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# The Effect of Different $Na^+$-Substitutes on the Intestinal Absorption of Glucose

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The effect of the replacement of $Na^+$ by Tris, $K^+$, $Li^+$ or mannitol on the intestinal absorption of 2.77 mM glucose is studied *in vivo* in the rat.

On using these substitutions minimal inhibition is seen with Tris and inhibition increases progressively with $K^+$, $Li^+$ and mannitol respectively. On a return to solutions with 154 mEq $Na^+$/l, inhibition practically disappears if the substitute is Tris. The recovery is also high in the case of $K^+$, whereas with $Li^+$, and more so with mannitol, a certain period of time is necessary to attain normal levels of absorption. Inhibition of absorption by a substitution for $Na^+$ cannot be explained as due to the mere absence of this cation but also to the intervention of other factors depending on the properties of the replacement substances, which affect the epithelium with a variable intensity for more time or less. The greatest alterations are seen with mannitol.

It is well known that active transport of different sugars by the intestine depends on the $Na^+$ concentration in the mucosal side (3, 4). *In vivo* experiments on the rat showed that absorption of galactose (8) or glucose (10) depends on the $Na^+$ concentration in the intestinal lumen when sorbitol or mannitol is used to mantain osmolarity. On the other hand, the concentration of $Na^+$ has been found to have either no effect, or very weak effect on the absorption of L-arabinose or D-fructose (10). It has also been described that after a period of absorption of glucose in the presence of mannitol with the $Na^+$ level practically nil, the absorption of glucose in successive periods in the presence of 154 mEq $Na^+$/l needs some time for recovery (10).

BOSACKOVA and CRANE (2) observed that the transport capacity of rings of hamster intestine preincubated in media with mannitol was noticeably reduced when later placed in a saline medium with $Na^+$, a phenomenon which was not produced on preincubation in Tris. The active transport of amino acids by rat intestine *in vitro* becomes inhibited after preincubation in the absence of $Na^+$, replaced by $K^+$, $Li^+$, Tris or choline (11).

Moreover, when $Na^+$ is replaced by mannitol or $Li^+$ the oxygen uptake of intestinal mucosa of strips is inhibited (1, 13); this is due not only to the lack of operation of the sodium pump but also to metabolic alterations in the tissue. Incubation of strips of rat intestine in different $Na^+$-free media also affects oxy-

gen uptake. However, after preincubation in the same media the respiration in a saline medium with $Na^+$ is found to be inhibited only on having used during the preincubation certain replacement substances (6).

The present work studies the effect of $Na^+$ replacement by Tris, $K^+$, $Li^+$ or mannitol, on the intestinal absorption of glucose in the rat *in vivo* and analyses the possibility of recovery of the process with time.

## Materials and Methods

The technique of SOLS and PONZ of successive absorptions (12) was used in adult 130-250 g Wistar rats anesthetized with urethane. The intestinal loops were 20 cm long with a filling pressure of 8 cm water and the animal temperature kept constant. Four consecutive periods of absorption, each lasting 20 min, were studied in each animal. The solutions used in the intestinal lumen in the first, third and fourth periods were 2.77 mM D-glucose in 0.9 % NaCl. In the second period enough Tris-HCl, KCl, LiCl or mannitol were added to make isosmotic a 2.77 mM glucose in 0.05 % NaCl solution. The same solutions, but without glucose, were respectively used to wash the intestinal lumen just before each absorption period.

Glucose determinations were made by the Nelson-Somogyi method (9). Absorptions are expressed as micromoles of sugar/cm intestine (14). Tables show mean values including standard error.

## Results

On substituting other substances for $Na^+$ the absorption of glucose is always inhibited although to a variable degree depending on the kind of replacer (Table I). Inhibition increases with Tris, $K^+$, $Li^+$ and mannitol in ascending order. There is a recovery of the process of absorption of sugar in the 3rd and 4th periods, when 154 mEq $Na^+$/l is again present, but notable differences are also seen here. When Tris is used in the 2nd absorption the following absorption is very nearly normal and completely so in the 4th period. Recovery is very similar in the case of $K^+$. When $Li^+$ is the replacer there is still a notable inhibition in the 3rd period which is then not seen in the following one. Least recovery is found with the use of mannitol, when even in the 4th period residual inhibition clearly persists.

