SITE OF INTESTINAL ABSORPTION OF ASCORBIC ACID IN GUINEA PIGS AND RATS

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SUMMARY: The site of absorption of ascorbic acid by the small intestine was studied in vivo in guinea pigs, normal and hypophysectomized rats after oral application of ^{14}C-ascorbic acid. A species-specific difference was revealed. The site of absorption in the guinea pig was located in the duodenal and proximal small intestinal wall, whereas the rat showed highest absorption in the ileum. Hypophysectomy in rats caused a shift of the absorption site from the ileum to the jejunum. No absorption was observed in the duodenum and ileum. A regulatory role of the pituitary gland in the absorption of ascorbic acid by the small intestine is discussed.

Guinea pig small intestine was shown to absorb ascorbic acid (AA) in vitro by an active transport mechanism (1). On the contrary, studies in rats indicated that AA is not absorbed against a concentration gradient (1,2). From studies with everted sacs of small intestine from rats, it was concluded that no accumulative transport of AA occurred which is consistent with a diffusion process for absorption (3). However, no data exist on the in vivo absorption of AA by the small intestine. Neither has the site of absorption of AA by the gastrointestinal tract been determined. Results from clinical studies suggest that in man AA is mainly absorbed from the proximal small intestine (4,5). In guinea pigs AA is excreted into the stomach and small intestine after intraperitoneal injection and no absorption from the lower portion of the intestine takes place (6). The highest absorption activity for AA along the small intestine was found in the ileal regio (1).

The kinetics of uptake of AA by the small intestinal wall of guinea pigs and of rats which, contrary to guinea pigs, do not require AA as a vitamin, were investigated after oral administration of (^{14}C)AA. Since hypophysectomized rats show a depressed AA biosynthesis (7), such animals were included in this study.

MATERIALS AND METHODS

L-(1-^{14}C)AA (specific activity 4.8 mC/mmole) was dissolved in water to a final concentration of 5 μC/100 μl (183 μg AA). Guinea pigs (Himalayan spotted) weighing 240-250 g, received a powdered diet (Nutritional Biochemicals), supplemented with 2 g AA/kg of diet. Rats (Füllinsdorf Albino) were fed a commercial diet (Nafag 199 by Nafag, Gossau, Switzerland). Normal rats weighed 180-220 g,

hypophysectomized rats 140-160 g. Hypophysectomy was carried out by the parapharyngeal approach (8). After the weights of the operated animals had stabilized, the rats were taken into the experiments.

The radioactivity was given in an emulsion (9). The radioactive solution (100 µl) was mixed with the emulsion (4 ml) containing 13 mg unlabelled AA, and was applied by stomach tube. Guinea pigs and rats were fasted from 15 h prior to dosage until termination of the experiments. Hypophysectomized rats were maintained on the normal diet. Always 2 animals were used for each time interval (2, 4, 8, 24 and 72 h). After decapitation of the animal, the abdomen was opened, the small intestine was removed and the contents were rinsed out with 20 ml water. The intestinal wall was cut into segments (1 cm) which were processed for counting of radioactivity. Solubilization of the tissue was achieved using Soluene-100 solubilizer (Packard) (10). Radioactivity was determined by means of a Nuclear Chicago Mark II scintillation spectrometer (counting solution: mixture of 4 ml C_2H_5OH, and 15 ml C_6H_5-CH_3 containing Butyl-PBD (Ciba) (8g/l)).

RESULTS

Approximately 8-10 % of the administered (^{14}C)AA was absorbed by the small intestinal wall 2 h after application. Thereafter, a rapid decrease in total radioactivity occurred with progressing time after dosage. Three days after administration only 1-2 % of the given dose were found to be still present in the wall of the small intestine.

The site of absorption of AA by the small intestine is significantly different in guinea pigs, rats and hypophysectomized rats (Figs.1 and 2). The total radioactivity located in the wall of the small intestine was taken as 100 % for each of the time intervals studied, respectively. The duodenum and the proximal part of the jejunum have to be considered as the site of intestinal absorption for AA in the guinea pig. The ileal region did not exhibit a noteworthy absorption of AA.

An even uptake of AA by the intestinal wall was observed in the rat 2 h after oral application. This pattern differed at 4 and 8 h, whereafter AA was mainly accumulated by the distal part of the small intestine. However, an absorption was also indicated in the duodenal region (Fig.2).

A relatively low uptake of AA by the duodenum occurred in the hypophysectomized rat. The main site of absorption was located in the jejunum as a sharply outlined zone (Figs.1 and 2). No accumulation was found in the distal region, as was the case in the normal rat. At 72 h the remaining radioactivity in the intestinal wall was evenly distributed, as could be expected from the kinetics observed 24 h after application of (^{14}C)AA (Fig.2).

