Dietary Regulation of Galactose-Metabolizing Enzymes: Adaptive Changes in Rat Jejunum

Abstract. *The effects of dietary galactose, sucrose, fructose, glucose, casein, and fasting upon the activity of four galactose-metabolizing enzymes (galactokinase, galactose-1-phosphate uridyltransferase, uridine diphosphate galactose 4-epimerase, and galactose dehydrogenase) were studied in the jejunum of rats. Galactose produced the greatest increase in enzyme activity, fructose and sucrose produced effects intermediate between galactose and glucose, and casein produced a greater activity increase than fasting, but less than the sugars.*

Dietary sucrose increases jejunal sucrase and maltase (*1*) and specific dietary sugars increase specific jejunal glycolytic enzymes in man and rat (*2*). We now report the effect of dietary galactose upon galactose-metabolizing enzymes.

Dietary galactose alters the hepatic galactokinase activity of young rats (*3*). The prolonged administration of galactose produced a slight but significant rise in liver galactokinase activity in adult rats (*3*). We have studied the effects of dietary galactose, sucrose, fructose, glucose, casein, and fasting upon the activity of galactose-metabolizing enzymes galactokinase (E.C. 2.7.1.6), galactose-1-phosphate uridyltransferase (E.C. 2.7.7.12), uridine diphosphate galactose 4-epimerase (E.C. 5.1.3.2), and galactose dehydrogenase (E.C. 1.1.1.48) in adult rat duodenum, jejunum, and ileum.

Twenty-four male Holtzman rats (320 to 400 g) were housed in individual cages with free access to water. Six groups of four rats each were fasted for 3 days. One group was killed after fasting. Each of the remaining five groups were fed as follows. The basic diet for the 3 following days consisted of (by weight) casein, 17 percent; Crisco, 10 percent; salt mixture, 4 percent; and NBC vitamin diet fortification mixture, 1 percent. To this diet we added 68 percent of sucrose for group 1; 68 percent of fructose for group 2; 68 percent of glucose for group 3; 68 percent of galactose for group 4; 68 percent of casein for group 5 (the total casein for this group being 85 percent). No significant differences were noted in the weight gains and food consumption of the five groups fed after fasting. The average total food consumption and standard error for each group during refeeding were: group 1, 65.5 ± 3.8 g; group 2, 62.5 ± 3.1 g; group 3, 64.0 ± 2.3 g; group 4, 59.9 ± 5.3 g; group 5, 57.0 ± 6.3 g.

The rats were stunned by cervical fracture; and portions of the duodenum, jejunum, and ileum were collected. The intestinal segments were slit longitudinally, and the mucosa was scraped with a glass slide. After storage at − 85°C, the mucosa was homogenized in a Kontes-Duall grinder with 20 volumes of a tris-ethylenediaminetetraacetic acid buffer, *p*H 7.5, containing 120 m*M* KCl, 20 m*M* tris, 5 m*M* $MgSO_4$, and 0.1 m*M* ethylenediaminetetraacetic acid (Na_2). The homogenates were centrifuged at 104,000*g* for 60 minutes in a Spinco model L2 ultracentrifuge at 4°C. The resulting supernatant fraction was used for the enzyme assays.

Activities of galactokinase (*3*), galactose-1-phosphate uridyltransferase (*4*), uridine diphosphate galactose 4-epimerase (*4*, p. 185), and galactose dehydrogenase (*5*) were determined for each sample, and protein was determined by the method of Lowry *et al.* (*6*).

Activities of the individual enzymes in the jejunum are shown in Table 1. These data show that galactose produces the greatest increase in enzymes with decreasing effects from fructose, sucrose, glucose, and casein, respectively.

In *Escherichia coli*, a single operon (the gal operon) contains the linked structural genes for the three enzymes specific for galactose utilization: galactokinase, galactose-1-phosphate uridyltransferase, and uridine diphosphate galactose 4-epimerase (*7*). These three enzymes, which are collectively called the gal enzymes, are coordinately induced by D-galactose (*8*). Our data show that the same three enzymes are also adaptively increased in jejunum of galactose-fed rats. The activities of galactokinase and uridyltransferase were increased eightfold, and the epimerase activity was increased fourfold, as compared to the activities in fasted rats. These observations are consistent with the idea of coordinate control of the three enzymes in the jejunum of rats fed galactose. Though consistent, this neither proves nor disproves the existence of a gal operon in rat jejunum.

