necessary for equilibration to be reached. With increased extracellular volume the time for equilibration to be reached is longer and the time for post-infusion urine collection becomes extremely long, making the inulin space determinations impractical under such conditions.

No satisfactory explanation can be offered to account for the difference in inulin recovery in our hands and those of Gaudino and Levitt. The possibility that light anesthesia significantly altered the rate of diffusion of inulin in our experiments has not been excluded.

Summary. 1. Inulin recovery was found to be incomplete in five hours after the termination of a two-hour inulin infusion. Since the unrecovered inulin amounted to an average of 15% of the inulin present in the dog at the time of equilibration, an equivalent error was introduced into the calculation of the inulin space by the method of Gaudino and Levitt. 2. By administering a solution made from pure, accurately weighed, dry inulin, and determining the inulin content of the urine excreted during the infusion, the amount of inulin remaining in the dog can be computed with an average error of only 3.8%. In addition, the time needed for urine collection is greatly reduced.

Received May 13, 1951. P.S.E.B.M., 1951, v77

Role of Fat on Utilization of Lactose in Milk by Rats and Rabbits. (18857)

SACHCHIDANANDA BANERJEE.

From the Department of Physiology, Presidency College, Calcutta, India.

The role of fat on the utilization of lactose or galactose by rats was reported by several workers(1-5). Rats cease to grow and die if lactose or galactose is the only source of carbohydrate. Growth is continued and death is averted if fat is included in the diet(1). Rats fed skim milk excrete galactose in urine and addition of fat prevents this loss in urine (2). The percentage of ingested galactose excreted in the urine is inversely proportional to the percentage of fat in the diet(4). It is, however, not clear how fat helps in the utilization of galactose. In a normal animal ingested galactose is converted into glycogen in the liver(6). It is probable that in the absence of fat in the diet liver can not convert galactose into glycogen. Glycogen content of the liver of skim milk-fed and whole milk-fed rats was, therefore, determined. As galactose is mainly present in the nervous tissue as a constituent of cerebroside it was of interest to study whether skim milk-fed rats could utilize galactose for the synthesis of cerebroside of the brain. To study if fat is necessary for the metabolism of galactose in other species of animals the effect of feeding skim milk on urinary excretion of galactose by rabbits was also studied.

Experimental. Effect of feeding skim milk and whole milk on urinary excretion of galactose and on glycogen deposition in the liver of rats. Male rats weighing between 68 and 98 g were taken. Eight rats were fed *ad libitum* mineralised(2) skim milk for 15 days. Each rat was kept in a metabolism cage and the urine was collected under toluene. For the next 2 days the rats were fed daily 80 cc of skim milk per rat. Nine rats were fed mineralised whole milk for 15 days and for the next 2 days each of the rats received daily 40 cc of whole milk isocaloric with 80 cc of skim milk. The rats also received 2 drops of a concentrate of vit. A, D and K twice a

1. Guha, B. C., *Biochem. J.*, 1931, v25, 1385.

2. Schantz, E. J., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1938, v122, 381.

3. Geyer, R. P., Boutwell, R. K., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1945, v162, 251.

4. Nieft, M. L., and Deuel, Jr., H. J., *J. Biol. Chem.*, 1947, v167, 521.

5. Zialcita, Jr., L. F., and Mitchel, H. H., *J. Nutrition*, 1945, v30, 147.

6. Bell, D. J., *Biochem. J.*, 1936, v30, 1612.

TABLE I. Urinary Excretion of Galactose and Glycogen Value of Liver of Rats Fed Whole Milk or Skim Milk for 15 Days. Whole milk-fed rats received isocaloric amount of milk as consumed by skim milk-fed rats for last 2 days of experiment.

	No. of rats used	Wt at death, g	Ingested galactose excreted in urine, %	Glycogen per 100 g liver, mg
Skim milk-fed rats	8	89 (82–100)	22.5 (16.2–32.4)	12.6 (9–17)
Whole milk-fed rats	9	98 (84–120)	0	776 (405–1847)
Diff. of means				763.4
Std error of diff.				161
t				4.7*

* Highly significant.

week. Sugar excreted in the urine was estimated with Benedict's quantitative reagent and calculated as galactose after standardizing the reagent against pure galactose. The sugar in the urine was identified as galactose(2). On the eighteenth day of experiment the rats were stunned by a blow on the head, neck veins were cut and livers were removed. Glycogen in the liver was estimated by the method of Grattan and Jensen(7). Percentage of the ingested galactose excreted in the urine on the last day of experiment and the glycogen value of the liver are given in Table I.

