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Commentary

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We thank Prof. H. Murer for reiterating the classic work of Prof. Diamond and his colleagues, as well as those of Dr. G. Esposito and others on effect of starvation and of Profs. H. Murer and G. Semenza themselves, related to D-glucose transport in addition to that cited in our paper. We give below the various experimental details, which we had to only indicate through chosen references, owing to space limitations.

Earlier experiments on D-glucose and amino acid transport during starvation were done up to 3 day starvation. We have extended our experiments for 6 days in our effort to model a possible physiological 'end point'. While we cannot discount the possibility of 6 days starvation being 'non-physiological', we think it is unlikely, because the changes both in transport and fluidity are seen to be monotonic with respect to the period of starvation (see Fig. 3 and Table II of our paper). Therefore, it does not appear likely that it is 'non-physiological'.

Both inverted intestine and membrane vesicle preparations were used to study D-glucose transport [1,2]. We have used intact inverted intestine for D-glucose transport study because (a) intact intestine pieces are closer to the in vivo condition; (b) there may be a change in the membrane protein profile during starvation; (c) there is no chance of contamination as in the case of membrane vesicle preparations; and (d) others (see reference 22 in the paper) have used inverted intestine for transport studies with success.

Since intact intestine could not be used for membrane fluidity experiments by pyrene incorporation, we prepared membrane vesicles according to the method of Kessler et al. (reference 8 in the paper). We checked the contaminants in the brush border membranes (BBM) vesicle preparations by enrichment of alkaline phosphatase (given in the paper) and by freeze-fracture and negatively stained membrane preparations by electron microscopy.

An overall increase in D-glucose transport was observed in the inverted starved rat intestine compared to normal well fed rat. This increase is independent of (a) Na^+ dependent D-glucose transport (data given in the paper); (b) temperature sensitive transport systems (there was higher transport of D-glucose in fasted compared to well-fed rats at 25°C or 37°C).

In the presence of ethanol, D-glucose transport decreases due to many factors [3]. In our study, we have also seen the decrease in both cases, however, fasting intestine showed higher transport compared to well fed control values. It may require further work to pinpoint the factors involved in higher D-glucose transport during fasting in the presence of ethanol.

As regards morphology, the decrease in surface area in microvilli may occur up to 48 h of fasting (Misch et al. [4]; Karasov and Diamond [5]). We have taken well-fed, six-days starved, and 6 h refed after 7 days starved rat intestine pieces for electron microscopic studies of microvilli. We have mentioned that atrophy of villi and also that the surface area of microvilli increase 2.6-fold per unit area in the six-days fasted rat compared to the well-fed one. We have computed our data by measuring the surface area of about 200 well-defined microvilli in each case, taken from 4 individual rats in each set.

Table II of the paper contains (1) the eximer–monomer ratio of pyrene incorporation of well-fed, starved and refed rats intestine BBM; (2) D-glucose transport data as measured in intact inverted intestine in Hank's buffer containing Na^+ (as described in section 2).

In Fig. 4, data presented were obtained by incubation of intestinal pieces with [^{14}C]D-glucose in Hank's buffer with and without Na^+. With regard to the question of Na^+-independent transport pathway, we have not addressed this issue in this work. Separate experiments using phlorizin might indeed be helpful.

We have attributed the high transport of D-glucose in fasting animals to (a) fluidity; (b) surface area of microvilli. This, we believe, is a new idea specific to the topic of the paper, and one that will stand scientifically critical, and not polemical, scrutiny. However, at this stage, besides these two factors, we do not know any other factors responsible for an increased transport of D-glucose during prolonged starvation.

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