Effects of sugars on the absorption of copper from the small intestine of rat

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Copper as an important nutrient in the feeding of livestock has been widely acclaimed. Anemia in cattle, pigs and sheep, rickets in young calves, ataxia or 'swayback' in lambs, fibrosis of the myocardium in cattle, cardiac hypertrophy in pigs and scouring in calves, are accompanied with copper deficiency. These diseases have been shown to respond to the administration of copper. Toxicity of copper has also been reported^{1b}. Little is known of the minimum requirements of copper for growth and production for different species, and about the factors influencing the absorption of copper at the mucosal level of the intestine. Several dietary factors have also shown to influence the intakes and utilization of copper. Knowledge on the mechanism of absorption, its transport and metabolism is limited.

Previous communications from this laboratory, work has been presented on the effects of amino acids⁶, and of fatty acids⁷, on the absorption of copper from the small intestine of the rat. In this paper the effects of some monosaccharides and disaccharides on the absorption of copper from the small intestine of the rat are described.

Methods and Materials

Male and female rats weighing 200 to 300 g used in this experiment were of the Wistar strain. The rats were raised and maintained on a commercial pellet and tap water ad libitum, and fasted for 24 hours prior to the experiments. The animals were anaesthetized by the subcutaneous administration of nembutal, 0.08 ml per 100 g body weight. The abdomen was

Fig. 1. *In vitro* intestinal perfusion apparatus

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The intestinal segments were washed with physiological saline solution to get rid of the intestinal contents, blotted gently and then used for the experiment. For each experiment seven segments were used from an animal. Seven sets of apparatus were set up of which three were controls. The intestinal perfusion apparatus developed in this laboratory and used for this experiment is shown in Fig. 1^{10,11}.

The mucosal and serosal solutions were made up of KREBS RINGER bicarbonate pH 7.4, 0.5% polyethylene glycol 4,000 and 2.5 $\mu\text{g}/\text{ml}$ copper ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$). In addition, the mucosal solution contained the sugar under study. The monosaccharide, *i.e.* glucose, galactose, sorbose and fructose, and the disaccharide, *i.e.* lactose and sucrose at different concentrations, were tested for their effects on copper absorption. The apparatus containing 50 ml each of the mucosal and serosal solutions were placed in a water bath at 37.4°C. The serosal solution was oxygenated prior to the fixing of the intestinal segments. During one hour of circulation adequate aeration with 95% O_2 and 5% CO_2 gas mixture was provided. After an hour circulation, the solution remaining in the intestine and the circulatory system was carefully emptied into the mucosal tube. Fifteen ml of this solution was taken and diluted with 15 ml 2 N HCl and centrifuged for 10 minutes at 3,000 r.p.m. Final analysis of copper was done using the atomic absorption flame spectrometer (Nippon Jarrel Ash). The average of the control experiments was taken as 100 and this index was used for all calculations.

Results

Figure 2 indicates the effects of 0, 0.2%, 2.5%, 5.0%, 7.5% and 10.0% glucose. At 2.5% concentration of glucose there was an enhanced absorption rate of copper. It was also seen that with the increase in the concentration of glucose above 5.0% there was a gradual decline in the rate of absorption. The lowest was seen at 10.0% concentration. Thus it was evident that a concentration above 5.0% had a detrimental effect on the rate of absorption of copper.

Fig. 2. Effect of different concentrations of glucose on copper absorption

Table 1 shows the data on the effect of galactose on the absorption of copper. Galactose at 0.2% concentration showed a slight enhancing effect but at 2.5% there was no enhancement of copper absorption. But with a high concentration (10% galactose) it showed an adverse effect which was comparable to that of glucose at the same concentration.

