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Iron Absorption in the Everted Chicken Intestine

J. Sánchez and J. Planas

Departamento de Fisiología Animal
Facultad de Biología
Universidad de Barcelona
Barcelona - 7 (Spain)

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Everted chicken intestines from 2-4 week old males were prepared according to the Wilson and Wiseman technique. The sacs were kept in a glucose ionic balanced solution, containing different cold iron concentrations (0.02; 0.01; 0.5; 1 mM Fe) and ^{59}Fe was added as a tracer.

The mucosal iron uptake and the iron transfer in the serosal fluid were determined at different intervals (1; 5; 10; 20 min) and in three main segments. The viability of the intestinal sacs was controlled by manometric O_2 evaluation.

The iron mucosal uptake was proportional to the iron concentration but the serosal iron transfer presented a saturation level, with similar behaviour for both the duodenum and the jejunum. Iron transfer decreased with time and this could suggest a serosal to mucosal reflux. No effect was noted from the presence of metabolic inhibitors (0.01 mM sodium azide or 0.5-2 mM 2-4-dinitrophenol).

Intestinal iron absorption in mammals has been studied frequently and various reviews have been published (1, 3, 4, 13, 14).

Interest in this aspect of iron metabolism is high, as the homeostasis of this metal is regulated through intestinal absorption and the excretion in the majority of animals is very reduced (9).

In birds, iron absorption has not been analyzed and in our laboratory, a close circuit perfusion technique for chicken intestinal loops, *in situ*, was set up to observe the influences of age, sex and laying on absorption (10).

In order to complete this study and to amplify our knowledge on this theme, an *in vitro* technique was used when the absorption capacity of the chicken intestine at different iron concentration and times, was not influenced by the high plasma levels during the laying period.

Materials and Methods

Young male chickens (2-4 weeks old), New Hampshire strain, weighing 100-250 g were used. Commercial food (Piensos Flotats, Barcelona), containing 185 ppm Fe and tap water were administered *ad libitum*.

The hematocrit concentrations were determined in heparinized blood samples, taken from the wing vein before intestinal extirpation. A microcentrifuge, «Haemofuge» Heareus for hematocrit was used. The hemoglobin concentration was determined by the cyanmethemoglobin technique. The plasma iron was assayed according to ICSH (7).

The intestinal loops were obtained following the WILSON and WISEMAN (7) method; they were washed twice with cold NaCl 7% and the mesentery and pancreas were removed. The loops were later everted with a glass rod and kept in balanced ion solutions. These solutions, used to fill up the sacs, contained: NaCl 120 mM; KCl 5 mM; $CaCl_2$ 0.1 mM; Tris-ClH 20 mM, pH 6.2. Glucose (1 g/l) was added in some ionic solutions. The solutions were phosphorous-free as this element interferes with iron absorption.

$FeCl_3$ solutions were prepared with ascorbic acid and L-histidin, in order to mantain the Fe^{2+} in a chelated form (10). The following proportions were used: A mM Fe; A mM Ascorbate and A M L-histidine.

Iron, $^{59}FeCl_3$ in HCl 0.1 N (Radiochemical Centre) with a specific activity of 3-20 mCi/mg Fe on the cold-iron solution, was added.

The everted sacs were put into vials, containing 4 ml ionic solution plus 2 ml glucose ionic solution; all the flasks were kept in a water thermostated bath (Gallenkamp) at 41° C and at a 80 movement/min shaking rate.

At scheduled time, the sacs were removed from the vials and the absorption procedure was stopped by a double immersion in 100 ml cold solution EDTA 0.01 M. The intestinal sac was carefully dried with filter-paper; the inner fluid was poured into a counting vial and the intestine, after a triple wash with EDTA, was placed into another vial. The ^{59}Fe in all the vials was measured by means of a Phillips well-type counting unit PW 4630-4620, 4313.

The viability of the tissue was checked by the manometric technique of WARBURG (15), where portions of the intestines were analyzed in separate flasks, with or without glucose, for 1 h at 41° C. The exclusion of tripan blue excretion from the enterocytes was also used as a test of viability (11).

