Physiol. Res. 44: 31–37, 1995

Elucidation of Intestinal Transport Results as a Function of Age

T. MARTÍ, E. GONZALEZ, M.P. VINARDELL

Department of Human Physiology and Nutrition, Faculty of Pharmacy Barcelona, Spain

Received June 12, 1995
Accepted September 13, 1995

Summary

Duodenal, jejunal, ileal and caecal morphometrics were determined in chickens from hatching to the age of 15 weeks. The ratios of fresh weight/surface area and dry weight/surface area showed age-dependent changes in all the intestinal segments studied. The percentage of mucosa in each segment was also age-dependent in the first three weeks of life with a higher participation in the proximal intestine. Transport of 3-oxy-methyl-D-glucose was determined in the jejunum and ileum using a perfusion technique *in vivo* and expressed as a function of fresh weight, dry weight and surface area. The age-dependent differences observed in weight and mucosa composition of the intestine make it difficult to interpret the results of transport capacity during development. The optimum expression of results in the field of intestinal absorption may be the surface area, since it involves the smallest errors.

Key words

Chick – Surface area – Dry weight-surface area coefficient – Fresh weight-surface area coefficient – Mucosa contents – Monosaccharide transport – *Intestinal transport – Age dependence*

Introduction

In studies of intestinal absorption *in vivo*, the results have generally been expressed in different ways. In earlier studies, an absorption coefficient was used, defined as the amount of substrate absorbed per 100 g body weight per hour (Cori 1925); this coefficient presupposed a relation between body weight and mucosal surface area, and it could only be employed in animals of similar body weight. Some authors have determined the surface area (Fisher and Parsons 1950), others have related the surface area with the fresh or dry weight of the tissue. Beside these, the following morphometric parameters have also been used in expressing the results: intestinal length (Eckanauer 1978), weight of the scraped mucosa, serosal surface area and amount absorbed by the whole intestine (Levin 1967).

Intestinal transport has been demonstrated to be an age-dependent process (Buddington and Diamond 1989, 1990, Obst and Diamond 1992) and the interpretation of the results may be affected by the expression of these processes, since the mucosal composition of the intestine at different ages is different.

Since studies on changes in the dimension of the intestinal mucosa in animals of different ages have been performed in rats (Forrester 1972), we have chosen the domestic fowl, a species frequently used in intestinal absorption experiments to study these structural differences with age.

Material and Methods

Animals and diets. Newly hatched male Leghorn chickens (commercial line Shaver-Starcross-28) were obtained from a commercial hatchery (Granja Gibert, Cambrils, Tarragona, Spain) on the same day. They were kept in a battery-type brooder during the first week and then transferred to larger cages in the same battery (Prolabor, Barcelona, Spain). Birds were maintained in a controlled environment with a 12 h photoperiod (0800–2000 h). The animals were fed two different standard chicken chows (Iniciarina to age 3 weeks and Gordina, Gallina Blanca Purina, Barcelona, Spain), and had access to tap water *ad libitum*. Eighteen hours prior to the assay, the animals were fasted. Experiments were performed on animals at the following ages: 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 10 weeks and 15 weeks.

Experimental procedure. After the animals had been anaesthetized with sodium pentobarbital (Ministerio de Sanidad, Madrid, Spain) (40 mg/kg) in the tibiometatarsial vein, a laparotomy was performed and a loop of the jejunum (yolk sac region) and ileum were isolated between two L-shaped glass cannulae secured to the intestine with silk suture and connected to a perfusion system equipped with a peristaltic pump (Gilson Minipuls 2). The length of the loops was from 6 to 15 cm depending on age. Anaesthesia was maintained during the whole absorption trial and animals were kept in a chamber with controlled environmental conditions.

After rinsing with NaCl 0.9 g l^{-1} (Merck, Darmstadt, Germany), the intestinal segment was perfused for 30 min with Krebs-Henseleit containing unlabeled 5 mmol l^{-1} 3-O-methyl-D-glucose (3-OMG)(Sigma, St Louis, MO, USA) and ^{14}C-labelled 3-OMG with a specific activity of 315 mCi $mmol^{-1}$ (New England Nuclear, Boston, USA). After rinsing, the same intestinal segment was perfused with the same solution in the presence of phloridzin (Sigma) at a concentration of 1 mmol l^{-1} to inhibit the carrier transport process (Vinardell 1992). The perfusion rate was 2 ml/min. 50 μl aliquots were taken at 1, 5 10 15, 20, 25 and 30 min and the monosaccharide concentration was determined by the scintillation method. Absorption was expressed as a function of the surface area, the fresh and dry weight.