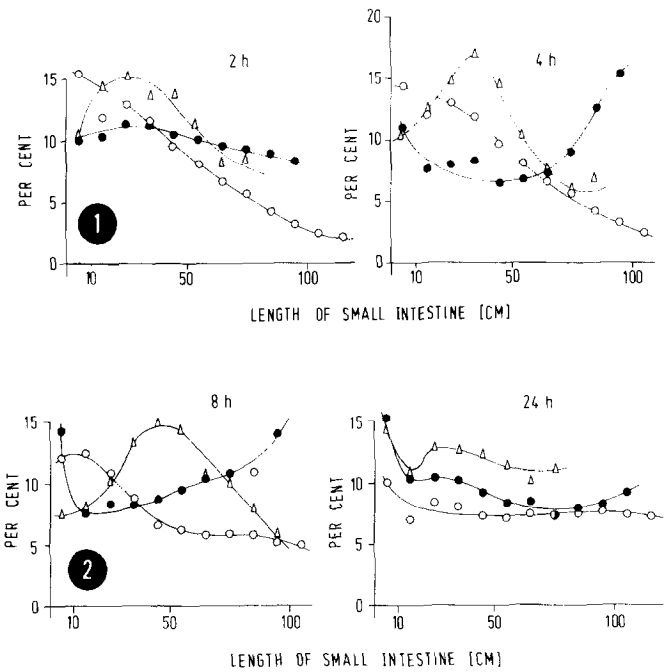

Fig.1: Distribution and site of absorption of ascorbic acid in the small intestinal wall of guinea pigs (o—o), normal rats (●—●) and hypophysectomized rats (Δ) at 2 and 4 h after oral administration of 5 μC L-(1-^{14}C)ascorbic acid. The rate of absorption is expressed as per cent of the total amount of radioactive material present in the wall of the small intestine. The average values for ten segments (1 cm) were always plotted.

Fig.2: Distribution and site of absorption of radioactivity in the small intestinal wall at 8 and 24 h after dosage of 5 μC L-(1-^{14}C)ascorbic acid. For details refer to Fig.1.

DISCUSSION

In vivo studies on the site of absorption of AA were carried out in the small intestine of guinea pigs as well as of normal and hypophysectomized rats. A species-specific difference in the site of in vivo absorption of AA in normal rats, which synthesize AA endogenously, and in guinea pigs, which require AA as a vitamin, was established. The fast absorption of the vitamin in the upper part of the small intestine (duodenum and proximal jejunum) of the guinea pigs may be interpreted as a physiological means of getting most of a limited supply of an exogenously needed nutrient. In accordance with this view is the fact that AA is absorbed in vitro in the small intestine by an active transport mechanism (1,2). In vitro, the absorption activity of the guinea pig intestine was reported to be highest in the ileal region (1). On the other hand, the absorbing capacity for AA in the different parts of the small intestine showed no remarkable dif-

ference, with the exception of the duodenum (2). The latter finding would be consistent with our observations: the transport system is located in the proximal part of the small intestine, whereas the distal region has only a limited facility for absorbing AA, most probably by a diffusion mechanism. It is further suggestive that AA is not absorbed in the large intestine.

The absorption pattern along the small intestine of the normal rat was revealed to be completely different. Whereas 2 h after oral administration the radioactivity in the intestinal wall was evenly distributed, a more distinct accumulation of label had occurred after 4 and 8 h (Figs.1 and 2). Since the ileal region exhibited the highest absorption, the rat might also absorb AA from the caecum. It is uncertain whether the uptake in the duodenal wall at 8 and 24 h after dosage was due to absorption of AA, since it did not occur initially. Studies with bile-duct cannulated rats (11) have shown that after oral application of (^{14}C)AA radioactivity was excreted with the bile, most probably as ascorbic acid 2-sulfate. Because the peak of biliary excretion was noticed 8-10 h after dosage, the presence of radioactivity in the duodenal wall might actually be due to ascorbic acid 2-sulfate, which is known to be absorbed by the lymph (11).

In a recent investigation (10) the pituitary gland was suggested to exert a regulatory role on the uptake behaviour of AA by various tissues of rats. In the hypophysectomized rat the concentration of AA in the portal blood was reported to be higher than in the normal rat. This was thought to be indicative of a more pronounced absorption of AA from the small intestine in the hypophysectomized rat (10). Hypophysectomy caused a shift in the site of absorption from the ileal region in the normal rat to the jejunum in the hypophysectomized rat (Figs.1 and 2). No explanation is available for this alteration. One could postulate, however, that the pituitary gland regulates the absorption of AA by the small intestine of the rat in such a way that an active transport mechanism, similar to that in the guinea pig, is depressed by action of the pituitary gland to such an extent that only diffusion will be important. Removal of the gland by hypophysectomy would then trigger the active mechanism. In order to contribute to this problem the mechanism for the absorption of AA should be investigated *in vitro* in hypophysectomized rats.

REFERENCES

1. Stevenson, N.R. and Brush, M.K., Amer.J.Clin.Nutr. 22, 318 (1969).
2. Kubo, H., Shikoku Acta Medica 22, 34 (1966).
3. Spencer, R.P., Purdy, S., Hoeldtke, R., Bow, T.M. and Markulis, M.A., Gastroenterology 44, 768 (1963).
4. Nicholson, J.T.L. and Chornock, F.W., J.Clin.Invest. 21, 505 (1942).
5. Stewart, J.S. and Booth, C.C., Clin.Sci. 27, 15 (1964).

6. Reid, M.E., Proc.Soc.Exp.Biol.Med. 68, 403 (1948).
7. Salomon, L.L. and Stubbs, D.W., Ann.N.Y.Acad.Sci. 92, 128 (1961).
8. Zarrow, M.X., Yochim, J.M. and Mc Carthy, J.L., in: Experimental Endocrinology, chapter 10-1, Academic Press, New York, 1964, p.308.
9. Gallo-Torres, H.E., Weber, F. and Wiss, O., Int.J.Vit.Nutr.Res. 41, 504 (1971).
10. Hornig, D., Gallo-Torres, H.E. and Weiser, H., Int.J.Vit.Nutr.Res. 42, 487 (1972).
11. Hornig, D. and Gallo-Torres, H.E., Scand.J.Clin.Lab.Invest. 29, Suppl.126, 31-4 (1972).