THE INFLUENCE OF LACTATION ON L-PROLINE ABSORPTION FROM SMALL INTESTINE IN THE ALBINO RAT

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Summary : Intestinal absorption of L-proline was studied in control and lactating rats from jejunum and ileum by *in vivo* method and presented per unit dry weight and per unit length of the respective segment. L-proline absorption was found to be significantly reduced in lactating animals as compared to the virgin controls. The results were discussed in light of serosal to mucosal ratio. By *in vitro* method also jejunal and ileal uptake of L-proline were found to be significantly reduced in lactating animals as compared to the virgin controls.

Key words : intestical absorption, L-proline, lactation, jejunum, ileum, *in vivo* method, *in vitro* method, serosal to mucosal ratio

INTRODUCTION

It is very well-known that the small intestine in rat undergoes remarkable adaptive changes during lactation: it increases in length and weight (11). There is hyperplasia and hypertrophy of the intestinal mucosa and the muscle tissue shows marked hypertrophy (7). Cell proliferation studies revealed decrease in cell transit time (2). However, the studies on absorptive functions are inconclusive.

Using *in vivo* technique Penzes and Simon (10) showed an increase in DL-methionine absorption from small intestine of lactating rats, while Craft (3), using the same technique reported a fall in the rate of glucose and glycine absorption. Cripps and Williams (5) on the other hand showed a decrease in the absorption rate of glucose and leucine in the small intestine of lactating rats, although using perfusion technique, the authors reported an increase in the total absorptive capacity of the gut for glucose and leucine.

The aim of the present investigation was to study the absorptive functions of rat small intestine during lactation more thoroughly and also to study the segmental variation in absorptive function during lactation.

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MATERIAL AND METHODS

The study was carried out on adult virgin female and lactating albino rats of Charles-Foster starin, weighing between 180-220 g. Lactating rats were sacrificed on 21 day of lactation. The age of the virgin and lactation rats at the time of sacrifice was same (about 4 months). The animals were maintained on Hindustan Lever Rat Diet and had free access to food and water. All the rats were kept on fasting for 12 hours before sacrifice, but had free access to water. All the lactating rats were nursing the first litter.

Transport studies

(a) In vivo methods : The animals were anaesthetised by sodium pentothal (40 *mg/kg* body weight, I.P). The abdomen was opened by a midline incision and one loop each 10-15 cm in length was prepared from the proximal jejunum and the distal ileum following the procedure of Crampton *et al.* (4) with some modifications. A known amount of 200 mM L-proline solution was injected into the loops through a fine needle attached to a one - *ml* tuberculin syringe. Filled sacs were replaced into the abdominal cavity and the body temperature of the animal was maintained at 37±1°C for an absorptive period of 10 min. The animal was then killed, bled and loops were excised from the abdomen and washed with normal saline The resulting solution was analysed for L-proline content. The absorption rate of L-proline was caiculated from the difference between the total amount of L-proline introduced into the lumen of the jejunal and ileal loops and the total amount of L-proline recovered at the end of the experimental period from the respective loops. The results are expressed in terms of the dry weight and the length of the loop in centimeters. The tissue dry weight was measured after drying the tissue at 110°-115°C for 3 hours.

(*b*) *In vitro methods* : The rats were anaesthetised with sodium pentothal (40 *mg/kg*, body weight, I.P) and the abdomen was opened by a midline incision. The proximal jejunal and the distal ileal segments were washed free of luminal contents with normal saline (37°C) and the animal was bled and killed by opening the thorax and cutting the heart. The washed segments of the small intestine (proximal jejunum and distal ileum) were stripped off their mesentery and removed from the abdoenm and transferred immediately into ice cold Kreb - Ringer bicarbonate buffer solution (8). The intestinal segments were cut separately into slices of about 2-3 *mm* length (1). Two samples (17-20 slices each having 300-500 *mg* wet weight) of intestinal slices from each segment were separately incubated with 5 *ml* of Kreb - Ringer bicarbonate buffer (pH 7.4), containing in addition 1 mM L-proline in an atmosphere of 95% O_2-CO_2 for 10 minutes at 37°C and a uniform mixing was achieved by using a shaker (60 oscillations/min, amplitude 1.5 - 2.0 *cm*). At the end of the incubation period, the incubating medium was assayed for the residual proline. L-proline uptake rate (in micromoles per *gm* dry tissue weight per hour) was

calculated from the difference in the concentration of L-proline in the medium before and after incubation.

