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# Short Communications

## Glucose Accumulation by Rat Small-Intestinal Mucosa after Depletion of Intracellular Adenosine Triphosphate

By H. J. LEESE and J. R. BRONK
*Department of Biology, University of York, Heslington, York YO1 5DD, U.K.*

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Glucose is absorbed from the lumen of the small intestine by an active-transport process that has been postulated to involve ATP, and in particular the $Na^+ + K^+$-stimulated adenosine triphosphatase (Kimmich, 1970). The present study was designed to determine whether there was any correlation between the actual ATP content of the mucosa of the small intestine and the extent to which the tissue can accumulate glucose.

Slices of mucosa from the jejunum of normal Wistar male rats (200–250g body wt.) were prepared by the method of Bronk & Parsons (1965). The slices (approx. 4–5mg dry wt.) were preincubated in glucose-free Krebs–Ringer bicarbonate buffer, pH 7.4 (Bronk & Parsons, 1965), at 37°C with continual stirring for either 30s or 4min, after which time glucose was added to a concentration of 11.1mM (2mg/ml). The accumulation of glucose in the tissue after various time-intervals was monitored by rapidly taking up a 0.5ml portion of medium, containing the mucosal slices, into a 2ml plastic syringe already containing 0.5ml of cold saline (0.9% NaCl). The fluid and tissue were then ejected from the syringe into 50ml of cold saline contained in a reservoir above a Millipore filter (pore size 0.6$\mu$m). Suction was applied, and the slice, which was trapped on the filter, was removed with a glass slide and added to 2ml of 6% (w/v) $HClO_4$. The separation procedure was completed in about 15s, and had the effect of diluting 100-fold any glucose from the original medium adhering to the tissue. The slice, in $HClO_4$, was homogenized and centrifuged, and the supernatant was neutralized with 40% (w/v) $K_2CO_3$ and assayed for glucose enzymically by an automated fluorimetric method (Leese & Bronk, 1972). The pellet from this extraction was weighed to give the tissue wet weight, which was then related to the tissue dry weight from the ratio (dry weight)/(wet weight) = 0.16±0.0031 (24) (mean±S.E.M.) determined in separate experiments. The values for glucose accumulation per mg dry wt. were then expressed as $\mu$mol of glucose/ml of tissue water on the basis that 1mg dry wt. of tissue was associated with 5.25$\mu$l of tissue water. It should be noted that this calculation may represent an underestimate of the true intracellular substrate accumulation, since part of the total tissue water will be due to the extracellular space.

In the experiments designed to measure ATP content, the incubation was stopped at various time-intervals by the addition of $HClO_4$ to give a final concentration of 5% (w/v) in the medium, and the combined tissue and medium were then assayed for ATP (Leese & Bronk, 1972). The extraction procedure was similar to that described above and the results are expressed as $\mu$mol of ATP/g dry wt. of tissue. Samples of medium removed at intervals were found not to contain ATP, which indicated that in these experiments the ATP was contained wholly in the tissue. However, in some experiments, in which 1mM-ADP was added to the incubation medium, subsequent samples of medium were found to contain ATP (probably formed by myokinase that had leaked into the medium from the tissue). To determine the true amount of cellular ATP in these situations a portion of medium was rapidly removed and the remaining tissue plus medium were deproteinized as above. The sample of medium was immediately added to a further quantity of $HClO_4$-deproteinized tissue plus medium, whose ATP content was known, and the increment of ATP due to the added medium was determined. From these measurements the ATP content of the original tissue was calculated by difference, and it should be noted that the experimental procedure was as far as possible designed so that tissue and medium samples were treated identically.

Fig. 1 shows the relationship between the ATP content of the mucosa in the presence and in the absence of glucose in the incubation medium. In the first 4min of incubation the ATP content declined from an initial value of about 3$\mu$mol/g dry wt. to 1$\mu$mol/g dry wt. and remained at or below this very low value at 8min. The addition of glucose 30s after the incubation had been started did not arrest the decline in ATP content. However, on the addition of 1mM-ADP after 4min the ATP content rose rapidly both in the presence and in the absence of glucose, reaching a value in the presence of glucose that was approximately double that at 30s and 5 times that at 4min, and remained at over twice the 4min value throughout the second 4min period. The

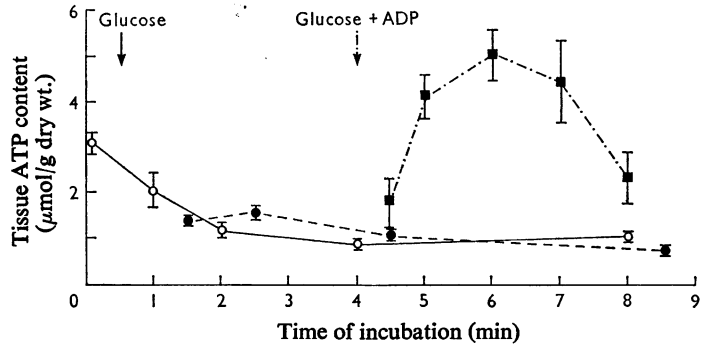


Fig. 1. *ATP content of slices of rat small-intestinal mucosa after various times of incubation*

Experimental details are given in the text. o, Control; ●, glucose (11.1 mM) added after 30s preincubation; ■, glucose (11.1 mM)+ADP (1 mM) added after 4 min preincubation. Values are means±S.E.M. of six determinations.

