Effects of Tolbutamide on Intestinal Glucose Absorption and Blood Glucose Levels.* (24223)

JOHN E. KLIMAS, JR.† AND GORDON W. SEARLE (Introduced by H. M. Hines)
Department of Physiology, College of Medicine, State University of Iowa

Apparent inhibition of intestinal glucose absorption by oral or intravenous carbutamide (1) has been attributed to possible effects of this compound on gastrointestinal motility (2) since some of its congener sulfonamides have been shown to affect visceral smooth muscle motility(3). The work with carbutamide suggests particularly a delay in passage of fluid from the stomach(1,2). Experience with still another member of this group, tolbutamide, lends support to this view. Thus, in the present study, with glucose absorption confined to the small intestine of the rat, tolbutamide introduced directly with the glucose solution into the gut lumen had no significant effect upon absorption of the sugar in acute experiments. At the same time, the amount of tolbutamide absorbed was sufficient to affect blood sugar levels measured at the end of the absorption period.

Methods. Adult, male rats‡ weighing from 170 to 278 g were used. The experiments were done on paired rats with all procedures including analyses being done at the same time for each pair. In each of the 9 pairs used, one rat served as "control" and the other as "test." The pairing was done by weight so that no more than 8 g difference existed and this difference was randomly distributed so as to favor neither "controls" nor "tests." The rats fasted 24 hours before each experiment, and were anesthetized with sodium pentobarbital (30 mg/kg, I.P.). The small intestine was reached through a midline incision in the ventral abdominal wall. The common bile duct was ligated and 2 glass cannulae were used to isolate the entire small intestine and connect its lumen with 10 ml syringes at both ends. The loop thus made was rinsed with warmed normal saline. When these washings were clear, a final rinse was made with a 1% glucose solution. This was flushed out with air in the same manner as the fluid in the system would be flushed out at the end of the absorption period in each experiment. Immediately following these preliminary steps, the solutions for absorption were introduced. One of the loop systems ("control") was filled with 1% glucose solution and the other ("test") received a solution of 1% glucose and 0.1% tolbutamide.§ The distal syringe barrel, without plunger, served as a means of establishing and maintaining a uniform hydrostatic pressure of 7 cm of the solution during the absorption period. Solution was added as necessary from the proximal syringe. After 45 minutes each system was emptied as described above. Both the solutions introduced and those removed, as well as tail vein blood samples taken before and at the end of the absorption period, were analyzed for glucose by the Somogyi-Nelson method(4). Net glucose absorption was estimated as the difference between the quantity introduced and that recovered from each loop system.

Results. Average net glucose absorption values are shown in Table I. The difference between the means is not significant ($t = 0.3$; $p > 0.7$) by the method of group comparison analysis(5). The mean difference between initial and final tail vein blood glucose concen-

TABLE I. Net Intestinal Glucose Absorption without ("Control") and with ("Test") Tolbutamide.

Group	n	Net glucose absorbed, mg
Control	9	152 ± 16*
Test	9	150 ± 14

* Mean ± stand. error.

* Supported in part by a grant from the U. S. Public Health Service.

† Public Health Fellow. Present address: Fairfield Univ., Fairfield, Conn.

‡ Hooded rats from the colony maintained by the Dept. of Psychology.

§ Tolbutamide (Orinase-Upjohn) was kindly supplied by the Upjohn Co.

TABLE II. Mean Difference between Initial and Final Blood Glucose Concentration in Control and Test Rats.

Group	n*	Mean difference, mg %
Control	8	85 ± 14.2†
Test	8	51 ± 4.8

* Blood glucose analyses were not obtained in one exp. pair included in Table I.
† Mean ± stand. error.

trations is shown in Table II. The increase is significantly greater ($t = 2.24$; $p<0.05$) by group comparison analysis(5) in the control group. For all the rats in which blood glucose concentrations were obtained, mean initial value was 94 mg %. Although not quantitatively measured, the peristaltic activity as reflected by oscillations in the fluid level in the distal syringe barrel appeared to be about the same in both control and test systems.

Discussion. It is apparent that under the test conditions in the rat, both tolbutamide and glucose are readily absorbed from the same solution. The net glucose absorption data support this directly in the case of glucose. The significantly smaller increment of blood glucose rise over the period of equivalent net glucose absorption is indirect evidence that tolbutamide was absorbed in sufficient quantity to exert its typical effect on blood glucose in normal rats. The apparent inhibition in absorption of glucose in the experiments with carbutamide previously cited(1) may be due to prolongation of gastric emptying time but this awaits direct proof. Structural differences in this family of compounds are too well known to be associated with widely varying physiological action to allow conclusions to be drawn by analogy from tolbutamide to carbutamide.

Summary. In rats glucose absorption from the small intestine was not affected by the presence of tolbutamide in the glucose solution used. The tolbutamide absorbed in these experiments was sufficient to produce a relative suppression of the alimentary hyperglycemia present at the end of the absorption period.

1. Friedlich, T. L., Ashworth, M. A., Hawkins, R. D., Haist, R. E., *Canad. M. A. J.,* 1956, v74, 973.
2. Haist, R. E., Hawkins, R. D., Ashworth, M. A., *Diabetes,* 1957, v6, 21.
3. Kleibel, F., *Lancet,* 1954, v1, 882.
4. Somogyi, M., *J. Biol. Chem.,* 1952, v195, 19.
5. Snedecor, G. W., *Statistical Methods,* The Iowa State College Press, Ames, 1946.

Received June 30, 1958. P.S.E.B.M., 1958, v98.

Survival of Unfertilized Mouse Eggs During Freezing and Thawing.* (24224)

J. K. SHERMAN AND TEH PING LIN (Introduced by Emil Witschi)
American Foundation for Biological Research, Madison, Wisc.

Preservation by freezing has been possible only with certain cells, others being susceptible to lethal factors, usually associated with ice formation. Long-term storage of germ cells at sub-freezing temperatures was given impetus by introduction of glycerol as a protective agent(1). Today, many of our dairy cattle are bred artificially with semen so preserved(2), and the technic has had successful clinical application in insemination of humans(3).

Similar success has not been realized with the mammalian female gamete, although reports on fertilized rabbit eggs and eggs of lower forms are encouraging. Eggs of the sea urchin reportedly were fertilized upon rewarming from a medium of sea water plus glycerol which had been frozen at −10°C(4). Formation of ice in the jelly coats of fertilized eggs of the frog did not prevent their subsequent normal development into tadpoles(5). Some hydrated eggs or early embryos of the brine shrimp proceed to hatch into free-swimming larvae after thawing from their frozen

* This study was supported in part by a research grant from the Nat. Inst. of Health, P.H.S.