Chemical estimation : L-proline estimations were made according to the method of Wren and Wiggall (12). L-proline was obtained from Sigma Chemical Company, U.S.A. All the chemicals used for the estimation of L-proline were of Analar grade of BDH Laboratory Chemical Division, England.

Procedure for separation of mucosal layer from serosal layer : The rats were killed by cervical dislocation and the abdomen was opened by a midline incision. The small intestine was removed, flushed with isotonic saline and cut open lengthwise along its mesenteric border. The mucosal layer was separated from the serosal layer by the technique of Dickens and Weil-Malherbe (6).

RESULTS

In vivo intestinal absorption of L-proline is shown in Table I and II. When presented as micromole/*mg* dry weight/hour, the lactating animals show significant fall in L-proline absorption from jejunal and ileal segments (Table I). When presented per mm length/hour (Table II), it is observed that in lactating rats jejunum shows a significant fall, however the ileum in lactating animals showed slight increase in the absorption rate of L-proline as compared to the virgin control, but the difference was not significant.

TABLE I. *In vivo* intestinal absorption of L-proline in virgin control and 21 day lactating rats

(µmol/mg dry weight/hour)

	Jejunum (Mean±S.E.)	*Ileum (Mean±S.E.)*
Virgin	4.099±0.162 (9)	2.178±0.141 (9)
Lactating	2.347±0.081 (6)	1.680±0.091 (6)
P	<0.001	<0.02

Figures in parentheses indicate number of observations.

In the lactating animals the rate of L-proline uptake is significantly reduced in the jejunal and the ileal segments (Table III); fall being more pronounced in the jejunal segment, the uptake was only about 55% of the control value, whereas the ileal uptake in these animals was about 75% of the control value.

TABLE II : *In vivo* intestinal absorption of L-proline in virgin control and 21 day lactating rats.

(μmol/mm length/hour)

	Jejunum (Mean±S.E.)	*Ileum (Mean±S.E.)*
Virgin	4.466±0.147 (9)	2.082±0.108 (9)
Lactating	3.141±0.236 (6)	2.359±0.325 (6)
P	<0.001	>0.4

Figures in parentheses indicate number of observations.

TABLE III : *In vitro* jejunal and ileal uptake of L-proline in virgin control and 21 day lactating rats.

(μmol/g dry weight/hour)

	Jejunum (Mean±S.E.)	*Ileum (Mean±S.E.)*
Virgin	60.80±2.19 (11)	37.53±1.32 (12)
Lactating	34.33±2.52 (10)	28.06±1.38 (10)
P	<0.001	<0.001

Figures in parentheses indicate number of observations.

TABLE IV : Serosal to mucosal ratio (dry weight) of jejunum and ileum in virgin and 21 day lactating rats.

	Jejunum (Mean±S.E.)	*Ileum (Mean±S.E.)*
Virgin	0.848±0.053 (4)	1.113±0.061 (4)
Lactating	0.818±0.016 (4)	1.116±0.059 (4)
P	>0.6	>0.9

Figures in parentheses indicate number of observations.

DISCUSSION

The results cited in Table I support the earlier reports of Craft (3) who employed *in vivo* loop technique, similar to that used in the present study as well as of Cripps and Williams (5) who used *in vivo* perfusion technique to perfuse the whole small intestine.

Cripps and Williams (5) noticed that on day 21 of lactation, absorption per mm length of intestine was not significantly different from that of the controls. Craft (3) also could not find diference in absorption rate of glucose and glycine from identical size jejunal loops of 21 day lactating and the control animals. In the present study, however, a significant decrease per unit length of jejunum for L-proline was seen in lactating animals as compared to the virgin controls, though no significant difference was observed in the ileal segments.

