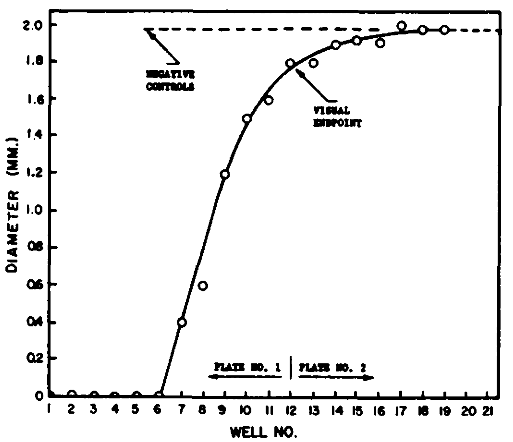

FIG. 1. Size of "buttons" as a function of concentration of toxin. A solution containing 0.42 μg of SEB/ml was carried through two assay plates in twofold serial dilution. At the *visual* end point, well no. 12, the solution has been diluted 1:2048, giving a "button" corresponding to that obtained from 2×10^{-4} μg of SEB/ml (or 1.54×10^{-5} μg of SEB actually in the well). Diameters were measured with the aid of a stage micrometer.

to 99+% pure) and latex particles were tested without adverse indications. The method evidently is not dependent upon unique, coincidental properties of any of the reagents.

In the well representing the end point of a titration with sensitivity of 2×10^{-4} μg/ml, there are approximately 0.027 molecules of SEB/latex particle. This low ratio indicates that reaction conditions described in this report are essentially optimal.

Summary. Staphylococcal enterotoxin B (SEB) may be assayed by a simple and rapid latex-fixation test, with sensitivity improved by several orders of magnitude over conventional methods. Latex particles, coated with specific antitoxin under carefully controlled conditions, were exquisitely sensitive indicators of toxin. The minimal detection limit, 2×10^{-4} μg of SEB/ml of sample, equivalent to 1×10^{-5} μg/well in the assay plate, can apparently be extended to an even lower value with instrumented reading of end points. Titrations require only 100 μl of sample.

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The Free Amino Acids of Brain and Liver during Fetal Life of *Macaca mulatta** (33365)

THEO GERRITSEN AND HARRY A. WAISMAN

Joseph P. Kennedy Jr. Laboratories, Department of Pediatrics, University of Wisconsin Medical Center, Madison, Wisconsin 53706

The relative concentrations of all free amino acids which can be part of protein molecules of animal tissue probably influence the rate of protein synthesis at the cellular level. The other ninhydrin positive compounds in the cell detected during free amino acid analysis should indicate the metabolic activity of these low-molecular weight compounds.

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In this report the free amino acid levels in the brain and the liver of fetal monkeys at different stages of gestation and shortly after birth are given. These data are part of a study of fetal development in a subhuman primate, whose placenta is structurally comparable to the human placenta. Other parts of this developmental study referring to free amino acids in the serum during pregnancy and fetal life of the *M. mulatta* have been reported by Kerr (1).

Although several studies can be found in the literature on free brain amino acids in young animals, fragmentary data on only a small number of fetal brains are available, and then mostly as part of another study. Carver *et al.* (2) compared free amino acids in fetal rat brain at 19 and 21 days gestation. Dravid *et al.* (3) determined some free amino acids in dog brain during development, but only a part of this study was concerned with the analysis of a whole fetal brain. No investigation into the free amino acid content of fetal liver of any animal or human has come to our attention.

Materials and Methods. The design of the fetal study and information on fetal specimens of known gestational age obtained by cesarean section have been described in detail by Kerr (1). Surgery was performed under local anesthesia 8–12 hr after the last feeding. Pregnancies were interrupted after 75, 100, 125, and 150 days, and the fetus was immediately killed by decapitation; the liver and brain were promptly removed and weighed, and frozen on Dry Ice. Several infant monkeys were sacrificed at 7–10 days of age after normal term delivery to obtain specimens 175 days after conception (the normal gestation period of the rhesus monkey is 168 $\pm$ 4 days).

