Effect of Scurvy on Active Transport of Glucose by Small Intestine *in vitro*. (29205)

SACHCHIDANANDA BANERJEE AND SAMBHU DAYAL VARMA
Department of Physiology, Sardar Patel Medical College, Bikaner, India

After glucose feeding blood glucose of scorbutic guinea pigs attained a markedly higher peak and showed a delayed fall compared with that of normal guinea pigs(1). The disordered carbohydrate metabolism observed in scorbutic guinea pigs was suggested to be due to associated insulin deficiency(2-5). Increased active transport of glucose by everted small intestine of alloxan diabetic rats had been reported(6-7). Adrenal cortical hormones promoted intestinal absorption of glucose(8) and increased adreno-cortical activity was observed in scorbutic monkeys and guinea pigs(9). In view of the above observations it was of interest to study the effect of vit. C deficiency on intestinal absorption of glucose in guinea pigs.

Materials and methods. Selection of guinea pigs, feeding of the scorbutogenic diet, division of the animals into different groups and treatments given have been described(10). In the fourth week of the experiment, the normal, scorbutic and insulin-treated scorbutic guinea pigs were fasted for 24 hours and killed by decapitation. Animals which were allowed to recover from scurvy after they were fed a daily dose of 10 mg of ascorbic acid for 4 weeks were also killed similarly. Removal of the intestine, preparation of the everted sacs of small intestine and study of the intestinal absorption of glucose was as described previously(7).

The sacs containing about 0.4 ml of Krebs-Ringer-bicarbonate saline with a 0.3% glucose concentration and a bubble of oxygen were incubated in a 50 ml stoppered and siliconized conical flask containing 6 ml of the same solution, gassed for 30 seconds with oxygen, for one hour in a shaking incubator at 37°C. Sacs were then removed, weighed, fluid drained in a watch glass, the empty sac weighed again and the final volume of the fluid in the sac obtained. The sac was dried to constant weight at 60°C. Glucose was estimated in the fluid inside the sac and in the fluid of the incubation medium by the method of Hagedorn and Jensen(11), and transference of glucose calculated.

Results. The results are given in Tables I and II. Mean transference of glucose from mucosal to serosal side of the everted sacs per g of dry sac and per hour of incubation was 9.04 mg in normal guinea pigs. The transference increased significantly when the animals developed scurvy; the value was 19.14 mg. Insulin treatment of the scorbutic animals did not significantly change the intestinal absorption of glucose. When the animals recovered from scurvy the transference of glucose was 8.71 and was similar to that of normal guinea pigs.

Discussion. Glucose was absorbed from intestinal sacs prepared from the small intestine of normal, scorbutic, insulin-treated scorbutic and recovered guinea pigs against a concentration gradient showing that the major part of glucose transported across the mucous membrane was by means of active transport. Absorption of glucose by intestine of rats is also by active transport(6). There appears to be no species difference in the mechanism of absorption of glucose in rats and guinea pigs.

Intestinal absorption of glucose was enhanced in scorbutic guinea pigs as compared to normal guinea pigs. Utilization of glucose is diminished in scurvy(4,5) as evidenced by the diminished glucose tolerance. Intestine of scorbutic guinea pigs, therefore, might also metabolize less glucose than normal, thereby inducing a greater concentration of glucose in the epithelial cells and as a result more glucose possibly is transferred into the serosal fluid.

Insulin treatment of the scorbutic animals did not significantly alter the absorption of glucose but when the animals recovered from scurvy, intestinal absorption of glucose was at a similar rate to that observed with normal guinea pigs. Ascorbic acid, therefore, plays

TABLE I. Glucose Absorption by Everted Small Intestine of Guinea Pigs.

Condition of animals	No. of sacs	Initial glucose conc (mg %)	Final conc of glucose (mg %) after 1 hr incubation, in Serosal fluid	in Mucosal fluid	Dry wt of sac (g)	Transference of glucose, mg/g dry sac/hr incubation at 37°C
Normal	16	291 (266–309)	331 (288–480)	271 (218–299)	.041 (.031–.058)	9.04 (3.76–18.80)
Scorbutic	19	292 (282–318)	416 (307–563)	270 (187–298)	.031 (.021–.047)	19.14 (9.20–32.53)
Recovered from scurvy	20	304 (290–314)	358 (301–433)	275 (208–305)	.047 (.026–.084)	8.71 (3.00–14.00)
Insulin-treated scorbutic	10	296 (280–304)	499 (466–570)	236 (214–260)	.055 (.048–.066)	23.60 (13.80–38.60)

Figures in parentheses indicate range of values.

TABLE II. Mean Transference of Glucose by Everted Small Intestine of Guinea Pigs Under Different Treatments.

Condition of animals	Transference of glucose, mg/g dry sac/hr incubation	t values	
(A) Normal	9.04 ± 1.20		
(B) Scorbutic	19.14 ± 1.90	Between A and B	4.59*
(C) Recovered from scurvy	8.71 ± .22	" A and C	.21
(D) Insulin-treated scorbutic	23.60 ± 3.09	" B and D	1.23

Values = Mean ± standard error. * This value of t is only significant.

a part in intestinal absorption of glucose. The action of the vitamin seems to be a direct one on the intestinal epithelium and is possibly not mediated through the insulin deficiency associated with scurvy. In studies with alloxan diabetic rats, Crane(6) showed that increased transference of glucose by diabetic intestine was not affected by insulin treatment of the animals. The mechanism of increased transference of glucose in the intestine in scurvy and in diabetes might be similar but the exact mechanism is not clear.