Effect of feeding skim milk and whole milk on cerebroside content of the brain and fat content of liver of rats. Weanling rats weighing between 31 and 56 g were divided into 2 groups. One group was fed *ad libitum* for 7 weeks skim milk prepared by mixing 10 g skim milk powder with 90 cc water in a Waring blender. The milk was mineralised(2). The second group of rats was fed for 7 weeks whole milk prepared by mixing 12 g of whole milk powder with 88 cc water. The rats also received 2 drops of a concentrate of vit. A, D and K twice a week. Rats were weighed on alternate days and galactose in urine was estimated daily. Skim milk-fed rats excreted galactose at the later stage of the experiment. The whole milk-fed rats did not excrete any galactose. The animals were killed after 7 weeks and brain and livers were removed for the estimation of cerebroside and fat respectively.

Estimation of cerebroside. The brain was weighed and spread on a washed filter paper of 9 cm diameter. The filter paper was rolled and placed inside a glass thimble with perforated walls, which was suspended from the lower end of a condenser fitted into a 125 cc conical flask with a ground glass joint. The brain was defatted by extraction with acetone in a boiling water bath for 4 hours and then extracted with aldehyde-free ethanol for another 4 hours. The ethanol extract was transferred into a 50 cc centrifuge tube and kept in a refrigerator overnight. The precipitate was discarded and the supernatant was made up to a definite volume with absolute ethanol. Cerebroside was determined in an aliquot of the extract by the method of Brand and Spery(8) slightly modified. The results are given in Table II. *Estimation of liver fat.* Fat content of livers of rats was estimated according to the method described by Chattopadhyay, Nandi and Banerjee(9). The results are given in Table II.

Relation of fat to utilization of lactose by the rabbit. Adult rabbits did not consume milk when supplied exclusive of other diets and died of inanition. Rabbits given milk from birth learn to drink it and gain in weight. Six rabbits born in this laboratory were given milk from birth, 3 received skim milk and the rest whole milk for 2 months. The rabbits were housed in individual metabolism cages and urine was collected under toluene. Sugar in the urine was estimated daily. None of the

7. Grattan, J. F., and Jensen, H., *J. Biol. Chem.*, 1940, v135, 511.

8. Brand, F. C., and Spery, W. M., *J. Biol. Chem.*, 1941, v141, 545.

9. Chattopadhyay, H., Nandi, N., and Banerjee, S., *Indian J. Physiol. and All. Sci.*, 1950, v4, 65.

TABLE II. Cerebroside Content of Brain and Fat Content of Liver of Rats Fed Whole Milk or Skim Milk *ad libitum* for 7 Weeks.

	No. of rats	Wt at death, g	Gain in wt per week, g	Cerebroside per 100 g brain, mg	Fat per 100 g liver, g
Skim milk-fed rats	9	96 (80–106)	8 (6–10)	741 (412–982)	5 (4.2–6)
Whole milk-fed rats	8	132 (111–161)	12 (8–16)	676 (502–876)	6.8 (6.1–7.6)
Diff. of means			4	65	1.8
Std error of diff.			1.18	83	.35
t			3.4	.8*	5.2

* Except for this value, all values of *t* are highly significant.

rabbits excreted sugar in the urine.

Results. Skim milk-fed rats excreted galactose in urine. The glycogen values of their livers were significantly lower than the values obtained in rats fed isocaloric amounts of whole milk. Considerable amount of fat was present in the liver of skim milk-fed rats. The cerebrosides content of brains of whole milk and skim milk-fed rats did not differ significantly. Skim milk-fed rats grew at a lesser rate than the whole milk-fed rats. Rabbits fed skim milk for 2 months did not excrete sugar in the urine.

Discussion. The diminished glycogen values of the liver of skim milk-fed rats possibly indicate that galactose is not converted into glycogen in the liver of rats in the absence of fat in the diet. The galactose, therefore, goes into the blood stream and being a low threshold substance is excreted by the kidney. Galactose, however, can be partly utilized in the absence of liver(10). The normal cerebrosides content of skim milk-fed rats indicate that galactose is partly utilized by these rats in the formation of cerebrosides. The presence of considerable amounts of fat in the liver of skim milk-fed rats might be due to partial conversion of galactose into liver fat. Fat is not necessary for the utilization of lactose by the rabbits.

Summary. (1) The glycogen and fat contents of the liver are significantly diminished in the rats fed skim milk. (2) The cerebroside content of the brain of the skim milk-fed and whole milk-fed rats did not differ significantly. (3) Urinary excretion of galactose by the skim milk-fed rats might be due to non-conversion of galactose into glycogen in the liver. (4) The skim milk-fed rats utilized galactose to form cerebrosides of the brain and fat of the liver. (5) Unlike rats, rabbits fed skim milk for 2 months did not excrete galactose in urine.

10. Bollman, J. L., Mann, F. C., and Power, M. H., *Am. J. Physiol.*, 1935, v111, 483.

Received May 21, 1951. P.S.E.B.M., 1951, v77.