The data presented in Tables 2 & 3 show the effects of the two ketohexose, fructose and sorbose, on copper absorption. In the case of fructose it was seen that 0.2% and 2.5% did not have any beneficial effect when compared to the control. At 0.2% level there was a suppression of the absorption rate of copper. With the increase in the concentration of

Table 1. Effect of galactose on Cu^{++} absorption

Galactose concentration %	No. of intestinal segments	Index of copper absorption rate*
0	7	100.0 $\pm$ 22.3
0.2	4	119.9 $\pm$ 31.2
2.5	7	108.7 $\pm$ 25.1
10.0	4	44.5 $\pm$ 14.3

* Mean $\pm$ Standard deviation.Table 2. Effect of fructose on Cu^{++} absorption

Fructose concentration %	No. of intestinal segments	Index of copper absorption rate*
0	7	100.0 $\pm$ 7.9
0.2	3	75.8 $\pm$ 36.6
2.5	8	94.9 $\pm$ 27.6
10.0	4	23.2 $\pm$ 3.9

* Mean $\pm$ Standard deviation.Table 3. Effect of sorbose on Cu^{++} absorption

Sorbose concentration %	No. of intestinal segments	Index of copper absorption rate*
0	9	100.0 $\pm$ 38.4
0.2	7	84.3 $\pm$ 39.3
1.0	6	99.2 $\pm$ 28.0

* Mean $\pm$ Standard deviation.Table 4. Effect of sucrose on Cu^{++} absorption

Sucrose concentration %	No. of intestinal segments	Index of copper absorption rate*
0	3	100.0 $\pm$ 24.1
1.0	3	89.0 $\pm$ 7.9
10.0	3	55.4 $\pm$ 4.0

* Mean $\pm$ Standard deviation.Table 5. Effect of lactose on Cu^{++} absorption

Lactose concentration %	No. of intestinal segments	Index of copper absorption rate*
0	5	100.0 $\pm$ 19.1
1.0	3	84.3 $\pm$ 16.3
10.0	7	80.4 $\pm$ 2.6

* Mean $\pm$ Standard deviation.

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opened and segments approximately 8 cm in length were excised from the jejunum and ileum. fructose to 10.0%, the index of copper absorption rate dropped to a mean of 23.2, showing that the ketohexose fructose behaved similarly to the aldohexoses at high concentrations.

Sorbose did not show any effect on copper absorption either at 0.2% or 1.0% concentration levels, and there was no significant difference between 0.2% and 1.0% sorbose.

Tables 4 and 5 give the data on the effects of lactose and sucrose on copper absorption. Lactose at 1.0% and 10.0% concentrations showed a decreased absorption of copper but there was no significant difference between the control and the 10.0% level of concentration.

In the case of sucrose at 10.0% a low rate of absorption was observed, ($p < 0.05$) compared with the control. Sucrose and lactose at 1.0% concentration did not remarkably alter the gastrointestinal absorption of copper.

Discussion

Glucose forms the structural unit of important polysaccharides, while galactose is a component of lactose and many polysaccharides. The effects of these two sugars, and ketohexose, fructose and sorbose, were tested among the monosaccharides, and sucrose, and lactose among the disaccharides. Lactose occurs in the milk of mammals and gives a mixture of glucose and galactose on hydrolysis by acid or the enzyme in the small intestine. Sucrose on the other hand is found in all photosynthetic plants and is an important carbohydrate in the natural feed of animals. On hydrolysis by acid or the enzyme of the intestine it gives a mixture of glucose and fructose. These sugars which are of great biochemical interest were studied.

By comparison of the six sugars on the influence on copper absorption, it was demonstrated that only glucose had a beneficial effect on copper absorption by rat intestine studied in '*in vitro*'. From the data presented in Figure 2, it may be said that a concentration below 5.0% enhanced copper absorption. The enhanced action of glucose on copper absorption may be attributed to the fact that energy from glucose was used up for the active transport of copper. Earlier papers have presented evidence that copper absorption is dependent on the respiratory mechanism of the mucosa¹¹. Glucose as an effective substrate for ion transport in toad bladder⁴) and rabbit ileum^{8,9}) has been reported. The observation that glucose has an enhancing effect on copper absorption from the gastrointestinal tract of the rat is a new finding. Though galactose did not show such a favourable influence on copper absorption as glucose, at 0.2% it showed a slight enhancement. The two aldohexoses at a high concentration of 10.0% had an adverse effect on the absorption of copper, while at a low concentration it was apparent that glucose was a good substrate for copper absorption than galactose. It has been reported that galactose is absorbed more rapidly than glucose²) and also that glucose is metabolised immediately after absorption while galactose to be metabolised at a very slow rate¹²). In the present experiment it showed that galactose had a lower enhancing effect than glucose which may be due to the fact that galactose was metabolised more slowly compared to glucose and thus less energy available for the absorption of copper.