Most samples were analyzed immediately for free amino acids according to the Gerritsen *et al.* (4) modification of the method of Spackman *et al.* (5) using a Beckman/Spinco automatic amino acid analyzer. As internal standards homocitrulline, β-thienylalanine and α-amino-γ-guanidino-butyric acid were used. If, due to technical difficulties inherent in automatic amino acid analysis of tissues, the separation between two adjacent peaks on the chromatogram was insufficient for proper calculation, these values were not reported.

Results and Discussion. Tissues from 20 fetal and four 1-week-old monkeys were available for this study. In Table I, free amino acid content of the brains expressed in mg per 100 g of fresh wet tissue are reported. It will be seen that a wide range in the concentration of some amino acids is present, and therefore it was thought more correct and informative to report mean values with ranges instead of the standard deviation. In Table II, the liver weights and free amino acid content are similarly reported.

The concentrations of the majority of amino acids in the brain during fetal development did not change essentially. The concentrations of aspartic acid, glutamic acid, glutamine and γ-amino-butyric acid increased, the highest levels being found shortly after birth. However, the concentrations of threonine, proline, and possibly alanine decreased during the period under study. Ornithine, which was present in the 75- and 100-day samples could not be detected at a later stage. Cystathionine, an amino acid present in high amounts in human brain but with an unknown function, was found at varying levels in monkeys throughout the fetal period. 3-Methyl-histidine was either absent, or present in traces only during early development, while low levels were found after 125 days. Taurine determinations were variable, but showed an apparent upward trend during fetal life.

The change in amino acid concentrations in the liver during fetal development showed more variation than in the brain. Only serine showed a rather consistent concentration *in utero,* but a sudden increase after birth. Aspartic acid, threonine, glutamic acid, proline, alanine, and cystathionine showed a generally downward trend during fetal development, extending into the first days of life. The following nine amino acids demonstrated the interesting pattern of a decreased concentration during the life of the fetus, but a sudden sharp increase in concentration during the first days of life: glutamic acid, glycine, valine, methionine, isoleucine, leucine, tyro-

TABLE I. Free Amino Acids (mg/100 g of wet tissue) in Monkey Brain during Fetal Develo

	75-day (5)[a]	100-day (4)	125-day (5)	150-day (6)
Brain wt. (g)	5.05 (4.55–5.84)	17.69 (17.14–18.47)	37.71 (36.40–40.06)	48.39 (44.51–51.20
Taurine	90.3 (87.8–92.8)	100.0 —	93.1 (83.1–103.1)	65.5 (24.8–112.8)
Aspartic acid	12.0 (8.5–15.2)	10.5 (8.4–14.8)	8.7 (6.8– 10.5)	14.7 (6.0– 25.2)
Threonine	5.1 (2.9– 8.2)	5.1 (3.3– 7.4)	4.0 (2.3– 4.9)	7.2 (3.3– 14.2)
Serine	9.6 (8.5–11.5)	11.1 (7.4–16.5)	8.6 (6.5– 10.8)	11.2 (7.4– 16.4)
Glutamine	43.7 (38.3–59.0)	50.6 (32.9–61.3)	48.7 (20.0– 67.0)	69.3 (48.5– 82.8)
Proline	7.3 (7.0– 7.6)	3.3 (3.1– 3.4)	3.1 (2.5– 3.9)	2.8 (2.1– 3.6)
Glutamic acid	73.7 (48.2–97.0)	71.1 (65.9–78.9)	75.5 (61.7– 91.9)	95.8 (78.1–113.3)
Glycine	5.9 (3.8– 8.6)	6.5 (5.2– 8.0)	5.9 (3.8– 10.2)	6.6 (3.5– 9.4)
Alanine	9.8 (5.9–14.1)	10.7 (8.7–13.2)	8.8 (5.1– 15.5)	9.4 (7.2– 11.2)
Valine	2.8 (1.4– 4.3)	3.2 (2.9– 3.5)	2.6 (1.9– 3.3)	3.4 (2.8– 4.8)
Cystathionine	—[b] (0.9– 7.2)	1.9 (1.0– 3.0)	1.8 (0.7– 2.8)	6.5 (4.3– 8.9)
Methionine	tr[c] (0.4– 1.8)	tr (0.8– 1.0)	tr (0.3– 1.0)	1.4 (0.6– 2.1)
Isoleucine	1.4 (1.0– 1.7)	1.3 (0.9– 1.7)	1.3 (0.8– 2.0)	1.5 (1.0– 2.0)
Leucine	2.4 (1.2– 3.5)	2.3 (1.7– 2.9)	2.3 (1.7– 3.7)	2.5 (1.7– 3.5)
Tyrosine	1.4 (0.5– 2.0)	1.7 (1.6– 2.1)	1.6 (1.1– 2.4)	2.1 (1.1– 2.5)
Phenylalanine	1.5 (0.7– 2.1)	1.6 (1.3– 2.0)	1.3 (0.8– 1.8)	1.5 (1.0– 2.3)
GABA	4.3 (1.1– 5.6)	6.6 (5.1–10.1)	7.7 (5.4– 9.8)	15.9 (10.3– 22.5)
Ornithine	1.9 (1.5– 2.2)	1.1 (0.8– 1.6)	tr (0.3– 0.5)	tr (0.3– 0.7
Ethanolamine	— (3.3– 7.2)	1.9 (1.4– 2.3)	— (0.3– 11.1)	2.5 —
Lysine	2.6 (2.0– 3.2)	3.4 (3.1– 3.9)	2.7 (1.2– 4.7)	3.0 (1.8– 5.0)
3-Methyl-histidine	tr[c]		2.1 (1.6– 3.0)	3.6 (2.5– 6.3)
Histidine	1.2 (1.0– 1.4)	2.3 (2.2– 2.6)	2.4 (2.0– 3.2)	2.7 (1.6– 3.8)
Arginine	1.5 (0.5– 2.3)	1.6 (tr – 1.6)	1.0 (0.9– 1.1)	tr (0.5– 1.4)

