aortic tissue samples was 86.2% of that observed for normal tissue of the same arterial specimens.

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Enhancement of Glucose Absorption by Oleic Acid.* (31361)

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Recent *in vitro* studies have indicated that bile salts inhibit intestinal absorption of glucose(1,2,3). The present experiments were designed to establish the validity of this observation *in vivo* and to study the reciprocal effect of glucose and oleic acid upon their intestinal absorption in the presence of bile salts.

Methods. Adult, male, mongrel dogs were prepared with 6 to 8 inches long Thiry-Vella fistulae constructed from the jejunum distal to the ligament of Treitz. Steel Gregory cannulae were placed in both ends of the loop, wrapped with omentum and brought through the abdominal wall. Each dog was allowed 3 weeks' convalescence before experiments were begun. Before each experiment, the loop was perfused with 100 to 200 ml isotonic saline at 38°C to evacuate cellular debris. An attempt was made to recover all this solution by gentle suction through a polyvinyl tube inserted into the loop. The omental adhesions surrounding the cannulae prevented loss of fluid from the loop.

Test mixtures were made in Krebs-Ringer bicarbonate solution, pH 7.4, with 0.5% polyethylene glycol (PEG). Concentrations of substances contained in the various test mixtures were as follows: glucose 168 mEq/l, sodium taurocholate (NaTC) 8 mEq/l, oleic acid either 20 mEq/l or 5 mEq/l, and 3-0-methylglucose (3-0-MG) 156 mEq/l. In each experiment, 25 ml of a test solution was introduced into the loop for 30 minutes. The fluid recovered at the end of that period was centrifuged at 2000 rpm for 15 minutes, and the supernate analyzed. After each experiment the loop was again rinsed with 100 to 200 ml of warm saline solution. Analysis of this rinse showed no measurable PEG and less than 1% of the total unabsorbed glucose.

Glucose and 3-0-MG were measured by the anthrone method described by Seifter *et al*(4). Oleic acid determinations were done by a modification of the method of Itaya and Michio(5). Cholate was determined by the method of Irvin *et al*(6). Sodium was measured by flame photometry. PEG was determined by a modification of the method of Hyden(7). Maximum turbidity was read 16 minutes after addition of trichloroacetic acid, rather than in 5 minutes noted in the original method. The estimated volume of fluid unabsorbed from the loops was calculated from the concentration of PEG in the fluid recovered at the end of each experiment. The volume of fluid recovered from the loop was usually 1 to 5 ml less than the estimated final volume calculated on the basis of PEG concentration. This discrepancy was greatest in experiments utilizing NaTC, for in these experiments residual fluid was nearly twice as great as when glucose alone was used. The total amount of each solute unabsorbed by the loops after 30 minutes was estimated from

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TABLE I. Effect of NaTC and Oleic Acid on Absorption of Glucose and Na^+.

Test solution*	No.	No. of studies	Glucose absorption mEq ± S.D.†	Glucose absorption % Total	Na^+ absorption mEq ± S.D.
Glucose	1	10	1.37 ± .26	32.7	1.22 ± .29
	2	10	1.72 ± .29	41.0	1.51 ± .25
	3	10	1.44 ± .19	34.4	1.29 ± .27
	4	10	1.68 ± .23	40.2	1.57 ± .21
	5	10	1.83 ± .21	43.7	1.72 ± .20
	6	8	1.49 ± .19	35.6	1.11 ± .19
Glucose and NaTC	1	10	1.52 ± .21	36.2	.80 ± .26
	2	10	1.46 ± .31	35.1	.88 ± .28
	3	10	1.67 ± .33	40.0	.97 ± .29
	4	10	1.55 ± .28	37.0	1.20 ± .39
Glucose and NaTC and oleic acid (20 mEq/l)	1	10	2.71 ± .39	64.9	.71 ± .23
	2	10	2.60 ± .28	62.0	.43 ± .35
	3	10	2.53 ± .36	60.5	.60 ± .22
	4	10	3.06 ± .36	67.4	.81 ± .37
	5	10	2.69 ± .26	64.5	.69 ± .26
	6	10	2.60 ± .32	56.5	.43 ± .30
Glucose and NaTC and oleic acid (5 mEq/l)	4	10	2.81 ± .44	73.2	.93 ± .28
	5	10	2.95 ± .37	70.5	.89 ± .31
	6	5	2.36 ± .37	62.2	.48 ± .22

* In each experiment, 25 ml of test solution was placed in the loop; this volume contained 4.18 mEq glucose, 3.75 mEq Na, and (where indicated) 125 or 500 μEq oleic acid and 0.2 mEq NaTC.