In rat liver, the activities of galactose dehydrogenase and galactokinase are of similar magnitude (*9*). Based upon the relative activities of these two enzymes, our data suggest that galactose is metabolized chiefly by galactokinase in the rat jejunum. The ratios of the activities of galactokinase to galactose dehydrogenase was 1.8 : 1 in fasted rats and 4.5 : 1 in galactose-fed rats.

Comparisons of enzyme activities in different sites of the small intestine (Table 2) showed that the jejunum was the most active metabolic site in the galactose-fed rats. The finding of highest enzyme activity in the jejunum coincides with the fact that the jejunum

Table 1. Effect of diet upon galactose-metabolizing enzymes in rat jejunum. Enzyme activity was measured in nanomoles per minute per milligram of protein.

Diet	Enzyme activity (mean ± S.E.)			
	Galactokinase	Uridyl-transferase	Epimerase	Galactose dehydrogenase
Galactose	20.77 ± 1.14*	75.94 ± 3.08	41.10 ± 2.99	4.66 ± 0.07
Fructose	11.61 ± 0.72	39.81 ± 2.88	31.08 ± 1.60	2.96 ± 0.08
Sucrose	9.98 ± 0.42	34.60 ± 0.42	29.95 ± 0.51	3.05 ± 0.17
Glucose	6.90 ± 0.48	26.80 ± 0.87	24.19 ± 0.95	2.38 ± 0.08
Casein	4.69 ± 0.18	17.66 ± 0.54	18.22 ± 0.83	1.84 ± 0.09
Fasting	2.68 ± 0.29	8.99 ± 0.29	11.09 ± 0.96	1.53 ± 0.10

* Data for 4 rats.

Table 2. Effect of diet upon galactose-metabolizing enzymes in different sites of the small intestine of rats fed galactose.

Small intestine site	Enzyme activity (mean ± S.E.) (nmole min^{-1} mg^{-1})			
	Galactokinase	Uridyl-transferase	Epimerase	Galactose dehydrogenase
Duodenum	12.76 ± 0.18*	73.39 ± 0.53	30.98 ± 0.44	4.14 ± 0.09
Jejunum †	20.77 ± 1.14	75.94 ± 3.08	41.10 ± 2.99	4.66 ± 0.07
Ileum	10.38 ± 0.44	64.68 ± 1.14	22.26 ± 0.35	3.96 ± 0.09

* Data for four rats. † The data for the jejunal enzymes are taken from Table 1.

is the most active site of absorption of monosaccharides in the small intestine (*10*).

Our data demonstrate the adaptive nature of several galactose-metabolizing enzymes (galactokinase, galactose dehydrogenase, uridyltransferase, and uridine diphosphate galactose 4-epimerase) in the jejunum of rats. These results, coupled with previous observations on certain jejunal glycolytic enzymes (*2*), provide a convenient model for studying the regulation of intestinal enzymes in vivo.

FRED B. STIFEL, ROBERT H. HERMAN
NORTON S. ROSENSWEIG
U.S. Army Medical Research and Nutrition Laboratory, Denver, Colorado 80240

References and Notes

1. N. S. Rosensweig and R. H. Herman, *J. Clin. Invest.* **47**, 2253 (1968).
2. N. S. Rosensweig, F. B. Stifel, R. H. Herman, D. Zakim, *Clin. Res.* **16**, 291 (1968).
3. P. Cuatrecasas and S. Segal, *J. Biol. Chem.* **240**, 2382 (1965).
4. E. S. Maxwell, K. Kurahashi, H. M. Kalckar, *Meth. Enzymol.* **5**, 179 (1962).
5. P. Cuatrecasas and S. Segal, *J. Biol. Chem.* **241**, 5910 (1966).
6. O. H. Lowry, N. J. Rosenbrough, A. D. Farr, R. J. Randall, *ibid.* **193**, 265 (1951).
7. G. Buttin, *J. Mol. Biol.* **7**, 164 (1963); E. Jordan, M. B. Yarmalinsky, H. Kalckar, *Proc. Nat. Acad. Sci. U.S.* **48**, 32 (1962); E. L. Lederberg, in *Microbial Genetics*, W. Hayes and R. C. Clowes, Eds. (Cambridge Univ. Press, London, 1960), p. 115.
8. H. Kalckar, K. Kurahashi, E. Jordon, *Proc. Nat. Acad. Sci.. U.S.* **45**, 1776 (1959).
9. P. Cuatrecasas and S. Segal, *Science* **153**, 549 (1966).
10. R. K. Crane and P. Mandelstam, *Biochim. Biophys. Acta* **45**, 460 (1960).