[a] Number of animals in group.
[b] Values ranged so widely that calculation of a mean is not justified.
[c] tr = trace; indicates a mean value of <0.5 mg.

TABLE II. Free Amino Acids (mg/100 g of wet tissue) in Monkey Liver during Fetal Develo

	75-day (5)	100-day (4)	125-day (5)	150-day (6)
Liver wt. (g)	1.80 (1.44–2.00)	5.65 (4.72–6.17)	8.95 (7.75–9.83)	14.70 (13.30–16.03
Taurine	58.1 (49.6– 66.6)	114.8 —	93.6 (85.9–104.1)	79.8 (55.6–93.0)
Aspartic acid	45.9 (37.8– 56.6)	37.5 (33.8–42.3)	23.3 (16.8– 26.2)	21.0 (15.1–28.9)
Threonine	12.0 (9.2– 15.1)	10.9 (8.3–12.4)	9.1 (5.8– 13.6)	9.5 (6.9–13.7)
Serine	28.0 (25.4– 29.6)	32.1 (23.2–44.7)	28.5 (19.3– 34.1)	34.8 (21.8–52.7)
Glutamine	61.5 (57.6– 69.0)	62.0 (42.6–80.2)	61.3 (45.8– 71.7)	54.4 (38.1–85.1)
Proline	24.1 (23.6– 24.6)	21.0 (17.2–27.4)	17.2 (15.9– 20.2)	9.8 (4.5–11.9)
Glutamic acid	115.1 (81.3–140.0)	80.8 (65.6–99.8)	64.2 (50.3– 90.1)	48.9 (33.1–78.0)
Glycine	41.0 (26.7– 48.4)	29.4 (23.9–33.6)	21.6 (16.1– 34.0)	17.1 (10.9–29.4)
Alanine	35.1 (30.3– 38.0)	33.3 (24.4–45.3)	29.5 (24.1– 43.0)	32.5 (24.4–39.2)
Valine	7.3 (6.4– 8.1)	5.0 (4.2– 5.7)	3.5 (1.2– 5.0)	4.6 (3.3– 8.3)
Cystathionine	12.1 (9.6– 14.6)	16.3 (12.4–20.2)	— (0.6– 6.4)	3.2 (1.0– 6.2)
Methionine	3.6 (tr – 4.6)	1.7 (tr – 2.4)	1.1 (0.1– 2.1)	tr (0.3– 1.6)
Isoleucine	4.1 (2.2– 5.8)	2.8 (2.1– 3.4)	2.4 (1.8– 3.1)	2.1 (1.4– 2.8)
Leucine	9.1 (7.4– 10.2)	5.3 (4.7– 6.7)	4.8 (3.9– 6.9)	4.3 (3.0– 5.9)
Tyrosine	8.3 (7.2– 9.4)	4.0 (2.3– 5.2)	4.1 (3.1– 5.6)	2.9 (1.8– 4.1)
Phenylalanine	6.1 (5.3– 7.0)	3.4 (3.0– 3.8)	2.2 (1.8– 2.6)	2.0 (1.1– 2.8)
Ornithine	10.8 (8.8– 14.8)	7.5 (5.8–10.0)	7.4 (4.4– 13.6)	4.8 (3.0– 7.9)
Ethanolamine	9.3 (6.5– 12.1)	11.7 (5.1–19.4)	— (1.2– 9.4)	12.7 (10.0–14.2)
Lysine	13.0 (10.2– 15.5)	9.8 (7.8–12.9)	8.3 (7.6– 10.2)	9.2 (5.7–16.1)
3-Methyl-histidine	1.6 (1.4– 1.7)	3.0 (1.7– 4.1)	3.4 (1.9– 6.9)	3.0 (2.4– 6.2)
Histidine	5.5 (3.6– 9.2)	7.5 (3.8–10.2)	9.0 (6.9– 12.1)	10.4 (7.4–14.5)
Arginine	tr	tr	tr (0.9– 1.0)	tr (tr – 1.4)