In some experiments, different metabolic inhibitors, such as DNP at 0.5 mM, sodium azide, 0,01 M, were added to the absorption solution.

The statistical analysis of the mean values was carried out according the students' «t».

Results

The hematocrit, hemoglobin concentration and the plasma iron determination, in a lot of 30 birds of the same age as the experimental group, were evaluated. The results showed normal conditions in their iron status and the average values were as following: Hematocrit: 23.3 ± 1.5 %; Hemoglobin concentration: 11.4 ± 1.2 g/100 ml blood; Plasma iron: 120.3 ± 10 μg Fe/100 ml plasma. In order to assure the normal iron-state of each specimen, the hematocrit and the hemoglobin concentration were assayed in blood samples before the experiment.

Viability of the intestinal sacs. The O_2 respiration of the everted sacs in a Warburg apparatus was used as a control of the viability of the intestine. The same O_2 consumption for the duodenum and jejunum, is observed and the ileum presented a lower O_2 consumption for gram dry intestine. However, in all three cases, a perfect lineality, during the 60 min experiment, was observed.

Iron absorption as a time function. The Fe accumulated in the duodenum, during the 20 min of the experiment, was propor-

Table I. *Intestinal iron uptake in everted chicken sacs.*
Each mean represents the average values (mean ± s.e.m.) of 3 sacs per bird, using 6 animals for each experiment.

Mucosal solution Fe mM	Intestinal uptake (μg Fe/cm intestine)											
	Duodenum				Jejunum				Ileum			
	1′	5′	10′	20′	1′	5′	10′	20′	1′	5′	10′	20′
0.02	0.06 ±0.01	0.14 ±0.02	0.16 ±0.01	0.18 ±0.01	0.06 ±0.01	0.12 ±0.01	0.12 ±0.01	0.14 ±0.01	0.03 ±0.00 ●● **	0.09 ±0.01 ●	0.12 ±0.01	0.16 ±0.00
0.01	0.35 ±0.03	0.43 ±0.06	0.66 ±0.03	0.73 ±0.05	0.31 ±0.03	0.52 ±0.03	0.56 ±0.04	0.66 ±0.08	0.21 ±0.01 ● *	0.25 ±0.03 ●● **	0.33 ±0.01 ●● **	0.37 ±0.05 ●● **
0.5	1.32 ±0.15	1.71 ±0.15	2.05 ±0.07	3.18 0.29	1.14 ±0.20	1.72 ±0.20	1.89 ±0.17	2.90 ±0.28	1.10 ±0.07	1.18 ±0.07 ● *	1.52 ±0.04 ●●	2.64 ±0.08 ●● **
1.0	3.34 ±0.24	3.96 ±0.32	5.10 ±0.62	7.64 ±0.51	3.40 ±0.28	3.69 ±0.18	4.56 ±0.36	5.64 ±0.39	2.17 ±0.10 ● *	2.28 ±0.09 ● *	3.09 ±0.23 ●	5.52 ±0.20 ●

p values: ●● $p < 0.001$ and ● $p < 0.01$ related to duodenum; ** $p < 0.001$ and * $p < 0.01$ related to jejunum.

Table II. *Transfer iron measured in the serosal fluid.*

Each mean represents the average values (mean ± s.e.m. of 3 sacs per bird using 6 animals for each experiment). No statistical differences between the different parts of the intestine have been shown.