Intestinal morphometrics. After decapitation of the animals, a section of the duodenum, jejunum, ileum and the whole caecum were taken out, washed with saline solution, dried with filter paper and weighed. The segments were cut longitudinally and the area was determined by planimetry without accounting for area amplification by villi and microvilli (Nys and Mongin 1982). The weight of the dry tissue was determined after drying for 48 h at 60 °C.

Two coefficients were calculated by dividing the fresh weight and the dry weight by the surface area and expressed as g/cm^2. The percentage of the mucosa weight in the different segments was also determined after removing the mucosa from the segment by gentle scraping with a glass slide.

Statistics. All values and figures represent means ± S.E.M. The analysis of variance from the use of linear models allowed us to calculate the F of Snedecor and to establish statistical differences among the different age groups. Differences were considered significant at $p<0.05$.

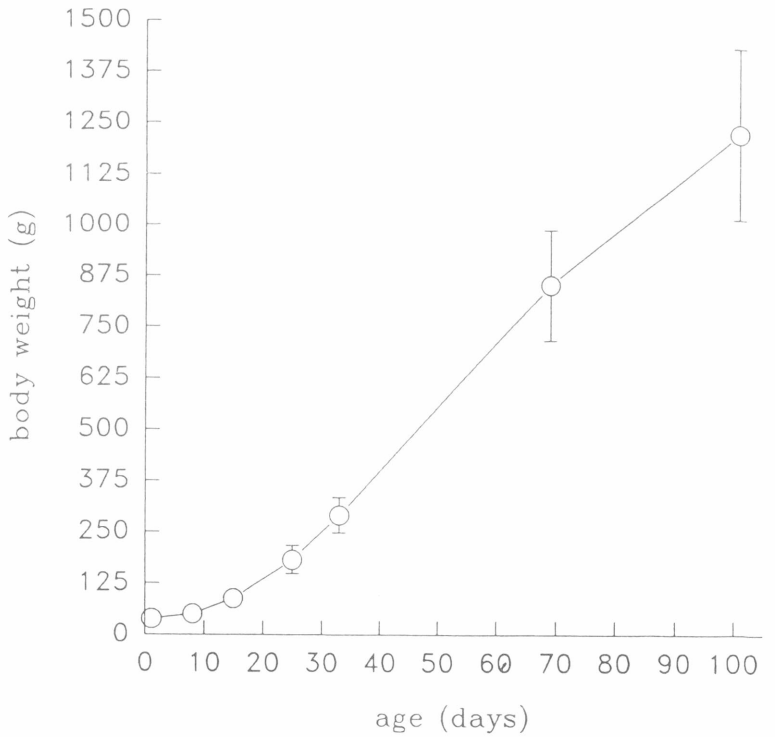

Fig. 1
Growth of body weight during the age period studied. Mean values ± S.D. Error bars smaller than symbols are not represented.

Results and Discussion

Fig. 1 presents the body weight growth as a function of age from 1 day to 15 weeks. The weight increase was rapid during the first days of life. From 2 weeks to 3 weeks this represents an approximately twofold increase, and from the first day to 15 weeks of the age, this increase is about 34-fold. These results are in agreement with other authors (Lilja 1983).

The ratio of fresh weight/surface area as a function of age in the segments studied is shown in Fig. 2. There is an increase in this parameter with age, in all the segments during the first 40 days of life, thereafter this value remains practically constant. This indicates that the evolution of the intestinal layers during the first 5 weeks of life is rapid and subsequently becomes stabilized (Vinardell and Martí 1992).