Other experiments were carried out in order to follow the recovery process in the presence of high $Na^+$ concentrations, during variable times. After the second period of absorption in the absence of

Table I. *Effect of $Na^+$ replacement on the absorption of glucose by the rat intestine in vivo.*

Four 20 minutes consecutive periods of absorption. 2.77 mM glucose in 0.9 % NaCl in the 1st, 3rd and 4th periods. 2.77 mM glucose in 0.05 % NaCl with Tris-HCl, KCl, LiCl or mannitol made to isosmolar solution, in the 2nd period.

| N.º exp. | Substitute in 2nd Abs. | 1st Abs. µM/cm/20 min. | Inhibition % | | |
|---|---|---|---|---|---|
| | | | 2nd Abs. | 3rd Abs. | 4th Abs. |
| 10 | Mannitol | 0.35±0.025 | 57.74±3.54 | 32.20±2.75 | 17.32±3.75 |
| 10 | LiCl | 0.38±0.018 | 48.74±2.27 | 23.94±2.81 | 5.31±2.05 * |
| 8 | KCl | 0.46±0.031 | 21.0 ±2.17 | 9.0 ±3.08 * | 4.0 ±1.88 * |
| 10 | Tris | 0.39±0.032 | 12.10±2.51 | 7.31±3.15 * | 1.88±1.05 * |

$P < 0.001$ for all the inhibition values excepting those marked with asterisk.

Table II. *Time for recovery of intestinal absorption of glucose after substituting $Li^+$ or mannitol for $Na^+$.*

Four periods of absorption as in Table I. 0.9 % NaCl solution was used for 30 or 60 minutes between 2nd and 3rd periods.

| N.° exp. | Time between 2nd and 3rd Abs. min. | 1st Abs. µM/cm/20 min. | Inhibition % | | |
|---|---|---|---|---|---|
| | | | 2nd Abs. | 3rd Abs. | 4th Abs. |
| | | LiCl in 2nd absorption | | | |
| 10 | — | 0.38±0.018 | 48.74±2.27 | 23.94±2.81 | 5.31±2.05 * |
| 8 | 30 | 0.49±0.038 | 46.6 ±3.92 | 7.0 ±1.50 | 1.8 ±0.80 * |
| 10 | 60 | 0.38±0.002 | 40.8 ±3.64 | 5.05±1.56 * | 0.5 ±0.36 * |
| | | Mannitol in 2nd absorption | | | |
| 10 | — | 0.35±0.025 | 57.7 ±3.54 | 32.2 ±2.75 | 17.32±3.75 |
| 6 | 30 | 0.40±0.033 | 56.8 ±4.05 | 12.8 ±1.96 | 2.5 ±1.16 * |
| 9 | 60 | 0.33±0.016 | 60.4 ±3.86 | 3.8 ±1.68 * | 2.8 ±1.22 * |

P < 0.001 for all the inhibition values excepting those marked with asterisk.

$Na^+$ (replaced by $Li^+$ or mannitol) and before the third period, the intestinal loop was filled with an isotonic NaCl solution and maintained so for 30 or 60 minutes. As it is shown in Table II exposures of 60 minutes caused a much better recovery than exposures of 30 minutes.

Another factor to be considered was whether the strong inhibition of the absorption of glucose when substituting mannitol for $Na^+$ was due to a cause limited to the intestinal segment used or whether other effects could be included. Hence experiments were done using contiguous segments of intestine of approximately the same length (15 cm). Four successive absorptions were carried out in the proximal segment, always using 2.77 mM glucose in isotonic NaCl solution, whereas the same solution without sugar was used in the distal segment during the 1st, 3rd and 4th periods and a 0.3 M mannitol solution in 0.05 % NaCl during the 2nd period. It can be seen from Table III that the presence of mannitol in a contiguous segment does not affect the active transport of glucose.

## Discussion

The concentration of 2.77 mM of glucose is approximately half that found in blood as a result of which sugar passes from the intestinal lumen into the blood against a gradient.