Cripps and Williams (5) expressed doubt over the validity of results on intestinal absorption presented in terms of the dry weight of the tissue. They suggested that the intestinal weight may not bear a direct relation to mucosal surface area due to a greater increase in the weight of the muscle layer per unit length of intestine as compared with mucosa. To clear the above point the serosal to mucosal dry weight ratio was calculated in jejunum and ileum from 21 day lactating rats and the control animals. No significant change was observed (Table IV) in serosal to mucosal ratio in the two groups of animals. These findings substatiate the earlier suggestions of Fell *et al.* (7), who observed that the increase in the thickness of muscular coat was proportionate to the mucosal changes, so that the normal relationship of the tissue remained undisturbed. Considering the absorptive function of small intestine, expressed as per unit dry weight, in light of the present finding on serosal to mucosal ratio, it may be appropriate to suggest that during lactation the intestinal absorption rate of nutrients like glucose and amino acids decrease significantly. Though, because of significant increase in intestinal length, the absolute absorption of the nutrient increases in lactation (5,10).

The present study also provides evidence to indicate that the absorption of amino acids across the gut is partially hampered at the initial stage of uptake of the nutrient by the absorptive epithelial cells.

The fall in absorption rate of nutrients from small intestine simultaneous to the increased absorptive surface area of mucosa in lactation may be due to rapid rate of passage of enterocytes along the villus length during lactation (2) so that the absorptive cells never attain full functional maturity because of shortened life span (9).

REFERENCES

1. Agar, W. T., F. J. R. Hird and G. S. Sidhu. The uptake of amino acids by the intestine. *Biochim. Biophys. Acta.*, **14** : 80-84, 1954.
2. Cairnie, A. B. and R. E. Bentley. Cell proliferation studies in the intestinal epithelium of the rat. Hyperplasia during lactation. *Exp. Cell. Res.*, **46** : 428-439, 1967.
3. Craft, I.L. The influence of pregnancy and lactation on the morphology and absorptive capacity of the small intestine. *Clin. Sci.*, **38** : 287-295, 1970.
4. Crampton, R.F., S.D. Gangoli, P. Simson and D.M. Matthews. Rates of absorption by rat intestine of pancreatic hydrolysates of proteins and their corresponding amino acid mixtures. *Clin. Sci.* **41** : 409-417, 1971.
5. Cripps, A.W. and V.J. Williams. The effect of pregnancy and lactation on food intake, gastro-intestinal anatomy and the absorptive capacity of the small intestine in the albino rat. *Brit. J. Nutr.* **33** : 17-32, 1975.
6. Dickens, F and H. Weil-Malherbe. The metabolism of intestinal mucous membrane. *Biochem. J.*, **35** : 7-15, 1941.
7. Fell, B.F., K.A. Smith and R.M. Cambell. Hypertrophic and hyperplastic changes in the alimentary canal of the lactating rat. *J. Path. Bact.*, **85** : 179-188, 1963.
8. Hanson, P.J. and D.S. Parsons. The utilisation of glucose and production of lactate by *in vitro* preparations of rat small intestine. Effects of vascular perfusion. *J. Physiol.*, **255** : 775-795, 1976.
9. Padykula. H.A. Recent functional interpretations of intestinal morphology. *Fed. Proc.*, **21** : 873-879, 1962.
10. Penzes, L. and G. Simon. Intestinal absorption and turnover of dl-methionine during reproduction in the rat. *Jap. J. Physiol.*, **18** : 288-296, 1968.
11. Poo, L.J., W. Lew and T. Addis. Protein anabolism of organs and tissues during pregnancy and lactation. *J. Biol. Chen.*, **128** : 69-77, 1939.
12. Wren, J. J. and P. H. Wiggall. An improved colorimetric method for the determination of proline in the presence of other ninhydrin-positive compounds. *Biochem. J.*, **94** : 216-220, 1965.