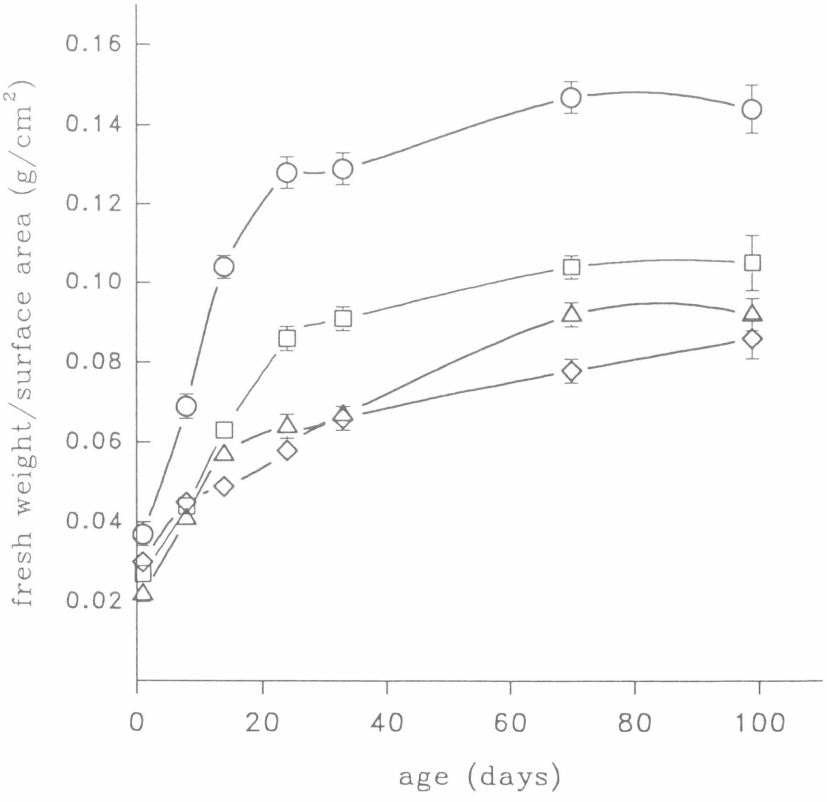

Fig. 2
Evolution of the ratio fresh weight/surface area of different intestinal segments as a function of animal age. Mean values ± S.E.M. (circles – duodenum, squares – jejunum, triangles – ileum, diamonds – caecum)

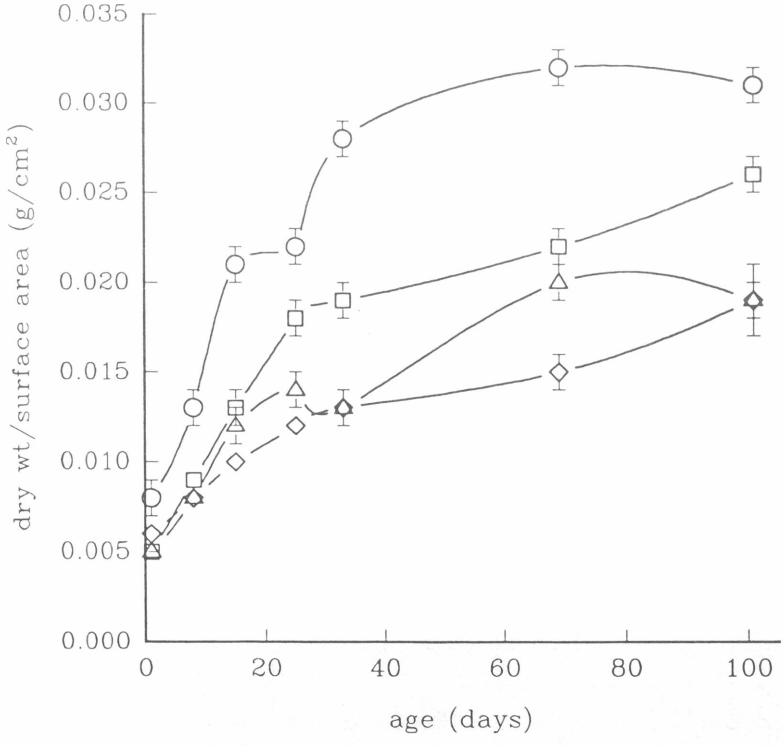

Fig. 3
Evolution of the ratio dry weight/surface area of different intestinal segments as a function of animal age. Mean values ± S.E.M. For further explanation see Fig. 1.

