with RNA 4 days before challenge were also lower than the controls. In 9 experiments of 24, however, the bacterial counts of both organs or one organ in the controls were lower than the counts in the RNA injected mice. The difference between the mean bacterial counts was slight and does not warrant the conclusion that RNA had enhanced the bactericidal ability of the fixed phagocytes.

On the day of challenge the mean leucocyte count of mice injected with RNA 30 minutes before irradiation was not significantly different from the mean control count. The former was 700 per mm^3 and the latter 500 per mm^3.

Summary. CF1 female mice 9-10 weeks old were injected intravenously with RNA 30 minutes before or on the 4th day after exposure to a sublethal dose of X-irradiation (550 r). Simultaneously an equal number of irradiated mice were injected intravenously with buffered saline to serve as controls. On the 5th day post-irradiation all mice were injected intravenously with *Ps. aeruginosa* followed one minute later by intravenous polymyxin or colymycin. The next day all mice were killed and heart's blood cultured. Livers and spleens were removed, ground separately in tissue grinders and appropriate dilutions plated. The results of the bacterial counts indicated that the mean number of pseudomonads in livers and spleens of mice injected with RNA were slightly lower than the mean number in livers and spleens of controls but the difference was not significant. Similar results were obtained among unirradiated mice injected with RNA 4 days before challenge with *Ps. aeruginosa.*

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1. Hammond, C. W., Anderle, S. K., Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 690.
2. Gordon, L. E., Cooper, D. B., Miller, C. P., *ibid.*, 1955, v89, 577.

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Reversibility of Intestinal Absorption of 3-0-Methylglucose.* (28630)

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The model amino acids, α-aminoisobutyric acid and 1-aminocyclopentanecarboxylic acid, reach much the same distribution between the contents of isolated loops of the small intestine and the blood serum of the rat, whether by absorption or by secretion(1). The distribution ratios reached are far higher than those attained across the walls of isolated intestinal sacs(2). These findings appear to represent the first demonstration and evaluation of "uphill" transport of amino acids from the intestine *in vivo*, and of the reversible action of this transport. We have now made the same demonstration with the unmetabolizable sugar, 3-0-methylglucose(3-6).

Bárány and Sperber showed that absorption of glucose from sulfate- or sorbose-containing solutions in isolated loops of the intestine of the rabbit could bring the concentration of sugar in the loop to approximately one-quarter to one-fifth of the level of the blood sugar(7). These experiments appear to show unequivocally that glucose absorption can occur against a considerable concentration gradient. Campbell and Davson(8) showed in 3 experiments that the 3-0-methylglucose level of the contents of the small intestine of the cat could be lowered by absorption to values in the range of 55 to 78% of simultaneous plasma levels, which were maintained by injection at 5-minute intervals.

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Neither of these investigations was designed to provide information about the existence or position of a steady state in the "uphill" absorption of monosaccharides. The present experiments indicate that the transport of 3-O-methylglucose is a reversible one and that the steady state distribution places the level in the lumen of the ileum of the rat at about one-fourth to one-eighth of the plasma level.

Materials and methods. Rats of the Sprague-Dawley strain were the gift of Upjohn Co. Males weighing 200 to 250 g were used, after first fasting about 24 hours. In contrast to the model amino acids, 3-O-methylglucose shows a poor renal tubular reabsorption, so that the rate of loss from the animal is a serious technical disadvantage. Because nephrectomized animals tolerated poorly the preparation of intestinal loops, we did not remove the kidneys, but instead injected enough of the methyl-labeled sugar (usually 20 μc = 40 mg) so that the large loss could be accepted. Urinary loss every 8 hours appeared to be roughly 90% of that present. About 16 hours were allowed for the sugar to distribute itself before filling 3 ileal loops with an isotonic solution containing 144, 100, 54 and 10 milliequivalents per liter respectively of Na^+, HCO_3^-, Cl^- and K^+. In the indicated cases 154 millimolar glucose, sorbose or mannitol replaced 77, 54 and 23 milliequivalents per liter respectively of Na^+, Cl^- and HCO_3^-. The operative procedure and the procedures for measuring ^{14}C have been described(1). Ileal loops were selected for technical reasons, even though the jejunum is known to show stronger concentrative transport of sugars *in vitro*.

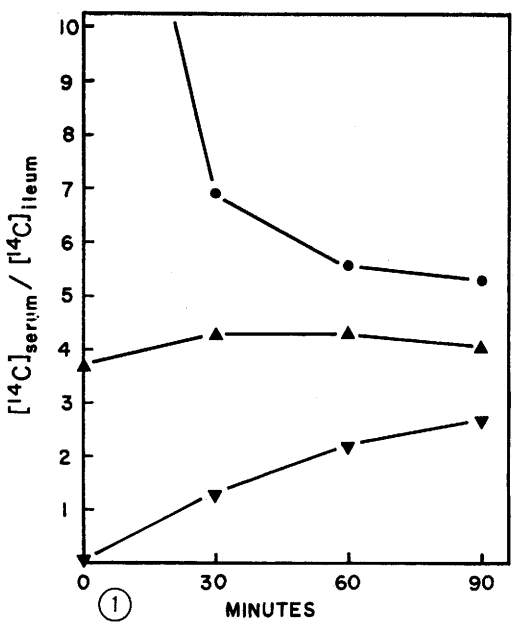
Just before or just after the ileal loops were filled, blood was taken from the tail, and at the end of the procedure a large sample of blood was taken from the heart of the animal. As an average the serum level of ^{14}C was halved during 2 hours, although the changes ranged from scarcely any to a quartering. A halving corresponds well with the mean rate observed over longer intervals. Because the values on tail blood were not considered reliable enough to justify calculating interpolated values for the serum radioactivity at each time interval, we calculated the results illustrated, using the final serum value as if it applied throughout the 90- to 120-minute experiment. This procedure for dealing with the inherent uncertainty as to the actual value for the plasma radioactivity at each point has a conservative effect on the mean value ascribed to the distribution ratio for ^{14}C between serum and ileal contents. The real value may well be about 50% greater than the values of 4 to 6 usually obtained.

