tissue produces phenylalanine. For example, the concentration of phenylalanine increased from 0.13 μmoles to 0.58 μmoles/g tissue. The increase in phenylalanine in the tissue without addition of sodium phenylpyruvate is minimal (0.07 μmoles to 0.13 μmoles/g). Other experiments in which the amount of sodium phenylpyruvate in the medium was 0.0089 mM resulted in a tissue phenylalanine increase from 0.10 μmoles to 1.4 μmoles/g. In addition, concentrations of aspartic acid, glutamic acid and glutamine were decreased in the experimental flasks. Although taurine decreased during the incubation, the other amino acids generally were the same in control and experimental tissue and fluid after incubation.

Discussion. The results presented herein are suggestive of a transamination of phenylpyruvic acid to phenylalanine with either glutamic acid, aspartic acid and/or glutamine serving as amino group donors. Such a concept has been previously proposed(6) on the basis of feeding L-glutamine to phenylketonuric patients. These investigators noted a decrease in excretion of phenylpyruvic acid such as might be anticipated in a transamination mechanism; an increase in blood phenylalanine levels was not observed, however. Other investigators(7) have also presented evidence which is consistent with this concept. In addition, the present experiments demonstrate in rat brain an increase in phenylalanine *in vitro* with a decrease in amino group donors in presence of sodium phenylpyruvate.

It is also of interest that part of the phenylalanine formed from phenylpyruvic acid is found in the incubation fluid at the end of experiment. This would suggest that some phenylalanine is not bound strongly to any cell structure and passes back into the incubation fluid during the experiment. Alternatively, it is also conceivable that there is a leakage of enzyme into the medium and transamination occurs there. However, a slice to medium ratio of approximately 2 was achieved which indicates a substantial concentration in the slice as opposed to the medium.

It would appear from the data that sodium phenylpyruvate does not influence the quantity of the other free amino acids of brain since no important changes, with the exception of taurine, were noted. Whether the other excretory products of the phenylketonuric, or whether phenylalanine itself, may have some effect is currently being studied.

Summary. Addition of sodium phenylpyruvate to rat brain slices incubated *in vitro* with glucose resulted in an increase in brain phenylalanine with a decrease in concentration of aspartic acid, glutamic acid and glutamine. The concentration of other brain amino acids was not affected.

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Studies on Carbohydrate Metabolism in Hypercholesteremic Rhesus Monkeys.* (27457)

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We have reported(1), increased plasma non-esterified fatty acids (NEFA) levels in rhesus monkeys in which hypercholesteremia was produced by feeding cholesterol along with different edible oils. Dole *et al.*(2) suggested that changes in blood NEFA concentration might provide a useful index of alterations in carbohydrate metabolism, as suppression of glucose utilization by adipose tissue was followed by a brisk release of NEFA into the blood and diminution of NEFA when glucose utilization was stimulated. Waddell *et al.*(3) observed diminished glucose tolerance in patients with hypercholesteremia although the fasting blood sugar was normal. A fall in plasma NEFA concentration in normal subjects after an injection of insulin(4) and a progressive rise in NEFA values in diabetic patients(5) suggested a possible relationship between plasma insulin and NEFA concentration. The mobilization of NEFA from adipose tissue by epinephrine was found by Shafrir and Steinberg(6) to be dependent on intact adrenals. It was, therefore, of interest to study the adrenal cortical and plasma insulin-like activities in hypercholesteremic rhesus monkeys and the relation between blood glucose and plasma NEFA of these animals after an oral dose of glucose. The results are presented here.

Experimental. Ten rhesus monkeys were fed a basal diet(1) for a week. They were fasted overnight and a fasting blood sample was collected next morning. Plasma insulin-like activity was determined in these samples using rat diaphragm as described by Willebrands *et al.*(7). 0.5 ml of plasma was used for the determination. The results have been expressed in terms of glucose uptake in mg by rat diaphragm per g of dry tissue per 90 min. incubation per ml of plasma and not in absolute insulin units.

After the above studies, the animals were divided into 5 groups and fed basal diet only; basal diet with 3 g cholesterol mixed with the diet; 3 g cholesterol and 30 g mustard oil mixed with the basal diet; 3 g cholesterol and 30 g coconut oil mixed with the diet and 3 g cholesterol and 30 g sesame oil mixed with the diet as described previously(1). The diets were fed for 8 months. The animals were fasted over night and after a fasting venous blood was collected, each animal was fed 50% glucose, 4 ml/kg. Samples of blood were withdrawn at intervals of 30 minutes for 2 hours. Blood glucose(8) and plasma NEFA were estimated(9) in the different blood samples. Plasma total cholesterol(10) and plasma insulin-like activity(7) were determined in the fasting blood samples.

The animals were then placed in metabolism cages for collection of urine. Twenty-four hour urine samples were collected for consecutive 3 days without addition of any preservative. An aliquot of urine was hydrolyzed with hydrochloric acid, extracted with carbon tetrachloride and 17-ketosteroid estimated by the Zimmermann reaction as modified by Callow *et al.*(11). The average of 3 days' excretion is given in Table I.

Results. The results are given in Fig. 1 and 2 and in Table I. *Glucose tolerance test.* All the animals had normal fasting blood sugar values. Blood sugar of monkeys fed

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