† S.D. = Standard deviation.

the calculated value for the final volume and the concentration of the various solutes chemically determined in the fluid actually recovered. Absorption of the various solutes in the test solutions was determined by subtracting the quantity of solute remaining in a loop at the end of an experiment from the amount known to have been present initially.

The statistical significance of the difference between the absorption of a given solute administered under different conditions was determined individually for each dog using Student's t-test.

Results. Effect of oleic acid and sodium taurocholate on absorption of glucose. Thirty minutes after instillation of 4.18 mEq of glucose into intestinal loops of 6 animals, an average of 1.59 mEq or 37.9% of the glucose was absorbed. Addition of NaTC to the solution did not significantly alter absorption of glucose in any dog. Under this condition, the average glucose absorption in 4 dogs was 1.55 mEq or 37.1%. Addition of oleic acid, 20 mEq/l, to a solution containing glucose and NaTC in concentrations similar to those present in control experiments resulted in an average absorption of 2.69 mEq or 60.9% of glucose instilled into the loops of 6 dogs. In 3 animals a solution of glucose, NaTC and oleic acid, 5 mEq/l, was used. In these experiments with the lipid completely in micellar solution, an average of 2.71 mEq or 68.6% of glucose was absorbed. In both groups of experiments, glucose absorption from solutions containing oleic acid was significantly greater ($P<.001$) for each dog than absorption from solutions without oleic acid (Table I). Had the estimation of glucose absorption in these experiments been incorrect, the calculations used in determining the amount of fluid and glucose remaining in a loop at the end of an experiment would have resulted in an underestimation of glucose absorption. This strengthens the significance of these results.

Net sodium absorption from the glucose solution was 1.40 mEq, or 37% of the total sodium load. When NaTC or NaTC and oleic acid were added to glucose, the average net sodium absorption was reduced to 0.96 and 0.68 mEq, respectively. Net sodium absorption in all experiments in which NaTC was present was significantly less ($P<.01$) for each dog than that which occurred from glucose solution without NaTC.

Cholate determinations indicated no sig-

TABLE II. Effect of Glucose on Jejunal Absorption of Oleic Acid.

Dog No.	No. of exp	Glucose present	Total initial lipid (μEq)	Lipid absorbed μEq	Lipid absorbed % Total
4	5	—	125	101 ± 6*	81
	10	+	125	106 ± 6	85
	5	—	500	242 ± 67	48
	10	+	500	275 ± 60	55
5	5	—	125	111 ± 8	89
	10	+	125	94 ± 11	76
	6	—	500	257 ± 49	51
	10	+	500	286 ± 62	57
6	6	—	125	91 ± 14	73
	10	+	125	113 ± 12	90
	5	—	500	322 ± 53	64
	10	+	500	307 ± 41	61

* ± S.D.

nificant absorption of cholate from the loops.

Effect of glucose on absorption of oleic acid. The influence of glucose on the absorption of oleic acid from intestinal loops was measured in 3 dogs. From a micellar solution containing 125 μEq of oleic acid, an average of 104 μEq or 84% of the oleic acid was absorbed in the presence of glucose, and 101 μEq or 81% in the absence of glucose. When the concentration of oleic acid was increased to 500 μEq, an average of 289 μEq or 58% was absorbed in the presence of glucose and 274 μEq or 54% in the absence of glucose. The absorption of oleic acid at the two concentrations studied was not significantly altered by the presence of glucose (Table II).