Table 1
Age changes on total transport and diffusion of 3-OMG by chicken jejunum after 30 min perfusion *in vivo* expressed as a function of the surface area, fresh weight and dry weight of the segment

	Total transport						
	1 day	1 week	2 weeks	3 weeks	4 weeks	10 weeks	15 weeks
Surface μmol/cm^2	2.79 ± 0.08 (6)	1.49 ± 0.06 (9)	1.38 ± 0.14 (8)	0.98 ± 0.14 (14)	0.70 ± 0.08 (8)	0.89 ± 0.14 (10)	0.69 ± 0.07 (11)
Fresh wt μmol/g	114.47 ± 17.47 (7)	41.05 ± 4.06 (9)	22.25 ± 1.82 (8)	12.15 ± 1.53 (14)	12.45 ± 3.30 (8)	5.65 ± 0.96 (9)	7.26 ± 1.22 (11)
Dry wt μmol/g	574.78 ± 81.45 (7)	212.23 ± 16.19 (6)	103.46 ± 10.49 (6)	52.46 ± 10.17 (8)	54.23 ± 6.74 (5)	23.53 ± 2.59 (8)	25.75 ± 2.70 (5)
	Diffusion						
Surface μmol/cm^2	1.46 ± 0.09 (7)	0.99 ± 0.09 (12)	0.68 ± 0.13 (7)	0.56 ± 0.10 (14)	0.43 ± 0.12 (7)	0.43 ± 0.08 (7)	0.28 ± 0.03 (9)
Fresh wt μmol/g	58.15 ± 13.37 (7)	25.86 ± 2.66 (12)	11.79 ± 2.74 (7)	8.75 ± 1.92 (15)	5.20 ± 1.23 (7)	4.32 ± 0.75 (7)	2.52 ± 0.29 (9)
Dry wt μmol/g	295.28 ± 67.77 (7)	123.76 ± 20.43 (6)	59.09 ± 14.15 (5)	46.43 ± 12.11 (9)	25.26 ± 10.21 (4)	15.18 ± 4.48 (6)	13.03 ± 1.69 (2)

Mean values ± S.E.M. Number of animals in parenthesis.

Table 2
Age changes on total transport and diffusion of 3-OMG by chicken ileum after 30 min perfusion *in vivo* expressed as a function of the surface area, fresh weight and dry weight of the segment

	Total transport						
	1 day	1 week	2 weeks	3 weeks	4 weeks	10 weeks	15 weeks
Surface μmol/cm^2	2.28 ± 0.20 (7)	1.24 ± 0.07 (6)	1.10 ± 0.13 (10)	1.03 ± 0.11 (11)	0.79 ± 0.16 (7)	0.70 ± 0.09 (9)	0.65 ± 0.07 (9)
Fresh wt μmol/g	74.90 ± 6.30 (8)	34.80 ± 6.09 (4)	17.28 ± 1.57 (10)	16.07 ± 1.95 (11)	11.64 ± 2.69 (7)	8.61 ± 1.06 (9)	7.50 ± 1.16 (9)
Dry wt μmol/g	385.27 ± 45.00 (6)	172.85 ± 26.42 (4)	86.65 ± 9.88 (6)	78.96 ± 13.45 (6)	81.07 ± 21.07 (3)	32.69 ± 2.67 (3)	45.98 ± 8.29 (8)
	Diffusion						
Surface μmol/cm^2	1.21 ± 0.08 (5)	0.81 ± 0.09 (8)	0.71 ± 0.11 (10)	0.61 ± 0.08 (9)	0.51 ± 0.09 (7)	0.48 ± 0.08 (8)	0.39 ± 0.04 (9)
Fresh wt μmol/g	83.74 ± 23.09 (9)	18.84 ± 4.22 (4)	10.63 ± 1.76 (10)	10.60 ± 2.07 (9)	7.54 ± 1.30 (7)	4.62 ± 0.90 (8)	4.33 ± 0.78 (3)
Dry wt μmol/g	245.83 ± 89.35 (5)	96.04 ± 22.70 (4)	58.24 ± 13.92 (7)	64.64 ± 10.79 (6)	40.41 ± 2.66 (4)	23.49 ± 7.21 (3)	29.91 ± 8.54 (8)