Mucosal solution Fe mM	Transfer Iron (μg Fe/cm Intestine)											
	Duodenum				Jejunum				Ileum			
	1′	5′	10′	20′	1′	5′	10′	20′	1′	5′	10′	20′
0.02	0.01 ±0.00	0.04 ±0.01	0.04 ±0.01	0.01 ±0.00	0.01 ±0.00	0.06 ±0.00	0.03 ±0.01	0.01 ±0.00	±0.01 0.00	0.03 ±0.01	0.04 ±0.01	0.02 ±0.00
0.01	0.11 ±0.02	0.11 ±0.01	0.12 ±0.01	0.07 ±0.02	0.10 ±0.02	0.08 ±0.01	0.11 ±0.01	0.10 ±0.02	0.10 ±0.01	0.09 ±0.02	0.12 ±0.01	0.08 ±0.02
0.5	0.48 ±0.04	0.33 ±0.04	0.33 ±0.02	0.15 ±0.03	0.48 ±0.05	0.39 ±0.05	0.36 ±0.03	0.34 ±0.04	0.33 ±0.02	0.25 ±0.02	0.28 ±0.05	0.29 ±0.07
1.0	0.87 ±0.14	0.34 ±0.06	0.36 ±0.03	0.24 ±0.04	0.82 ±0.09	0.45 ±0.07	0.49 ±0.07	0.50 ±0.09	0.56 ±0.04	0.45 ±0.07	0.41 ±0.12	0.48 ±0.14

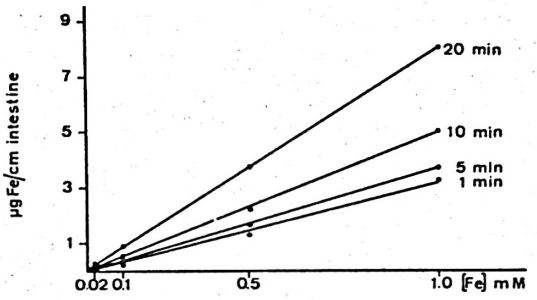

Fig. 1. *Iron uptake in duodenum at several Fe concentrations in mucosal fluid.* Each point is the average of 3 sacs per bird, using 6 animals for concentration and time.

tional *versus* time. The increased concentration of iron in mucosal fluid implied a small decrease in the percentage of iron absorption, from 1.6 % in the iron concentration of 0.02 mM to 1.1 % in a 0.5 mM. The 1.0 mM Fe only produced a 1.4 % absorption, because of a loss in the regulation mechanism for such high concentrations (16). The comparisons, among the three intestinal segments, showed only a slight difference between the duodenum and the jejunum (table I and II).

Iron absorption as a concentration function. The modification of the iron concentration in the mucosal fluid from 0.02 mM to 1.0 mM, produced a proportional mucosal uptake (fig. 1). Meanwhile, the iron transferred to the serosal side presented a saturation level which presuposed the existence of a regulatory step (table II). The duodenum and jejunum presented similar behaviour, but ileum showed significant quantitative differences (table I and II).

The effect of oxidative metabolism inhibitors. The addition of sodium azide (0.01 mM) to the mucosal fluid neither altered the Fe accumulation in the intestine nor the serosal transfer of the metal.

No effect in these parameters was observed in everted sacs, in the presence of 0.5 mM, DNP or 2.0 mM, either.

Discussion

The net iron absorption increased proportionally from 12 to 695 µM and also versus time from 15 to 160 minutes, in *in vivo* experiments in chickens (10). The mucosal iron uptake was lineal to iron concentration but increased slightly versus time.

In everted sacs, the iron transfer decreased with time (table I). This fact could suggest the presence of a reflux from the serosal to the mucosal side, which is contrary to the normal polarization of the absorption process (4). However, a similar phenomenon has been observed in rats (12), and GUY *et al.* (5) described an active serosal to mucosal transport *in vitro* experiments. LINDER and MUNRO (9) also observed the iron transport to the lumen in overloading experiments.

On the other hand, the mucosal iron uptake increased *versus* time and maintained a lineality against the exterior iron concentration, general in several species using high doses (100-2500 mg/kg), where the Fe is bound to nonspecific receptors (4). SHEEHAN (12) also found a lineality in a concentration range from 0.1 to 10 mM, similar to ours; WHEBY *et al.* (16) working with rats suggested a dose of 50 µg Fe, as a critical value, beyond which the regulatory capacity is exceeded.

