similar study using a serotonin releaser, like reserpine. Such an experiment could throw some light on the pathogenesis of steroid induced ulcers since 5-hydroxy-tryptophan, a serotonin precursor(14) and serotonin(15) were found to be ulcerogenic in rats.

It is interesting to note also that the animals of all groups gained weight equally during the pretreatment period with polymyxin B (Table I). When, however, these animals were fasted, whether in addition they received prednisolone or not, they lost more weight than their respective controls (not receiving polymyxin B). It would seem that previous depletion of histamine renders the animal less resistant to fasting in terms of body weight loss.

Summary. Administration of polymyxin B releases chiefly histamine from tissues. When rats are depleted of histamine by increasing doses of polymyxin B, the ulcerogenic property of prednisolone is unimpaired. This seems to rule out mediation of histamine in the pathogenesis of steroid induced ulcers, at least in the rat.

1. Feldberg, W., Talesnik, J., *J. Physiol.*, 1953, v120, 550.
2. Franco-Browder, S., Masson, G. M. C., Corcoran, A. C., *J. Allergy*, 1959, v30, 1.
3. Jasmin, G. Bois, P., *Rev. Canad. Biol.*, 1959, v18, 367.
4. Robert, A., Nezamis, J. E., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 443.
5. Selye, H., Jean, P., Cantin, M., *ibid.*, 1960, v103, 444.
6. Robert, A., Nezamis, J. E., *Fed. Proc.*, 1962, v21, 201.
7. ———, *Endocrinol.*, 1961, v65, 937.
8. Parratt, J. R., West, G. B., *J. Physiol.*, 1959, v137, 179.
9. Robert, A., Nezamis, J. E., *Fed. Proc.* 1961, v20, 182.
10. ———, *Am. J. Path.*, in preparation.
11. Code, C. F., *J. Physiol.*, 1937, v90, 485.
12. ———, *Physiol. Rev.*, 1952, v32, 47.
13. Wegelius, O., Teir, H., *Acta Endocrinol.*, 1958, v28, 197.
14. Haverback, B. J., Bogdanski, D. F., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 392.
15. Hedinger, C., Veraguth, F., *Schweiz. med. Wchnschr.*, 1957, v87, 1157.

Received January 5, 1962. P.S.E.B.M., 1962, v109.

Placental Transport of Model Amino Acids.* (27312)

BRUCE H. FELDMAN AND HALVOR N. CHRISTENSEN
Department of Biological Chemistry, University of Michigan, Ann Arbor

Amino acids are known to be concentrated in their transfer across the placenta(1). In exploring such transport processes, one needs to exclude irrelevant metabolic attack of the solute in question. For this purpose amino acids not susceptible to modification by the animal organism have proved useful. The present study explores the utility of such model amino acids for investigation of placental transport.

Experimental. Pregnant guinea pigs were used when estimated to be within one week of parturition. The weights of the fetuses on subsequent delivery were sufficiently uniform (65 to 75 g) to support this estimate. The gestation period is 68 days. Each animal was injected intraperitoneally with 10 μc of the 4 carboxyl-C^{14}-labeled amino acids. For α-aminoisobutyric acid this dose represented 0.22 mg, for l-aminocyclopentanecarboxylic acid, 1.3 mg, and for β-monofluoro and β,β,β-trifluoro-α-aminoisobutyric acid, about 3 mg. These amino acids have all been shown to yield extremely small amounts of radioactive CO_2 after injection into the mouse or rat; in the case of the first no other radioactive substance besides the injected amino acid has been detected in the urine(2,3). Therefore we assume that distribution of radioactivity measured distribution of the amino acid in the guinea pig.

* Supported in part by a grant of U. S. Public Health Service. Mr. Feldman, as a medical student, held a Dr. Henry Uriah Upjohn Memorial Fellowship during this work.

TABLE I. Illustrative Experiments with Each of 3 Model Amino Acids. Tissue levels are in cpm for 1 g of fresh tissue.

Amino acid inj.	Body wt, g	Plasma, cpm/ml		Liver, cpm/g		Muscle, cpm/g	
		Maternal	Fetal	Maternal	Fetal	Maternal	Fetal
AIB	1050	2,640	12,500	26,200	10,900	6,390	15,000
l-Aminocyclopentane-carboxylic acid	1300	3,220	21,500	126,000	19,000	10,900	20,600
Fluoro-AIB	1450	1,430	9,250	14,900	7,000	5,400	8,000

The animals were kept in metabolism cages for 24 hours without food, during which time the urine was collected. After the 24-hour interval, the abdomen was opened under ether anesthesia. Blood was then collected from the umbilical vein; this sample represents fetal blood that had just passed through the placenta. Maternal blood was collected from the mesenteric vein. The left lateral lobe of the liver of both mother and fetus was removed, after which the animal was sacrificed. Samples of thigh muscle were then excised from both organisms.

After separating it by centrifugation the plasma was treated with 4 volumes of 10% trichloroacetic acid, and the resulting precipitate removed. Liver and muscle samples were homogenized with a glass homogenizer in the presence of an equal mass of 0.1 *N* acetic acid. Nine portions of water were then added, and homogenization repeated. The resulting suspension was held in boiling water 3 minutes to coagulate protein, then centrifuged to eliminate the resulting precipitate. Two-tenths ml of the extract was added to 3 ml of absolute alcohol, followed by 6.8 ml of a toluene solution containing the phosphors, 2,5-diphenyloxazole (0.5%) and 2-(1-naphthyl)-5-phenyloxazole (0.02%), and the disintegrations counted in a Packard Tricarb liquid scintillation counter.