29 July 1968; revised 8 October 1968

Transmission of Experimental Kuru to the Spider Monkey (Ateles geoffreyi)

Abstract. *Clinical signs and pathological changes characteristic of kuru in man and experimental kuru in chimpanzees were observed in two spider monkeys,* Ateles geoffreyi, *after inoculation with brain tissue from a kuru-affected chimpanzee. The incubation period for one of the monkeys was 23 months, and 26 months for the other.*

A clinical syndrome remarkably similar to kuru in man (*1*) and experimental kuru in chimpanzees (*2*) appeared in two spider monkeys (*Ateles geoffreyi*) 23 and 26 months, respectively, after inoculation of each animal with brain tissue from a chimpanzee (*Pan satyrus*) with experimentally induced kuru. The syndrome, with progressive cerebellar ataxia, incoordination, and tremor, has not been seen as a spontaneous disease of any monkeys in our own or other laboratories. It closely mimicked the clinical pattern of kuru in man and the chimpanzee and progressed to severe incapacitation in 6 months. Similar inoculation of a suspension of brain from the same chimpanzee into nine rhesus (*Macaca mulatta*), one cynomolgus (*Macaca irus*), six African green (*Cercopithecus aethiops*), two squirrel (*Saimiri sciurea*), and one patas (*Erythrocebus patas*) monkeys has produced no disease after 30 months; inoculation into a chimpanzee produced kuru in 11 months.

On 9 February 1966, chimpanzee A-1 was killed in the advanced stage of experimental kuru, 30 months after intracerebral inoculation of a suspension of human brain from a patient who had kuru (see *2*). Chimpanzee brain tissue was ground to a 20 percent suspension in isotonic physiologic phosphate-buffered saline, *p*H 7.4, and the homogenate was clarified by centrifugation at 1500*g* for 30 minutes. Male (S-1) and female (S-2) adult spider monkeys were each inoculated with a 10 percent suspension of brain as follows: 0.2 ml intracerebrally into the left frontal cortex, 0.2 ml intravenously, and 0.1 ml subcutaneously.

In spider monkey S-1, apathy and decreased activity were first noted 23 months after inoculation. Over the next 3 months he became progressively more slow, deliberate, and cautious in his movements; during the 3rd month of clinical disease he developed intermittent, shivering truncal tremors and poorly coordinated hand movements, with bilateral dysmetria and coarse intention tremor of the upper extremities. By the 5th month of overt disease he had become thin and shabby. Incoordination of the extremities became more severe, and, although the animal could still use his hands, he was frequently observed to eat from the floor by leaning forward and taking food directly into his mouth. Gait was slow, swaying, and ataxic; he rarely attempted to climb and, while sitting, exhibited truncal titubation. No ataxia was noted in the prehensile tail.

During the animal's last week of life there was continual drooling of saliva. No other abnormalities were noted except for a left dorsal scoliosis. The facial expression was normal (without the lip droop seen in kuru-affected chimpanzees). Cranial nerve functions appeared normal. There was no focal weakness of the limbs or evidence of spasticity or rigidity; deep tendon reflexes were normal, and both plantar responses were flexor. Touching of the lips provoked a rooting response.

Throughout illness S-1 remained alert and friendly and had a good appetite. At no time was there evidence of any systemic illness. There were no convulsions or fever. Findings in hematological and chemical studies of blood did not differ significantly from normal human values. On 10 July 1968, 6 months after onset of illness, the animal was anesthetized and surgical biopsies of the frontal and occipital cerebral cortex were performed to obtain tissue for electron microscopic study; the animal was then killed by exsanguination.

The brain and other organs were grossly normal. Preliminary histopathological studies show intense, diffuse status spongiosus of cerebral gray matter with marked astroglial hypertrophy and neuronal loss with vacuolation of neurons—a picture remarkably similar to that in the chimpanzee with experimental kuru (*3*). More extensive neuropathological findings will be presented elsewhere.

Twenty-six months after inoculation, spider monkey S-2 was first noted to have become slow and clumsy. A month later she exhibited intermittent truncal titubation and fine tremors of the extremities. Disease continued to progress in essentially the same course as that of S-1. By the time she was killed, 4 months after onset, S-2 had severe ataxia of gait, with dysmetria, incoordination, and coarse intention tremors of all limbs and tail. She showed a marked startle in response to loud noise and light touch. Clinical signs were otherwise the same as those of S-1. Brain biopsies and autopsy were performed as for S-1, and the brain and other organs were grossly normal. Preliminary histopathological studies showed the same findings as those of S-1.

Until recently only two other spider monkeys were inoculated with kuru tissue: one, S-3, was inoculated 12