Proc. Soc. Exp. Biol. & Med., 1964, v115, 102.
3. Wong, S. C., Kilbourne, E. D., J. Exp. Med., 1961, v113, 95.
4. Pollock, M. E., Treadwell, P. E., Kenny, G. E., Exp. Cell Res., 1963, v31, 321.
5. Morgan, J. F., Morton, H. H., Parker, R. C., Proc. Soc. Exp. Biol. & Med., 1950, v73, 1.
6. Rappaport, C., ibid., 1957, v96, 309.
7. McLimans, W. F., David, E. V., Glover, R. L., Rake, G. W., J. Immunol., 1957, v79, 428.
8. Taylor, A. C., Exp. Cell Res., 1961, suppl. 8, 154.
9. Weissman, G., Dingle, J., Exp. Cell Res., 1961, v25, 207.
10. Fell, B., Thomas, L., J. Exp. Med., 1961, v114, 343.
11. Weiss, L., Exp. Cell Res., 1965, v37, 540.
12. Polet, H., ibid., 1966, v41, 316.
13. Gillette, R. W., Findlay, A., Conway, H., J Nat. Cancer Inst., 1961, v27, 1285.
14. Schryver, H. F., Exp. Cell Res., 1965, v40, 610.
15. Fand, I., Orlan, M., J. Morphol., 1962, v111, 89.
16. Ambrose, C. F., J. Exp. Med., 1964, v119, 1027.

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Intestinal Absorption of Sugars in Semi-Starved Rats.* (32117)

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The intestinal epithelium is constantly renewed in normal, well fed animals(1). After a period of starvation, however, this tissue undergoes morphological(2) and biochemical(3) alterations resulting in the reduction of absorption of glucose and fructose(4), as well as other changes. If the food intake is restricted for a period ("semi-starvation"), the animal loses weight and its intestinal wall becomes thin. Kershaw *et al*(5) reported that the active intestinal transport of sugars and amino acids increases markedly in semi-starved rats, both *in vivo* and *in vitro*. The increased absorbing ability of the intestine disappears rapidly, however, when the rats are fed in normal fashion.

The mechanism of enhancement of the intestinal transport in semi-starved animals is not clear. It is of particular interest to ascertain whether semi-starvation modifies the function of the intestinal carrier or of the pump, or whether the enhanced transport reflects the modification of the epithelial substrate metabolism. To gain insight into this problem, the rate of disappearance from the intestinal lumen of two sugars, glucose and 3-0-methylglucose (3 MG), was compared in normally fed and semi-starved animals. 3 MG is known to be transported in the intestine in the same way that glucose is, yet it is not metabolized(6,7).

Methods. Sprague-Dawley male rats, weighing about 200 g, were housed in individual cages. A standard pelleted laboratory diet (Purina rat and mice ration, Laboratory Chow) was fed either *ad libitum* or 8 g per day per rat. As the normal daily food intake of male rats weighing 200 g was found to be approximately 15 g, the latter group was semi-starved. In fact, these rats lost an average of 22% of their initial body weight during 15 days of feeding.

At the end of 15 days of special feeding, the animals were ready for absorption experiments. These were performed *in vivo*, perfusing a loop of the upper jejunum in urethan-anesthetized animals with 50 ml of isosmotic Na_2SO_4 solution containing either glucose or 3 MG. The absorption (disappearance of sugar from the perfusate) was calculated per gram dry weight of the perfused loop, assuming arbitrarily that the dry weight was proportional to the absorptive surface. The methodology was the same as Method I, as described by Csáky and Ho(8).

Results. Both glucose and 3 MG were present in the perfusing fluid in 2 initial concentrations: 50 mg % (about 3.1 mM or

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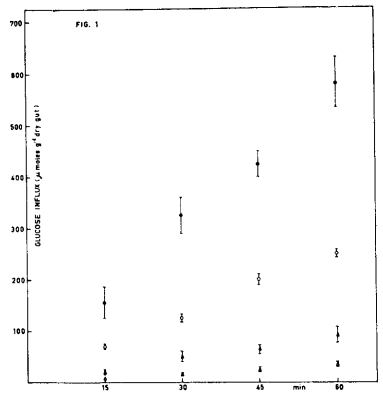