Liver and muscle radioactivities were calculated per g of cellular water on the assumption that the maternal liver contained 45% cellular water and 28% extracellular water; the fetal liver 38% cellular water and 31% extracellular water; the maternal muscle 60% cellular water and 17% extracellular water; and the fetal muscle 50% cellular water and 31% extracellular water. These values are averages of those obtained earlier(1).

Results. Table I presents illustrative results in terms of actual radioactive counts on individual experiments with each of 3 of the amino acids studied. Table II shows mean results with standard deviations for α-aminoisobutyric acid and l-aminocyclopentanecarboxylic acid, and calculated ratios of distribution between tissues and plasma for these amino acids and also for monofluoro-α-aminoisobutyric acid. The results show that these 3 model amino acids are strongly concentrated across the placental barrier. Trifluoro-α-aminoisobutyric was concentrated only by 1.3 times. The concentration of l-aminocyclopentanecarboxylic acid by the placenta and by maternal tissues is at least as strong as is that of α-aminoisobutyric acid. The fetal muscle and liver were very much less vigorous in concentrating these amino acids. The result contrasts strikingly in this single respect with those obtained previously for glycine, glutamate and the total of the amino acids excluding glycine and glutamine. These natural amino acids appeared to be more strongly accumulated by the fetal tissues than by the maternal(1).

The 47-fold concentration of AIB by the maternal liver is not astonishing when it is compared with the value for glycine in this species(1).

l-Aminocyclopentanecarboxylic acid is an acceptable transport model for intestinal(4) and renal(3) transport, where it is concentrated to about the same extent as valine or leucine. The present result indicates a similarly high degree of acceptability to placental transport, and to uptake by the maternal liver and muscle, but a surprising discrimination against it and the other amino acids with tertiary alpha-carbons, relative to normal amino acids, by the 2 fetal tissues.

The introduction of a single fluorine atom into the β-carbon of α-aminoisobutyric acid

TABLE II. Distribution Ratios of Test Amino Acids Among Fluids and Tissues in the Pregnant Guinea Pig. The recorded tissue levels are calculated per g cell water, relative to concentration in the appropriate plasma; *i.e.*, as the quantity $(F - mP)/nP$, where F represents epm per g fresh tissue, P represents epm per ml of plasma, and m and n are the number of g respectively of extracellular and cellular water per g of fresh tissue.

Amino acid	No. of animals	Fetal/maternal		Liver relative to its plasma	Muscle relative to its plasma
AIB	6	6.4 + 3.9	Maternal	47 ± 18	7.8 ± 7.6
			Fetal	2.1 ± 1.2	3.5 ± 0.4
1-Aminocyclopentanecarboxylic acid	6	8.7 + 2.1	Maternal	51 ± 35	8.0 ± 4.4
			Fetal	1.18 ± 0.08	1.7 ± 0.3
Fluoro-AIB	1	6.5	Maternal	22	6.7
			Fetal	1.18	1.44

appears not to have a strong effect on its distribution in the pregnant guinea pig (Table II). A CF_3 group, however, in place of one of the CH_3 groups of α-aminoisobutyric acid, largely abolishes the uphill transport. This result is not surprising since the structural change lowers the pK_a values to 0.5 and 5.9 for the carboxyl and amino groups respectively (to be published), thereby causing this amino acid to be an anion at neutral pH values. Comparatively slow transport is also seen for it in all other systems so far studied.

Summary. Three model amino acids resistant to metabolic alteration are, like normal amino acids, strongly concentrated across the placental barrier in the guinea pig. Accordingly these substances are suitable for study of this active transport. Although maternal tissues accumulated these 3 amino acids vigorously, the fetal tissues reached only moderately higher levels of the models than did the fetal plasma. Two fluorine-containing amino acids showed no advantages as models; one of them was only slightly concentrated by the placenta.

1. Christensen, H. N., Streicher, J. A., *J. Biol. Chem.*, 1948, v175, 95.

2. Noall, M. W., Riggs, T. R., Walker, L. M., Christensen, H. N., *Science*, 1957, v126, 1002.

3. Christensen, H. N., Jones, J. C., *J. Biol. Chem.*, in press, April 1962.

4. Akedo, H., Christensen, H. N., *ibid.*, 1962, v237, 113.

Received January 8, 1962. P.S.E.B.M., 1962, v109.

Synergistic Effect of Molybdenum and Fluoride on Dental Caries in Rat.* (27313)

GEORGE K. STOOKEY, ROBIN A. ROBERTS AND JOSEPH C. MUHLER
Department of Biochemistry, Indiana University Medical Center, Indianapolis

Adler has reported that the presence of molybdenum in the drinking water is associated with a decreased incidence of dental caries in humans(1). He has also reported a significant reduction in incidence of caries in rats which received 0.1 ppm molybdenum in drinking water(2). Other studies have shown that the presence of molybdenum in drinking water is associated with an increased skeletal fluoride retention in the rat(3), and that rate of fluoride absorption from the gastrointestinal tract of the rat is increased in the presence of molybdenum(4). This study was performed to learn whether fluoride and

* This study was supported in part by a grant from U. S. Public Health Service.