The hyper function of the adrenal cortex in scurvy(9) might also be responsible for the increased transference of glucose across the intestine as has been observed in experiments with adrenalectomized rats.

Summary. Intestinal transport of glucose *in vitro* was studied in normal, scorbutic, insulin-treated scorbutic and ascorbic acid-supplemented scorbutic guinea pigs by the use of everted small intestine sac technique. Glucose was absorbed from the intestine of the differently-treated animals against a concentration gradient, indicating that the major part of glucose was absorbed by active transport. Rate of absorption of glucose from the intestine of scorbutic guinea pigs was twice that of normal. When the guinea pigs recovered from scurvy after supplementation with ascorbic acid, intestinal absorption of glucose became normal. Insulin treatment of the scorbutic animals did not alter significantly the glucose absorption observed in the scorbutic condition. Ascorbic acid seems to play a role in intestinal transport of glucose. This does not seem to be mediated through the associated hypoinsulinism observed in scorbutic guinea pigs.

1. Banerjee, S., Divakaran, E. R., *Am. J. Physiol.*, 1958, v195, 291.

2. Banerjee, S., *Nature*, 1943, v152, 329.

3. ———, *ibid.*, 1944, v153, 344.

4. Banerjee, S., Ghosh, N. C., *J. Biol. Chem.*, 1947, v168, 207.

5. Banerjee, S., Biswas, D. K., Singh, H. D., *ibid.*, 1958, v230, 261.

6. Crane, R. K., *Biochem. Biophys. Res. Comm.*, 1961, v4, 436.

7. Varma, S. D., Banerjee, S., *Indian J. Med. Res.*, 1963, v51, 507.

8. Althusen, T. L., Anderson, E., Stockholm, M., PROC. SOC. EXP. BIOL. AND MED., 1939, v40, 342.

9. Banerjee, S., Singh, H. D., *Am. J. Physiol.*, 1957, v190, 265.

10. Banerjee, S., Bandyopadhyay, A., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 372.

11. Hagedorn, H. C., Jensen, B. N., *Biochem. Z.*, 1923, v137, 92.

Received November 27, 1963. P.S.E.B.M., 1964, v116.

Influence of Number of Suckling Young on Nucleic Acid Content of Lactating Rat Mammary Gland.* (29206)

H. ALLEN TUCKER (Introduced by Joseph Meites)
Department of Dairy, Michigan State University, East Lansing

Single litters of 8 rat pups per 12 mammary glands provided sufficient suckling stimulus to maintain deoxyribonucleic acid (DNA) content, a measure of cell numbers, to 24 but not 28 days of lactation(1). Suckling by foster litters, however, maintained mammary DNA to 61 days(2) and maintained lobule-alveolar system to 70 days(3). Ribonucleic acid (RNA) content and RNA/DNA ratio, measures of protein synthetic activity, were maintained to 21 days of lactation(1) but markedly declined thereafter despite continued suckling(2). On the other hand, teat ligation of 3 abdominal-inguinal mammary glands of rats decreased the DNA and RNA content and RNA/DNA ratio when compared with contralateral, nonligated glands or glands of normal rats lactating similar periods of time(4). Thus, suckling stimulus, without milk removal, did not prevent cellular loss nor maintain the protein synthetic activity of the mammary gland. The nucleic acid content, however, of contralateral, nonligated glands was greater than those of glands of normal rats(4) and mice(5) lactating the same length of time.

The present research was undertaken to determine whether or not varying numbers of suckling pups would influence the number of cells (DNA) and protein synthetic activity (RNA and RNA/DNA) of intact abdominal-inguinal mammary glands of rats.

Methods. The litter size of lactating primiparous Sprague-Dawley rats was adjusted to 4, 6, 8, or 10 pups per 12 glands (groups 4/12, 6/12, 8/12, 10/12, respectively) on day 3 of lactation. In a second experiment, teats of the 6 thoracic glands were ligated on day 3 of lactation and 2, 4, or 6 pups (groups 2/6, 4/6, 6/6, respectively) were allowed to suckle the 6 intact abdominal-inguinal glands. Litter weight gain from the 7th to 15th day after parturition was used as an index of lactational performance.

Mother rats were sacrificed by cervical dislocation on the 21st day of lactation, and the 6 abdominal-inguinal mammary glands of rats of both experiments were removed, washed in ice-cold 0.25 M sucrose, blotted on paper towels, weighed, and then stored in 0.25 M sucrose at —20°C until analyzed.

The 6 abdominal-inguinal mammary glands were suspended in ice-cold distilled H_2O (1 g to 20 ml) and homogenized 2 min in a Waring blendor at top speed in a cold room. Duplicate 2-ml samples of homogenate were suspended in 8 ml of 95% ethanol for 24 hr at room temperature. The samples were extracted for 24 hr with 9 ml of methanol: chloroform (2:1) and for 24 hr with 9 ml of anhydrous ether(6) with constant agitation. The samples were extracted twice with 5 ml of ice-cold 10% trichloracetic acid which was removed by washing with ice-cold ethanol saturated with sodium acetate. The samples were digested in 2 ml of 1 N KOH for 15 hr at 37°C, and the digest was acidified with 0.3 ml ice-cold 6 N HCl and 5 ml ice-cold 10% perchloric acid (PCA). The residue was washed twice with 5 ml of 5% PCA and combined supernatants were analyzed for RNA-ribose(7). The remaining residue was extracted with 5 ml of 5% PCA at 70°C for 15 min and washed twice with 5 ml of

* Journal article No. 3270 from Mich. Agri. Exp. Station.