Mean values ± S.E.M. Number of animals in parenthesis

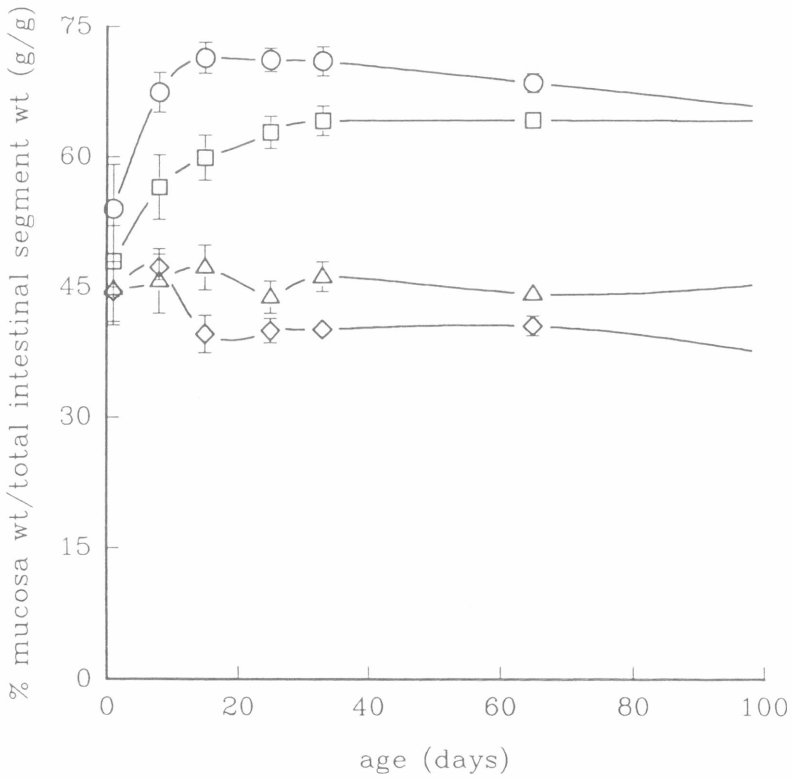

Fig. 4
Percentage of mucosa in different intestinal segments as a function of animal age Mean values ± S.E.M. For further explanation see Fig. 1.

Fig. 3 shows the ratio dry weight/surface area as a function of age in the segments studied. The behaviour of this parameter is similar to that observed above, since the percentage of water is similar in all the segments, around 80 %, and is independent of age.

The principal component of the intestinal wall is the mucosa. The percentage of mucosa varies with age in the duodenum and ileum during the first 3 weeks of life and remains constant thereafter (Fig. 4). The percentage of mucosa is highest in the duodenum. The order is duodenum > jejunum > ileum > caecum, decreasing in the caudal direction. The ileum and caecum exhibit similar percentages of the mucosa, but lower than the proximal intestine, around 45 % without changes in relation to age.

We compared the jejunal and ileal transport capacity of animals at different ages as a function of different parameters (Tables 1 and 2), and the differences observed could affect the correct interpretation of the results and introduce erroneous conclusions. It is important to clarify the parameter (s) used in the expression of intestinal absorption studies, especially when comparing animals of different ages, to avoid possible error in their interpretation.

An accepted expression is the term "physiological length", which is represented by I_{30g} x 0.6, where I_{30g} is the length of the intestinal segment removed from the abdominal cavity after being suspended with a 30 g weight. The 0.6 factor corresponds to an arbitrary value calculated from the relationship between I_{30g} and the length of the same segment under physiological conditions (Ponz *et al.* 1979).

The choice of the basis upon which to express intestinal absorptive function has always been a controversial issue, especially in animals under different hormonal and nutritional conditions (Levin 1982). Many years ago, it was proposed that a "functional diffusive area" could be measured by the absorption of a passively diffusing, non-metabolized substance such a thiourea in the rat intestine (Levin 1967). In the domestic fowl, this passive absorption of thiourea cannot be used as a simple index of the functional diffusive surface area of dietary-induced changes in the small intestine or age-induced changes.

Differences observed in intestinal absorption of sugars in studies performed at different ages (Penzes and Skala 1977, Vinardell and Bolufer 1984, Younoszai and Lynch 1974) could be attributed to the parameter chosen. Our results showed that the decrease in intestinal transport with age depends on how the results are expressed.