Results. Fig. 1 shows a result, typical of those obtained repeatedly, when 3 successive ileal sacs were filled with a saline solution containing ^{14}C-labeled 3-O-methylglucose (always of the same specific activity as that injected) either at a level substantially greater than (lower curve), or one nearly equal to the final serum level (as indicated by ^{14}C counting), or no sugar at all (upper curve). In the first case net absorption occurs, in the last case net release, to approach in this case a distribution ratio of about 4.

Fig. 2 illustrates results indicating that the release of radioactivity probably represents a specific transport, as shown for the release of 1-aminocyclopentanecarboxylic acid(1). The presence of an excess of glucose in the loop accelerated the release of radioactivity into the usual saline solution in the lumen, whereas the presence of sorbose or mannitol had no consistent effect. Radioactivity was released into a loop formed from the ascending colon at roughly the same rate seen for the ileum.

At the end of the experiment the ileal tissue contained, on a fresh weight basis, 2.8 ($\sigma = 0.7$) times as much radioactivity as the serum. To account for this result the radioactivity must presumably have been concentrated from the extracellular fluid into the cells by a mean factor larger than 4. The liver uniformly contained much less radioactivity per gram than the intestinal tissue, although the level estimated for the liver water was ordinarily somewhat above the serum level. This result is not taken necessarily to argue for "uphill" transport into this tissue, in view of the rapid decline of the body content of the sugar.

Summary. Although 3-O-methylglucose, because of its rapid excretion, is less satisfac-



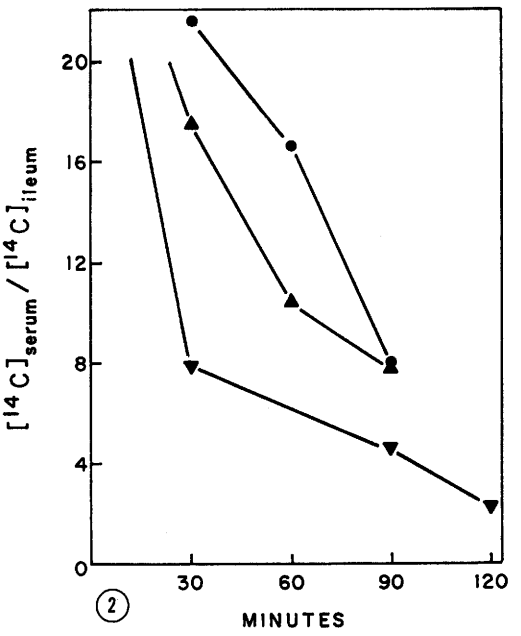


FIG. 1. Course of change of distribution ratio of 3-0-methyl (-^{14}C)-glucose between blood serum and contents of ileal loops. Uppermost curve represents one of 3 successive loops of lower ileum, which was filled with sugar-free electrolyte solution. The other 2 loops (in the same animal) were filled with solutions containing the sugar at about the serum level (middle curve) or at a level far above serum level.

FIG. 2. Release of 3-0-methyl (-^{14}C)-glucose into electrolyte solutions contained in 3 successive ileal loops. Middle curve, no sugar initially in solution in ileal loop. Upper curve, 77 millimolar sorbitol in solution in this loop; lower curve, 77 millimolar glucose in solution in this loop.

tory than the amino acids used as models for the study of the nature of the steady state of absorption across the intestinal wall, the present results indicate that in this case also, transfer occurs both into and out of the lumen, apparently by specific processes, and that the resultant steady state, although not precisely evaluated, yields a distribution ratio $[\text{methylglucose}]_{\text{serum}}/[\text{methylglucose}]_{\text{ileal lumen}}$ of somewhat above 4 to 6.

1. Christensen, H. N., Feldman, B. H., Hastings, A. B., *Am. J. Physiol.*, 1963, v205, 255.

2. Akedo, H., Christensen, H. N., *J. Biol. Chem.*, 1962, v237, 113.

3. Csaky, T. Z., *Z. Physiol. Chem.* 1942, v277, 47.

4. Campbell, P. N., Young, F. G., *Biochem. J.*, 1952, v52, 439.

5. Csaky, T. Z., Wilson, J. E., *Biochim. et Biophys. Acta*, 1956, v22, 185.

6. Csaky, T. Z., Glenn, J. E., *Am. J. Physiol.*, 1957, v188, 157.

7. Bárány, E., Sperber, E., *Skand. Achiv. Physiol.*, 1939, v81, 290.

8. Campbell, P. N., Davson, H., *Biochem. J.*, 1948, v43, 426.

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