Urea Cycle Enzyme Adaptation to Dietary Protein in Primates

Abstract. *Activities of the five hepatic enzymes of the urea cycle increased two- to threefold in monkeys on a 60 percent protein diet compared with levels in monkeys on an isocaloric 6 percent protein diet. Equal adaptation occurred in rats on the same diets. These enzyme activities in adult humans approximate those of monkeys on a similar protein intake.*

Schimke (*1*) has shown that rat liver adapts to an increase in the level of protein intake by an increase in the activities of the urea cycle enzymes. For studies of human protein nutrition, coma in liver disease, and genetic defects with hyperammonemia, it is pertinent to know whether this response occurs in primates. Therefore, we tested two species of macaques for urea cycle enzyme adaptation.

The five enzymes of the urea cycle—carbamyl phosphate synthetase (CPS), ornithine carbamyl transferase (OCT), argininosuccinate synthetase (AS), argininosuccinate lyase (AL), and arginase—were assayed in liver homogenates by the methods of Schimke (*1*), with use of conditions found optimal in primate liver (*2*). Activities of the five enzymes were linear throughout their incubation periods. We performed each assay at two enzyme concentrations; these duplicates were proportional in all experiments, with variances from 5 percent for OCT to 10 percent for AS.

After four rhesus macaques (*Macaca mulatta*) adjusted to oral–gastric tube infusion of a synthetic diet containing 20 percent casein, we fed two of them 6 percent and the other two 60 percent casein (*3*) for 8 days before performing liver biopsies (*4*). We then reversed diets between the two pairs of animals, repeating biopsies and assays after 8 more days. At 110 cal/kg per day, the monkeys gained 4 percent (average) of initial weight on the low casein diets and 2 percent on the high casein diets. Urinary ratios of urea to creatinine (in milligrams) stabilized by 4 to 5 days at average values of 9 on 6 percent casein and 92 on 60 percent. We fed four 150-g Sprague-Dawley rats the same low casein diets and another four the same high casein diets (measuring their uncontrolled intake) for 8 days before they were killed and the assays were performed.

In Table 1 the activities of the enzymes of the urea cycle are compared in monkeys and rats on the two diets. Adaptation of the five enzymes occurred in each monkey. The ratios of mean activities, with an isocaloric tenfold difference in casein intake, are similar to those of the rats. Ratios based on liver protein are lower than those based on wet weight, because of greater liver protein content in animals on the high casein diet (23.4 percent versus 18.9 percent). Rat arginase activity is higher, particularly on the low casein diet.

Total activities of liver CPS, OCT, AS, and AL per kilogram of body weight, calculated for monkeys (*5*), did not differ from those found in rats, even though the rats consumed 2.5 times more nitrogen per kilogram on both diets. Arginase was the exception, being twice as high in rats as in monkeys. Thus it appears that the rat's daily urea formation is greater than the monkey's, relative to a given level of these enzymes. Peak catalytic rates may be similar, however, as the monkeys received protein by three rapid infusions while the rats fed more steadily. Alter-

Table 1. Comparison of adaptation of the enzymes of the urea cycle to dietary protein in monkeys and in rats. Liver was homogenized in distilled water, and CPS, AS, and AL were assayed by coupled reactions supplemented with purified enzymes prepared from beef liver. Units are defined as micromoles of citrulline or urea formed per hour, and specific activities are expressed as units per gram wet weight or units per milligram of protein. Protein was measured by the Lowry method. Incubations were completed 80 minutes after obtaining fresh tissue. Concomitant assays on liver from rats on a 23 percent protein diet provided quality control.