The replacement of $Na^+$ by different

Table III. *The effect of mannitol in an intestinal segment on glucose absorption by a contiguous segment.*

Four periods of absorption of 2.77 mM glucose in 0.9 NaCl in the proximal segment. Simultaneously 0.9 % NaCl in the distal segment, except during the 2nd period when 0.3 M mannitol in 0.05 % NaCl was used.

| N.° exp. | Successive absorption (µM/cm/20 min.) | | | |
|---|---|---|---|---|
| | 1st Abs. | 2nd Abs. | 3rd Abs. | 4th Abs. |
| 4 | 0.47±0.053 | 0.45±0.067 | 0.46±0.049 | 0.44±0.058 |

substances keeping isosmolarity always inhibits the active transport of glucose, which confirms that this process depends on the presence of $Na^+$ in the intestinal lumen. However, the degree of inhibition varies considerably with the kind of replacer used in each case, from a maximum inhibition of 57.7 % with mannitol to only 12 % with Tris. Use of Tris revelas that, in the most physiological conditions in which *in vivo* techniques are used, the concentration of $Na^+$ in the intestinal lumen is not so decisive for sugar absorption as was deduced from *in vitro* experiments.

It might be thought that *in vivo* the $Na^+$ concentration in the intestine increases greatly due to its passage from the blood and tissues during the 20 minutes of absorption, but as was determined in a previous work (10), this amount of $Na^+$ only means a rise from 8.5 mEq/l initially to about 30-35 mEq/l at the end. It can be concluded that the inhibition of absorption cannot only be attributable to the lack of $Na^+$ but also to the intervention of other factors related to the nature of the substitute for $Na^+$, and whose importance may be nil in the case of Tris and increases progressively with $K^+$, $Li^+$ and mannitol.

The same could be said of the variable recovery seen after absorption with a very low $Na^+$ (2nd absorption), according to the kind of replacer used. With Tris, the recovery is practically immediate and the same can be said of $K^+$. However, in the case of $Li^+$ and even more so with mannitol absorption slowly returns to normal even though the $Na^+$ level in the 3rd and 4th absorptions is 154 mEq/l. A similar observation has been made with respect to $Li^+$ (5). These results show that $Li^+$ or mannitol substitution for $Na^+$ produces alterations of the absorption capacity for some time, which are inexplicable as being due to the simple influence of the $Na^+$ concentration on the active transport mechanism.

On the other hand, the fact that the presence of mannitol in a segment contiguous to another in which glucose is absorbed, has no influence on the rate of absorption, shows that the alterations observed when mannitol is substituting for $Na^+$ are of a local nature, affecting only the intestinal segment exposed to it. This is to be expected as mannitol either penetrates very little or does not penetrate at all.

The difficulty in recovery of the intestinal absorptive capacity found on placing a LiCl or mannitol solution in the intestinal segment is related to metabolic alterations produced by preincubation of intestinal strips in the absence of $Na^+$. After one hour of preincubation in media without $Na^+$, the intestinal oxygen uptake, measured in solutions with $Na^+$, is found to be variably inhibited according to the substitute used for $Na^+$ on preincubation. In the first hour there is no inhibition on preincubation with Tris, whereas this occurs when $Li^+$ (6) or mannitol (7) are used. Correspondingly, preincubation in media where $Na^+$ is replaced by mannitol decreases not only the later oxygen uptake whether in the presence or not of an exterior substrate, but also the capacity of glucose utilisation by the tissues with a major part of glucose metabolized to lactate (7).

The data show that on studying the dependence of the active transport of sugars, aminoacids or other substances on the $Na^+$ concentration, the nature of the substitutes utilised to maintain the solutions isosmolar, must be taken into account. Whether these substitutes are ionic or not may have a notable influence on changes in the composition of intracellular fluid. Other characteristics of the replacer may similarly explain the differences found between one and another, according to whether they more or less affect the intracellular enzyme systems or structures related directly or indirectly to the active transport mechanism. These peculiarities

of the different substitutes for $Na^+$ become very obvious when one considers the reversibility of the process with the presence of $Na^+$. With some substitutes the recovery is immediate whereas with others more time or less is needed.

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