It is universally accepted that the transfer of iron through the enterocyte is bound to some carrier, of which the exact identity is still questionable (4, 6, 9, 14). The carrier is the limiting factor in the transfer system.

In chicken's everted sacs, we observed the saturable character of this process (table II), as did EASTHAM *et al.* (2) in isolated membranes of rat enterocytes. In *in vivo* experiments with chickens, MARTI

et al. (10) found a saturation in the serosal transport from 695 μM Fe, but the mucosal uptake was lineal in the whole concentration range.

We did not observe any effect of the metabolic inhibitors on the iron absorption. This result could suggest that the process was not mediated by the oxydative metabolism. EASTHAM *et al.* (2) obtained similar results using isolated membranes of rat enterocytes and suggested that the thiol groups in the membrane were responsible for the iron transport.

SAVIN and COOK (11), working on isolated mucosa cells of rats saw that the iron uptake was not affected by the inhibitors of the cell respiration but was blocked by chelatant agents. Moreover, FORTH and RUMMEL (3), have demonstrated the continuity of the absorption process in isolated loops of rat intestine under a nitrogen atmosphere. In chickens, the anaerobic metabolism of isolated mucosa cells could be quantitatively important. A lineal production of lactate and CO_2 in a 2 h experiment on chickens isolated mucosa cells was observed (8).

The *in vitro* results contrasted to the *in vivo* response, where the connections between the cellular metabolism and the iron transport were not doubtful although the exact steps that needed energy were not identified (2, 3, 13).

The absorption process *in vitro* could be passive and also, it seems, non-dependent on the general iron status of the animal. Therefore, SHEEHAN (12), in iron deficient or in phenylhydrazine induced hemolitic anemia, did not find an absorption increase in everted sacs. Also SAVIN and COOK (11), in isolated mucosa cells, found that the iron status of the animal did no affect the iron absorption, which could be produced by passive difusion.

Classically, the iron absorption *in vivo*, was considered to be regulated by two factors: the iron concentration in the whole body and the erythropoiesis rate of the animal (1, 3, 9, 13, 14).

No significant differences were found in the absorption rate between duodenum and jejunum, although the ileum absorbed less. Mammals, man included, offered similar results *in vivo* (4, 13, 16). However, chickens (MARTI *et al.* personal communication) showed a clear supremacy of the proximal segment.

A minor stability of the specific iron binding in the brush border, could explain a lower absorption capacity (3). In our experiments, the 6.2 pH of the perfusion fluid was farther than the physiological pH in chicken ileum which is nearer to 7.2. Nevertheless, the slow intestinal flux and the long run in the ileum, could be compensated by its smaller absorption rate (3, 13).

The peculiar response of the intestinal iron absorption in evered sacs versus the *in vivo* behaviour, introduces new aspect to this process, that could alter the results, in spite of simplifying the phenomenon.

Resumen

Se estudia la absorción intestinal de hierro en sacos evertidos de intestino de pollos de 2-4 semanas de edad, según la técnica de Wilson y Wiseman. Los sacos se mantienen en una solución salina equilibrada, conteniendo glucosa, y a la que se añaden distintas concentraciones de hierro (0,02, 0,1, 0,5 y 1 mM); el Fe^{59} se utiliza como trazador.

La captación de hierro por la mucosa y el transporte de hierro serosal se determinaron a diferentes tiempos (1, 5, 10 y 20 minutos) en sacos preparados con los tres tramos principales del intestino. La supervivencia de los sacos intestinales fue comprobada por el consumo de oxígeno.

La captación de hierro por la mucosa es proporcional a la concentración, presentando el hierro transferido al lado serosal una saturación, tanto para el duodeno como para el yeyuno.

El hierro transferido disminuye con el tiempo, y ello sugiere la existencia de un reflujo serosal al lado mucosal. No se observa ningún efecto de los inhibidores metabólicos, azida

sódica, 0,01 mM, y 2,4-dinitro-fenol 0,5-2 mM, sobre la absorción intestinal.

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