Animals ($n = 4$)	Protein intake (grams per kilogram per day)	CPS	OCT	AS	AL	Arginase
			*Units per gram wet weight of liver**			
Rhesus	1.6	272 ± 47	7,100 ± 470	117 ± 12	243 ± 57	91,500 ± 25,000
monkey	16.0	900 ± 78	17,200 ± 3,200	359 ± 62	627 ± 94	212,000 ± 25,000
(Ratios)	(10)	(3.3)	(2.4)	(3.1)	(2.6)	(2.3)
Rat	4.0	246 ± 53	6,900 ± 1,850	142 ± 65	213 ± 57	200,000 ± 56,000
	40.0	841 ± 82	15,400 ± 2,900	500 ± 77	600 ± 127	330,000 ± 31,000
(Ratios)	(10)	(3.4)	(2.2)	(3.5)	(2.8)	(1.7)
			*Units per milligram of liver protein**			
Rhesus	1.6	1.52 ± 0.21	40.0 ± 3.5	0.66 ± 0.11	1.36 ± 0.30	516 ± 146
monkey	16.0	4.06 ± 0.21	75.5 ± 13.1	1.36 ± 0.30	2.84 ± 0.39	957 ± 88
(Ratios)	(10)	(2.7)	(1.9)	(2.5)	(2.1)	(1.9)
Rat	4.0	1.41 ± 0.14	39.6 ± 5.8	0.80 ± 0.27	1.22 ± 0.20	1,050 ± 97
	40.0	3.79 ± 0.40	68.9 ± 13.1	2.23 ± 0.35	2.69 ± 0.60	1,480 ± 160
(Ratios)	(10)	(2.7)	(1.7)	(2.8)	(2.2)	(1.4)

* The differences of the means between high and low protein intakes were significant with $P < .01$ for each enzyme in both monkeys and rats.

Table 2. Comparison of hepatic enzymes of the urea cycle in man and monkeys on a similar protein intake.

Animals ($n = 8$)	Protein intake (grams per kilogram per day)	CPS	OCT	AS	AL	Arginase
			Units per gram wet weight of liver			
Man	1.3*	279 ±65	6,600 ± 1,580	90 ± 12	220 ± 25	86,000 ± 9,300
Monkey	1.3	256 ± 63	6,600 ± 1,740	121 ± 14	248 ± 46	122,000 ± 34,000
			Units per milligram of liver protein			
Man	1.3*	1.91 ± 0.27	44.2 ± 7.7	0.62 ± 0.14	1.49 ± 0.26	579 ± 106
Monkey	1.3	1.36 ± 0.29	35.1 ± 8.8	0.64 ± 0.06	1.34 ± 0.25	637 ± 156

* Dietary protein intakes were calculated from hospital diets and are approximate values.

natively, the greater rate of urea synthesis in rats may reflect the greater metabolic rate and protein metabolism of small animals compared to larger ones (*6*).

In a similar experiment, four rhesus macaques and four stump-tailed macaques (*Macaca arctoides*) took isocaloric low casein (1.3 g/kg per day) and high casein (13 g/kg per day) diets for 14-day trials. Although caloric intake was less than 90 cal/kg per day and the animals lost an average of 5 percent of body weight, adaptation occurred in all eight monkeys. Enzyme activities did not differ by species, sex, or dietary sequence. The adaptive ratios, based on wet weight of liver, were: CPS, 2.7; OCT, 1.9; AS, 2.1; AL, 1.6; and arginase, 1.8. Differences of the means for each enzyme were significant at $P < .01$.

Stephen and Waterlow (*7*) reported that the liver argininosuccinase activities of 11 children with protein malnutrition averaged 1.06 units per milligram of liver protein and increased to 1.47 units with refeeding. In biopsies of normal livers from two men and six women, 30 to 70 years of age, undergoing abdominal surgery, we found activities matching those of the eight macaques on a comparable protein intake (Table 2). These two findings, along with the evidence that the five enzyme levels reflect protein nutrition in monkeys as in rats, imply that adaptation occurs in man. In testing for defects of urea synthesis, prudent interpretation of enzyme assays requires control data from normal subjects on the same protein and calorie intake.

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References and Notes

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2. P. J. Snodgrass, *Biochemistry* **7**, 3047 (1968); C. T. Nuzum and P. J. Snodgrass, in preparation. Dr. Sarah Ratner generously supplied purified beef liver OCT and AL.
3. Test diets (General Biochemicals, Chagrin Falls, Ohio) No. 69136 (20 percent casein), No. 69138 (6 percent casein), and No. 69126 (60 percent casein) were isocaloric, with sucrose content inversely proportional to protein. They contained complete vitamin and mineral supplements.
4. The New England Regional Primate Research Center, Southboro, Massachusetts, provided macaques, animal care, and surgical facilities. Biopsies were obtained under phencyclidine anesthesia.
5. The monkeys were not killed, but in autopsies of 15 other rhesus of the same size the liver averaged 3.9 percent of body weight.
6. H. N. Munro, in *Mammalian Protein Metabolism*, H. N. Munro and J. B. Allison, Eds. (Academic Press, New York, 1964), vol. 1, chap. 10, pp. 406–407.
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8. Research support was derived from PHS grant AM-0848. C.T.N. was supported by a Daland Fellowship of the American Philosophical Society.
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16 February 1971