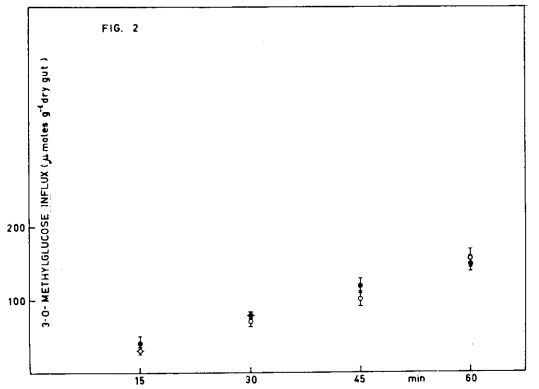

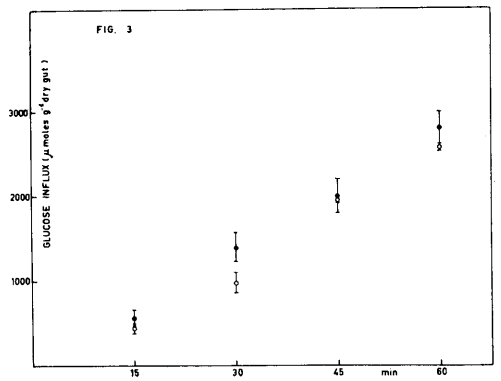

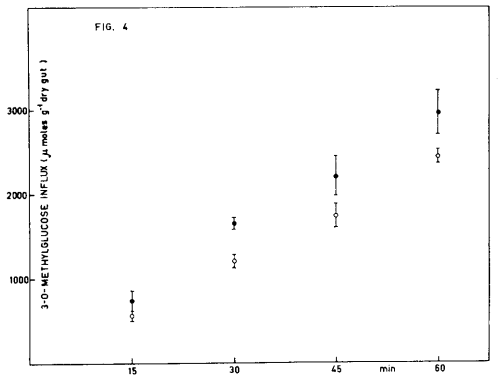

FIG. 1. Disappearance of glucose (50 mg% initial concentration in isosmotic Na_2SO_4) from intestine of well fed, controls 0; from intestine of semi-starved rats ●. The same in the presence of 10^{-4} M phlorizin in well fed △; and in semi-starved rats ▲.

FIG. 2. Disappearance of 3-0-methylglucose (50 mg% initial concentration in isosmotic Na_2SO_4) from intestine of well fed 0; and semi-starved ● rats.

FIG. 3. Disappearance of glucose (2.66% made isosmotic with Na_2SO_4) from intestine of well fed and semi-starved rats. Symbols as in Fig. 2.

FIG. 4. Disappearance of 3-0-methylglucose (2.8% made isosmotic with Na_2SO_4) from intestine of well fed and semi-starved rats. Symbols as in Fig. 2.

2.6 mM, respectively) and 150 mM. It can be assumed that at low concentration active transport is primarily involved in the absorption, whereas mediated diffusion primarily takes place at high initial concentration because of the very high lumen-to-blood gradient ("apparent facilitated diffusion," by Csáky(9); see also Csáky and Ho(10)).

Three to 6 individual animals were studied in each group. The results are reproduced on a graph showing the mean with standard deviation (vertical bars).

At low lumenal concentration, glucose disappears significantly faster from the intestine of semi-starved animals than from the controls (Fig. 1). Phlorizin in low (10^{-4} M) concentration very strongly inhibits glucose absorption from both the gut of the control rat and the semi-starved rat. Yet, absorption of the non-metabolizable sugar, 3 MG, is not at all influenced by previous semi-starvation (Fig. 2).

When sugar was offered in high (150 mM) concentration (Fig. 3 and 4) there was a slight increase in rate of absorption of both glucose and 3 MG from the intestine of semi-starved rats, but the statistical validity of this difference is highly questionable.

Discussion. The slight, statistically insignificant increase in the rate of glucose and 3 MG absorption in semi-starved rats could be attributed to the fact that the gut wall is thinner in these animals than in normal rats. A similar absorption increase was found in

germ-free animals, in which the intestinal wall is also thinner(11). The possibility that the actual decrease of dry matter in the gut relatively increases the amount of sugar absorbed per gut dry weight cannot be excluded either.

If glucose is present in the lumen at low initial concentration, however, its disappearance is significantly faster in semi-starved animals. The difference is much greater than could be accounted for by the change in gut dry weight. No such difference is observed, though, in the absorption of 3 MG. Both glucose and 3 MG are actively transported, and because of their mutual inhibition of each others absorption they most likely share a common carrier receptor(12). Consequently, it is unlikely that semi-starvation would increase the function of the carrier of the pump because in such instance the absorption of both sugars would have been equally affected, which was not the case. Since glucose is metabolized by the intestinal tissue and 3 MG is not, it is reasonable to assume that the semi-starvation increased the disappearance of glucose from the lumen by altering the metabolism in the epithelial cells. The latter assumption is also supported by the finding that the O_2 up-take of the semi-starved rat gut is increased(13). The enhancement of glucose absorption was also observed when the potassium content of the epithelium was increased (10,14). This causes augmentation of the turn-over of the mucosal glycogen and of the lactic acid production. As a result, glucose, but not 3 MG or galactose, disappears more rapidly from the lumen of the gut. There is, however, an obvious difference between the augmenting effect of high potassium and semi-starvation upon glucose absorption. The former is quite significant even at high lumenal glucose concentration, whereas the latter is more prominent if the glucose concentration is low and becomes insignificant at higher concentrations. It is thus likely that semi-starvation causes a type of metabolic change different from high K. Verzár(15) pointed out many years ago that intracellular metabolism has an effect on the rate of intestinal sugar absorption and he believed that this was the primary factor in the "preferential" absorption of glucose. Subsequent developments indicated that the mechanism was more complex and the significance of intracellular metabolism was gradually neglected. The present study, along with observations concerning the effect of potassium and with those reporting that low concentration of uranyl ion inhibits glucose but not galactose absorption(16), serves as a reminder that intracellular metabolic changes should not be neglected completely in intestinal absorption studies.