Effect of oleic acid and sodium taurocholic acid on absorption of 3-0-methylglucose. The effect of oleic acid and NaTC on absorption of a metabolically inactive sugar, 3-0-methylglucose (3-0-MG) was studied in 2 dogs. Thirty minutes after instillation of a solution containing 3.86 mEq of 3-0-MG, an average of 1.87 mEq or 48.4% was absorbed. Following instillation of a solution containing 3-0-MG in a micellar solution of NaTC with oleic acid, an average of 1.71 mEq or 44.2% of the 3-0-MG was absorbed. By contrast with glucose, the absorption of 3-0-MG was not enhanced by oleic acid. On the other hand, net absorption of sodium was as significantly reduced by NaTC in a solution of 3-0-MG as it was in a solution of glucose (Table III).

Discussion. Our data indicate that the presence of oleic acid enhances the absorption of glucose from the jejunum. This enhancement occurs whether oleic acid is present entirely in a micellar solution (5 mEq/l) or is present partly in micelles and partly as an emulsion (20 mEq/l). The transport of many sugars, glucose and 3-0-MG included, is reported to be dependent on the transport of Na^+ (8,9). Schultz and Zalusky have shown that the converse is also true; the simultaneous transport of certain sugars enhances Na^+ transport(10). The increased glucose absorption and decreased net Na^+ absorption when oleic acid and NaTC were present appears at first inconsistent with these observations. As the results in Table I indicate, addition of either bile or bile salt plus fatty acid decreased net Na^+ absorption; however, glucose absorption increased only when bile salts and oleic acid were administered together, and not in the presence of bile salts alone. This finding suggests that the observed enhancement of glucose absorption in the presence of oleic acid is not due to the maintenance of a higher Na^+ concentration alone, but must be due to some relationship between glucose absorption and the presence of lipid itself. In further support of this view,

TABLE III. Jejunal Absorption of 3-0-Methylglucose in Presence and Absence of Oleic Acid and NaTC.

Type study	Dog No.	No. of experiments	3-0-MG absorbed, mEq ± S.D.	Mean net mEq Na^+ absorbed
Control*	5	5	1.92 ± .21	2.11 ± .24
	6	5	1.83 ± .37	1.75 ± .26
Experimental†	5	4	1.70 ± .42	1.00 ± .31
	6	4	1.72 ± .29	1.03 ± .27

In each study, a total of 3.86 mEq was offered the loop initially.
* 3-0-MG in Krebs-Ringer.
† 3-0-MG in Krebs-Ringer plus 0.2 mEq NaTC and 125 μEq oleic acid.

the absorption rate of 3-0-MG, which is also Na^+-dependent, and which in many respects is comparable to that of glucose, was not increased by oleic acid and bile salt, although net absorption of Na^+ was similar to that observed when glucose was used.

When cholate determinations were performed on recovered samples, no absorption was found. The similarity of the initial and final cholate concentrations (initial concentration 8 mEq/l, final concentration range 8 ± 2.5 mEq/l) suggests that the test solutions containing bile salt or bile salt and oleic acid remained hypertonic throughout the test period. Since there is also a relationship between water and Na^+ transport (11), the apparent failure of water and sodium absorption in experiments with bile salts might be explained by the concomitant reentry of water and sodium into the lumen to achieve isotonicity. This explanation allows the assumption that the interdependence of glucose and Na absorption is unaltered in these studies.

Experiments in which glucose in Krebs-Ringer was replaced with 3-0-MG demonstrated that absorption of the non-metabolized analogue was similar to that of glucose. This is in accord with the findings of Fordtran *et al*(12). By contrast, absorption of 3-0-MG was not enhanced by addition of NaTC and oleic acid, as was glucose absorption. The transport mechanism for 3-0-MG is known to be similar to that of glucose: both sugars are poorly absorbed in the absence of Na^+; both enhance Na^+ transport; both are actively transported(9); and if one is added in the presence of the other, mutual transport inhibition is seen(12). These facts suggest that the two substances share a common intracellular carrier, and that increased glucose absorption in the presence of fatty acid is related to intracellular changes in glucose metabolism, due to absorption of fatty acids, rather than to a difference in the transport mechanism for the two sugars.