The two ketohexoses, fructose and sorbose, showed no beneficial effect on copper absorption. In contrast to glucose sorbose is reported to be passively transported³) and that only sugars with the OH radical attached to the second carbon atom to be actively transported⁵). Here we can assume that a lower amount of ketohexose is absorbed from the intestine than in the case of aldohexoses. Hence it may be said that energy from ketohexoses is not available

for copper absorption.

Definitive statements regarding the action of these two groups of sugars on the absorption of copper cannot be given from the present observations. All sugars tested showed a detrimental effect upon copper absorption at 10.0% concentration. This may be explained as to the formation of sugar chelates with the metal ion^{1,4,8,9)}, and it may also be due to the change in the osmotic pressure in the mucosal cells bringing about a physiological disturbance at the mucosal level at a high concentration of sugar.

Summary

1. The effects of sugars on the absorption of copper from the gastrointestinal tract of the rat was studied, in '*in vitro*' using the intestinal perfusion apparatus.
2. A concentration of 2.5% glucose enhanced the absorption of copper. A concentration above 5.0% glucose demonstrated a low rate of copper absorption.
3. The aldohexose showed a stimulating effect when compared with the ketohexose.
4. All sugars tested showed a decline on the rate of copper absorption at 10.0% concentration.

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ラットにおける銅の腸管吸収に及ぼす糖の影響

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本報告は、ラット小腸における Cu^{++} 吸収に及ぼす影響を、若干の単糖類、二糖類について検討を行なったものである。実験方法は、ウィスター系ラット小腸（十二指腸を除く）による *in vitro* 腸管灌流法を用いた。供試腸管は、体重 200~300 g のラットを約 24 時間絶食後、麻酔し開腹手術により摘出したもので、腸管長は約 8 cm、腸管膨圧は、4 cm H_2O 、還流流速は、15 ml/min である。供試溶液は、 Cu^{++} 25 $\mu\text{g}/\text{ml}$ と 0.5% PEG（容量指標物質）とを含む KRB 溶液を基調液とし、これに種々の濃度の各種の糖を添加した。吸収の程度は、粘膜側の Cu^{++} 量減少率（吸収率）を対照区（糖無添加）のそれを 100 とする指数で表わした。結果は、(1) グルコース 0.2%, 2.5%, 5.0%, 7.5%, 10.0% 添加した場合の Cu^{++} 吸収は、それぞれ平均 120.5, 154.3, 121.7, 82.6, 51.2 であった。(2) ガラクトース 0.2%, 2.5%, 10.0% 各添加区の平均 Cu^{++} 吸収は、それぞれ 119.9, 108.7, 44.5 を示した。(3) ケトース型の糖であるフラクトースは、0.2%, 2.5%, 10.0% の添加により、それぞれ平均 75.8, 94.9, 23.2 の Cu^{++} 吸収を示

した。またソルボースの場合は、0.2%, 1.0% の添加で、それぞれ 84.3, 99.2 であった。(4) 二糖類であるラクトースの場合は、1.0%, 10.0% 添加で、平均 Cu^{++} 吸収は、それぞれ 84.3, 80.4, シュークロース 1.0%, 10.0% 添加で、それぞれ 89.0, 55.4 を示した。

以上の結果から、グルコース 5% まで、およびガラクトース 0.2% での添加は、 Cu^{++} 吸収を、促進せしめているが、この事は、これらの糖が、 Cu^{++} の能動輸送のエネルギー源として利用されていることを示すものではないかと思われる。その他の糖では、添加によって Cu^{++} 吸収の促進はみられていない。これは、グルコースに比べて、これら糖自体の吸収され、そして利用される速度が著しく小さいことによるものではないだろうか。また、いずれの糖においても、非生理的な高濃度の添加は、 Cu^{++} 吸収に対して、抑制的に働らくと思われるが、これは、 Cu^{++} と糖との錯塩形成によるものに加えて、非生理的条件下における腸粘膜細胞の活性低下によるものではないかと考えられる。