Odor Differences between Enantiomeric Isomers

Abstract. *The enantiomers of* R- *and* S-*carvone,* R- *and* S-*carvotanacetone,* R- *and* S-trans-*dihydrocarvone, and* R- *and* S-cis-*dihydrocarvone were synthesized from* R- *and* S-*carvone, and all were subjected to gas-liquid chromatographic purification. Sensory analysis of the highly purified compounds revealed odor differences between enantiomeric pairs.*

Considerable controversy has surrounded the theories of odor perception (*1*). Claims of odor differences between enantiomeric isomers (*2*) have been open to question because of doubts concerning impurities, chemical resolution, and questionable methods of sensory analysis. Many biological systems such as enzyme-substrate interactions are highly dependent on, and subject to, enantiomeric stereospecificity; however, the degree to which odor perception depends on structure has remained unsettled.

Commercial *R*- and *S*-carvone have been described as having the odors of spearmint and caraway, respectively (*3*). Samples of each isomer, obtained commercially (*4*), were highly purified by gas-liquid chromatography (GLC). Each isomer was successively passed through at least two dissimilar GLC columns. The purified isomers were trapped in forms which were chemically "pure" to the limits of detectability (*5*). The enantiomeric carvotanacetones were synthesized (Fig. 1) from the respective carvones via hydrogen reduction (*6*). Enantiomeric *cis*- and *trans*-dihydrocarvones were synthesized by Wallach reduction of the respective carvones, and the diastereoisomers were

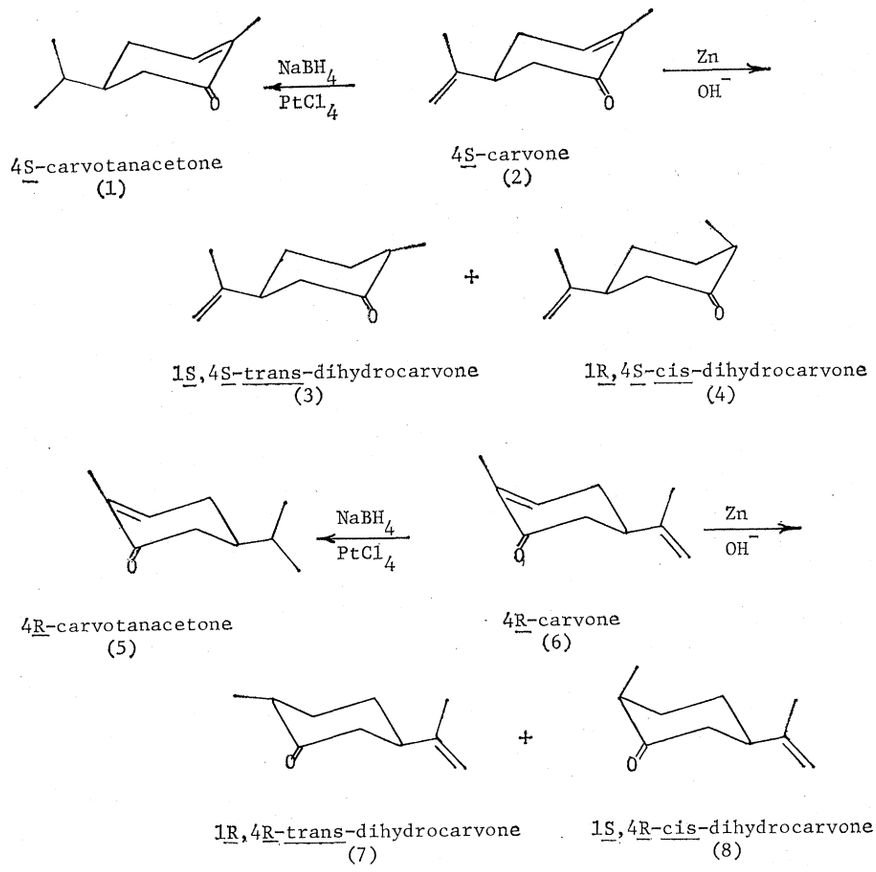

Fig. 1. Structures of carvones and related compounds.