The total transport of 3-OMG by the jejunum of 1- and 2-week-old chickens did not exhibit statistical differences if expressed as a function of the surface area, but was near double in 1-week-old chicken if expressed as a function of the fresh or dry weight ($p<0.0001$). We obtained similar results in the ileum, and when we compared the diffusive pathway at different ages.

The use of fresh or dry weight has the disadvantage of variations depending on the animal's age which hinders comparative studies in developing animals. Based on our results, we propose the surface area as the best parameter, because of the smaller error and simplicity of its determination. This area could be determined without difficulty by planimetric techniques or by the method described by Ponz *et al.* (1979). The latter approach implies that the intestinal diameter is constant along the whole segment, while this is generally true in the small intestine, although not in the caecum. It could thus be used only in the small intestine.

We propose the use of the surface area, determined by planimetry, in intestinal absorption studies, particularly when different stages of life are compared.

Acknowledgements

This study was supported by grant PB88-0219 from "Dirección General de Investigación científica y Técnica, Ministerio de Educación y Ciencia", Spain. We wish to thank Robin Rycroft of the "Servei Llingüistic de la Universitat de Barcelona" for language assistance.

References

BUDDINGTON R.K., DIAMOND J.M.: Ontogenic development of intestinal nutrient transport. *Annu. Rev. Physiol.* **51**: 601–619, 1989.

BUDDINGTON R.K., DIAMOND J.M.: Ontogenic development of monosaccharide and amino acid transporter in rabbit intestine. *Am. J. Physiol.* **258**: G544–G555, 1990.

CORI C.F.: Fate of sugars in the animal body. Rate of absorption of hexoses and pentoses from the intestinal tract. *Biol. Chem.* **66**: 691–715, 1925.

ECKANAUER R.: Analysis of the effects of food and of digestive secretions on the small intestine of the rat. III. Mucosal mass, activity of brush border enzymes, and in vivo absorption of galactose, sodium and potassium. *Gut.* **19**: 707–714, 1978.

FISHER R.B., PARSONS D.S.: The gradient of mucosal surface area in the small intestine of the rat. *J. Anat.* **84**: 272–282, 1950.

FORRESTER J.M.: The number of villi in the rats jejunum and ileum: effect of normal growth, partial enterectomy and tube feeding. *J. Anat.* **111**: 283–291, 1972.

LEVIN R.J.: Techniques, terminology and parameters in intestinal absorption. *Br. Med. Bull.* **23**: 290–312, 1967.

LEVIN R.J.: Assessing small intestine function in health and disease in vivo and in vitro. *Scand. J. Gastroenterol.* **17**: 31–51, 1982.

LILJA C.: A comparative study of postnatal growth and organ development in some species of birds. *Growth* **47**: 317–339, 1983.

NYS Y., MONGIN P.: Transport of electrolytes and water in the upper jejunum of the fowl in vivo perfusion. *Pflügers Arch.* **392**: 251–254, 1982 .

OBST B.S., DIAMOND J.M.: Ontogenesis of intestinal nutrient transport in domestic chickens (Gallus gallus) and its relation to growth. *The Auk* **109**: 451–464, 1992.

PENZES L., SKALA I.: Changes in the mucosal surface area of the small gut of rats of different ages. *J. Anat.* **124**: 217–222, 1977.

PONZ F., ILUNDAIN A. LLUCH M.: Method for successive absorptions with intestinal perfusion. *Rev. Esp. Fisiol.* **35**: 97–104, 1979.

VINARDELL M.P.: Phloridzin inhibition of 3-oxy-methyl-D-glucose absorption across chicken jejunum and caecum in vivo. *Med. Sci. Res.* **20**: 97–98, 1992.

VINARDELL M.P., BOLUFER J.: Age dependent changes on jejunal sugar absorption by rat in vivo. *Exp. Gerontol.* **13**: 73–78, 1984.

VINARDELL M.P. MARTÍ T.: Growth of the alimentary tract with aging in chickens. *Acta Physiol. Hung.* **79**: 367–370, 1992.

YOUNOSZAI M.K., LYNCH A.: In vivo intestinal absorption of hexose in growth-retarded suckling rat pups. *J. Nutr.* **104**: 671–677, 1974.

Reprint Requests

M.P. Vinardell, Department of Human Physiology and Nutrition, Faculty of Pharmacy, Av. Joan XXIII s/n, 08028 Barcelona, Spain.