Summary. The absorption of glucose and 3-0-methylglucose from the small intestine was examined in normally fed and semi-starved rats. Significantly more glucose, but not 3-0-methylglucose, was absorbed in the semi-starved rat if the sugar was in the gut lumen in low initial concentration. At high sugar concentration, no such difference was observed between controls and semi-starved animals. It is speculated that semi-starvation influences glucose absorption by modifying its intestinal metabolism, rather than by acting directly upon the transport mechanism.

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1. Leblonde, C. P., Stevens, C. E., Anat. Rec., 1948, v100, 357.

2. Sun, Y. P., ibid., 1927, v34, 341.

3. Pfeiffer, C. J., Debro, J. R., Arch. Int. Physiol. Bioch., 1966, v74, 97.

4. Verzár, F., McDougall, E. J., Absorption from the Intestine, Longmans, Green & Co., 1936, p149.

5. Kershaw, T. G., Neame, K. D., Wiseman, G., J. Physiol., 1960, v152, 182.

6. Csáky, T. Z., Fernald, G. W., Am. J. Physiol., 1960, v198, 445.

7. Csáky, T. Z., Wilson, J. E., Biochim. Biophys. Acta, 1956, v22, 185.

8. Csáky, T. Z., Ho, P. M., Proc. Soc. Exp Biol. & Med., 1966, v120, 403.

9. Csáky, T. Z., Fed. Proc., 1963, v22, 3.

10. Csáky, T. Z., Ho, P. M., J. Gen. Physiol., 1966, v50. 113.

11. Csáky T. Z., Gordon, H. A., unpublished.

12. Csáky, T. Z., Int. Abstr. biol. Sci. Suppl., 1958, v6, 79.

13. Esposito, G., Faelli, A., Capraro, V., unpublished.

14. Csáky, T. Z., Ho, P. M., Arch. gen. Physiol., 1966, v291, 63.
15. Verzár, F., Schweiz. Med. Wochschr., 1935, v65, 569.
16. Newey, H., Sanford, P. A., Smyth, D. H., J. Physiol., 1966, v186, 493.

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Detoxification of Endotoxin by Perfusion of Liver and Spleen.* (32118)

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This communication reports a study of the capacity of the liver and spleen to detoxify bacterial endotoxins introduced into the circulation. The evidence for detoxification is data showing 1, a correlation between survival rate and degree of accessibility of the liver to the endotoxin; 2, a loss of toxicity after perfusion through spleen, as determined by bioassay, and 3, disappearance of the toxic moiety after perfusion through spleen, as determined by immuno-assay. *In vitro* data showing detoxification of endotoxin interacted with splenic homogenate are also presented.

Experimental. A. *Effect on survival rate of a change in accessibility of the liver to the endotoxin.* Three groups of normal adult white rabbits were subjected to laparotomy under pentobarbital anesthesia (30 mg/kg) for exposure of a branch of the superior mesenteric vein. Each rabbit of the first group received one MLD/80 (1 mg/kg) of *Salmonella enteritidis* endotoxin into this vein. In the second group the same amount of endotoxin was injected into the renal artery instead of the superior mesenteric vein. In the third group the same amount of endotoxin was injected into a vein of the ear instead of the superior mesenteric vein. The laparotomy incisions were then closed and the survival rate, *i.e.*, the percentage alive and well 7 days later, observed.

Results. Table I shows that endotoxin injected *via* the superior mesenteric vein resulted in a survival rate of 85% as compared to 11% when it was injected *via* a systemic artery or vein.

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These results signify that the lethality of endotoxin is reduced or eliminated if it is given so that all or most of it must traverse the liver before reaching the systemic circulation. It follows that the normal liver can extract enough of an MLD/80 of endotoxin on its first passage through as to lower the circulating titer of the toxic moiety to a sublethal level. To determine whether the RE cells or the parenchymal cells in the liver, or both together, were necessary to achieve the detoxification, endotoxin was perfused through the spleen, which, apart from lymphocytes, contains only RE cells. The degree of detoxification of the endotoxin was determined as follows:

B. *Toxicity of bacterial endotoxin before and after perfusion through the dog's spleen.* An abdominal incision was made under ether anesthesia. Maximum accessibility of the splenic parenchyma to the endotoxin was achieved by abolishing vasoconstriction with 1% procaine injected into the splenic pedicle. Five mg of *S. enteritidis* endotoxin (labelled with Cr^{51}), suspended in 50 ml of normal dog plasma, was infused into a marginal artery in a period of 12 minutes, during which flow through the splenic artery was unimpeded. All of the venous effluent during this period and for 5 minutes thereafter was collected in a single pool in an ice bath, centrifuged in the cold, and its total plasma volume determined from its hematocrit. The spleen was then excised, washed free of blood with phosphate-buffered saline solution, and homogenized at 4°C in a volume of this solution equal to twice its own weight.

The homogenate was tested for toxicity by injecting 0.5 ml aliquots intraperitoneally into