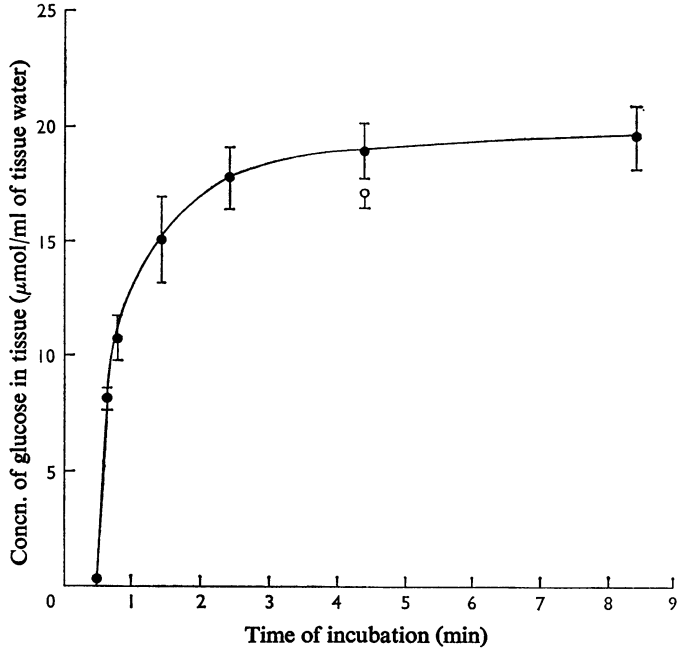


Fig. 2. *Accumulation of glucose* (11.1 mM) *by slices of rat small-intestinal mucosa*

Experimental details are given in the text. ●, Glucose added after 30s preincubation; o, glucose added after 4 min preincubation. Values are means ±S.E.M. of six determinations.

elevation in ATP content was also associated with a rise in the rate of $O_2$ consumption by the mucosal tissue.

The concentration of glucose in freshly prepared mucosal slices was 5.64±0.64 μmol/ml of tissue water (7) (mean±S.E.M.), which approximated that of rat blood. However, after 30s incubation the concentration was not measurable, and it is likely that some of the glucose in the tissue at zero time was extracellular in origin, possibly derived from traces of blood contaminating the preparation. Fig. 2 shows the rate of uptake of glucose when it was added to a final concentration of 11.1 mM after 30s preincubation. The curve has the form of a rectangular hyperbola

and reached a plateau at about 2–4min. After 4min the tissue glucose concentration reached a value of $18.79 \pm 1.23 \mu$mol/ml of tissue water (6), or about 1.7 times that of the medium. When glucose was added after 4min preincubation, initially in the absence of ADP the amount accumulated in the tissue after a further 4min was $17.20 \pm 0.65 \mu$mol/ml of tissue water (6). When glucose was added after 4min together with 1mM-ADP the concentration reached in the tissue after a further 4min was $18.36 \pm 0.85 \mu$mol/ml of tissue water (6). There were no significant differences between the extents of glucose accumulation in these cases. Although it is possible that the measured uptake of glucose might represent binding of the substrate rather than true intracellular accumulation, this is considered unlikely for the following reasons. (1) From the data of Diedrich (1968) it was calculated that there are approx. $2 \times 10^{19}$ glucose-binding sites/g of brush-border protein, or, assuming that the brush-border protein represents at most about 10% of the total epithelial-cell dry weight, approx. $2 \times 10^{18}$ glucose-binding sites/g of mucosal tissue. This value could account for less than one-thirtieth of the measured glucose accumulation, or about one-fifteenth of the extra glucose uptake above that which might be achieved by simple diffusion. (2) For a variety of glucose concentrations in the medium in the range 5.55–56mM the final tissue/medium concentration ratios were all at least 1.7, which is inconsistent with the accumulation being solely a binding phenomenon.

It therefore appears that glucose uptake by the mucosa of the small intestine is independent of the ATP content of the tissue. Our observation is in agreement with the 'sodium-gradient' hypothesis proposed by Crane (1962) for the active transport of the sugars in the gut. This hypothesis postulates that the driving force for glucose entry is a downward gradient of $Na^+$ from the extracellular to the intracellular phase of the epithelial cell. Although the gradient is thought to be maintained by the outward pumping of $Na^+$, which is dependent on ATP, there is no direct link between ATP hydrolysis and the actual accumulation mechanism (Schultz & Curran, 1970). This is supported by the fact that a diminished accumulation was observed with the present methods when the $Na^+$ in the external medium was replaced by $K^+$. It is also of note that Eddy *et al.* (1970) have observed the concentration of amino acids by yeast cells depleted of ATP, and suggested that ionic movements alone were sufficient to bring about the amino acid accumulation.

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