sine, phenylalanine, and ornithine. In this connection it is interesting to note that the average weight of the liver between 150 and 175 days only increased 1.3%. An increase in free amino acid concentration during this period could be explained by a general increase in metabolic activity and enzyme maturation. A decrease in concentrations of the TCA cycle related amino acids, glutamic acid and aspartic acid, may be related to the sharp increase in demand for energy, but no proof is available. Correlation between free amino acid concentrations in the brain and the liver of fetal monkeys is virtually absent. The four amino acids whose concentrations increase in the brain during fetal development do not show this trend in the liver. On the contrary, while the concentrations of aspartic acid and glutamine increase in the brain, they decrease in the liver. Glutamic acid levels in the brain and the liver showed the same sharp increase shortly after birth. The concentrations of threonine, proline, and alanine decrease both in brain and liver.

The free amino acid content of umbilical cord serum from the same fetal monkeys (1) failed to correlate with our data on the brain and liver amino acid concentrations. The free amino acid pool available during development of the fetus is undoubtedly a reflection of the free amino acid concentration in the plasma of the mother (6), but the influence of fasting of the mother monkeys for 8 hr prior to surgery on the amino acid levels in the organs of the fetus is unknown.

It is however, difficult to conceive that the wide range of values for some amino acids are due to dietary influences. Although all animals in this study were growing at the same rate despite the differences in free amino acid concentrations, it is doubtful that these levels in the organs of the fetus are a proper reflection of the rate of protein synthesis at the cellular level.

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Production of Antibodies against Insecticide–Protein Conjugates* (33366)

GERHARD J. HAAS AND ENRIQUE J. GUARDIA (Introduced by N. R. Rose)
Corporate Research Department, General Foods Corporation, Tarrytown, New York 10591

The increasing awareness to environmental pollution has resulted in widespread concern about means of detecting and minimizing contamination by pesticide residues. The work reported here constitutes an effort to apply immunologic methods for the assay of pesticide residues. Immune reactions are highly sensitive and specific and can be used as analytical tools without complex and expensive instrumentation. Hence, such a method might be particularly adaptable to field tests.

DDT [1,1,1,-trichloro-2,-2-bis(*p*-chlorophenyl)ethane] and Malathion[1] [*0,0*,-dimethyl-*S*-bis(Carboethoxy)ethylphosphorodithioate] are very widely used and represent two of the most important classes of insecticides: chlorinated hydrocarbons and organophosphates, respectively. These two compounds were chosen for the production of antibodies.

Small organic molecules are not antigenic

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[1] American Cyanamid.