Holt *et al* have shown that hamster jejunal slices utilize more glucose when low concentrations of oleic acid (4 mM/l) and bile salts are present(13). In addition, Isselbacher *et al* have shown that the conversion of fatty acids and glycerol precursors into triglycerides in the intestinal cell involves an energy-dependent esterification of the fatty acid(14, 15). Buell and Reiser(16) have shown that L-α-glycerophosphate is the principal glycerol precursor in this process, and it is known that intestinal cells contain the enzymes necessary for conversion of glucose to glycerophosphate. Thus, increased glucose absorption may result from its utilization as the source of energy for free fatty acid esterification and as a glycerol precursor for triglyceride formation. It is uncertain whether these pathways operate to enhance the absorption of glucose during fatty acid metabolism in the intestinal cell. These *in vivo* studies do indicate enhanced absorption of glucose in the presence of bile salts and oleic acid and do not support previous *in vitro* studies by others that indicate inhibition of glucose absorption by bile salts.

Summary. 1. NaTC alone exerted no effect on absorption of glucose from the jejunum. 2. Oleic acid (20 mEq/l and 5 mEq/l) in the presence of NaTC enhanced the absorption of glucose. 3. Oleic acid and NaTC did not increase absorption of 3-0-methylglucose. 4. Intestinal absorption of oleic acid in a solution of NaTC was not increased by the presence of glucose. 5. It is concluded that enhanced absorption of glucose from the jejunum by the presence of a micellar solution of oleic acid is possibly related to utilization of glucose in intracellular lipid metabolism.

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Effect of Insulin Pretreatment on Glucose and Lipid Metabolism of Liver Slices from Normal Rats.* (31362)

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The mechanisms controlling the dependence of hepatic lipogenesis on active carbohydrate metabolism continue to be the subject of investigation, which has inevitably involved consideration of the role of insulin in this relationship(1-4). These studies, however, have not dealt with the relative participation of glucose and insulin in the metabolism of hepatic tissue or with the effects of insulin and glucose on relative incorporation of glucose into its various metabolic pathways.

It has been suggested that elevated levels of plasma triglyceride observed in patients with the clinical syndrome of carbohydrate-induced hypertriglyceridemia(5) may result from increased hepatic production and secretion of triglyceride-rich lipoproteins(6). It is uncertain what role, if any, the abnormal glucose tolerance and elevated levels of plasma insulin found in these patients(7,8) play in altering hepatic lipid metabolism and plasma triglyceride levels. It is possible that increased hepatic triglyceride production may result from the effects of hyperinsulinemia and increased glucose concentration acting together on an otherwise normal liver.

To examine the relationship between glucose concentration and insulin action on the metabolism of glucose by the liver, the effect of insulin pretreatment and glucose concentration on the incorporation of glucose into CO_2 and various lipid fractions by liver slices from normal rats has been studied.

Methods. Male Sprague-Dawley rats weighing between 200-300 g were fed *ad libitum* until the time of sacrifice. One group was pretreated by subcutaneous injection of 1-2 units of ultralente insulin per kilogram of body weight, 20 hours prior to sacrifice. Liver slices were prepared using a Stadie Riggs hand microtome. All tissue was incubated in Krebs-Ringer bicarbonate buffer, pH 7.4. Medium glucose concentration was either 4.0 mg/ml or 0.4 mg/ml, and all incubations were carried out at both concentrations. For ease of expression, these concentrations will be designated as "high" and "low." Tracer amounts of glucose-$6^{14}C$ were added to the buffer so that the initial specific activity of the media was the same regardless of glucose concentration.

Three to four hundred mg of tissue from each rat were placed in 3 ml of media containing glucose at both "high" and "low" concentrations, and the amount of glucose-$6^{14}C$ recovered as CO_2 or lipid was determined for each animal. All studies were done in triplicate at each glucose level. Incubations were carried out for 2 hours in a metabolic shaker at 37°. Medium glucose was measured enzymatically (glucose oxidase)

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