Jejunal calcium permeability
in laying hens during egg formation

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Summary. The permeability of the upper jejunum to water, calcium, potassium, sodium and chloride was measured in the immature pullet and then in the laying hen before and during egg-shell calcification by an *in vivo* perfusion procedure. Jejunal calcium permeability was constant throughout egg formation. Thus, the increase of net absorption during shell calcification was not due to enhanced mucosal capacity for calcium translocation, but rather to a better solubilization of calcium carbonate in the upper digestive tract. However, this capacity increased at the onset of egg production, as shown by the difference between immature and mature birds.

Introduction.

Calcium absorption in the small intestine varies at sexual maturity and during egg-shell formation in the laying hen (Hurwitz *et al.*, 1973). Bar and Hurwitz (1972) have shown that its increase at maturity and at the onset of egg production is correlated with the concentration of calcium-binding protein (CaBP) in the duodenum. However, CaBP does not vary in that organ during the laying cycle, whereas calcium absorption almost doubles during egg-shell formation as compared to the status of no shell deposition (Bar and Hurwitz, 1975 ; Hurwitz and Bar, 1965 ; Itoh, 1967). In the first physiological situation, it seems logical that vitamin D and its metabolites would be involved in the regulation of calcium absorption, according to the classical model already described in chickens and rats (Haussler and Mc Cain, 1977). However, such a model apparently does not fit egg-shell formation in the laying hen, and another explanation must be found.

If, *a priori*, calcium translocation through the gut is a passive mechanism, the changes observed may be imputed to two main causes : (i) the force(s) driving calcium through the mucosa may increase, and (ii) for a given driving force, the passive permeability of the mucosa barrier to calcium may also augment. In both cases, calcium absorption would be greater. However, the non-absorbable indicator method used by Hurwitz and Bar, and the ^{45}Ca retention method used by Itoh, measure net absorption, and thus they cannot evaluate the respective roles of mucosa permeability and of the calcium gradient between the lumen and the plasma. Several observations support a role for the latter. Hydrochloric acid secretion in the proventriculus has

been found to increase during shell formation (Mongin, 1976b), and this secretion could enhance the dissolution of dietary calcium carbonate, thus elevating the calcium concentration in the liquid phase of the gut content. Mongin (1976a) confirmed this hypothesis, demonstrating that the calcium gradient through the mucosa increases during egg-shell formation.

In order to determine whether mucosal permeability also varies, the upper jejunum (the main site of calcium absorption : Hurwitz et al., 1973) was perfused in vivo before and during egg-shell calcification to estimate the passive calcium permeability of the mucosa. To test the method used, pullets were also perfused just before the onset of egg production when mucosal CaBP concentration and Ca absorption were low as compared to those of the laying bird.

Material and methods.

Birds. — The experiments were conducted on individually caged midweight laying hens (Warren). Twelve to 14-month old animals were used in the first trial, and 8 to 10-month old ones in the second trial. They had free access to water and were fed a commercial diet ad libitum containing 3.5 p. 100 of Ca. The poultry house was air-conditioned (temp. 20 ± 1 °C, RH 60 ± 5 p. 100) and equipped with an automatic oviposition recording system. The lighting schedule was 16 hrs of light and 8 hrs of darkness. In the first trial, one group of 6 birds was perfused just after oviposition when no shell calcification was in progress, and a second group of 6 birds was used 14 to 30 hrs later during egg calcification. In a second trial (8 birds), the perfusion started 3 to 5 hrs before the onset of calcification and lasted 7 to 8 hrs in order to detect any modification, in a given bird, resulting from the start of calcification. Four immature pullets was also used just before the onset of egg production.

Experimental procedure. — The birds were prepared for perfusion under Nembutal (trial 1) or Halothane (trial 2) anesthesea. The abdominal cavity was opened by lateral incision, and the upper jejunum tied off and cannulated 2-3 cm distal to the biliary duct and 20 cm before the diverticule of Meckel ; the length thus perfused was 21.4 ± 4.9 cm for trial 1, and 27 ± 5.2 cm for trial 2. The abdominal cavity was closed by suturing the muscle and skin separately. A wash period with warm physiological serum was followed by a 45 to 60-min equilibration period permitting a return to steady state. The jejunal segment was perfused by a peristaltic pump at a constant rate of 0.6 ml $\times$ min^{-1}. The effluent fluid was collected every 30 min by gravity drainage. The basic perfusion liquid used in trial 1 contained (mM $\times$ l^{-1}) : KH_2PO_4 : 2 ; $MgSO_4, 7H_2O$: 2 ; KCl : 40 ; NaCl : 0 ; $CaCl_2$: 4-12.5 or 20 (2 birds per concentration and physiological stage). Each bird was perfused with three solutions having the same solute concentration but different osmolarity adjusted to 200, 350 or 500 mosm by adding raffinose.

In the second experiment, the perfusion solution was as follows (mM $\times$ l^{-1}) : KH_2PO_4 : 2 ; $MgSO_4, 7H_2O$: 2 ; KCl : 23 ; NaCl : 30 ; $CaCl_2$: 12.5 or 20 (4 birds per concentration). The osmotic pressure was maintained constant at 350 mosm $\times$ l^{-1}.

Radioactive polyethylene glycol (PEG 4000) was used as volume marker (10 μCi and 3.3 g cold PEG l^{-1}) (Bindler and Skadhauge, 1971).

Chemical assays. — All chemical analyses were done in duplicate on both initial perfusion fluids and final effluent perfusates. Sodium and potassium were determined by flame photometry (Radiometer FLM 2) and chloride by chloridometry (Radiometer CMT 10). Calcium was determined by EGTA titration under ultraviolet light using calcein as an indicator. Osmotic pressure was measured in a Fiske osmometer. PEG ^{14}C was counted in a Nuclear Chicago liquid scintillation spectrophotometer. The scintillation fluid was toluene-triton X-100 (2 : 1) with PPO (4 g l^{-1}) and POPOP (0.05 g l^{-1}).

Calculation. — The following equations were used :

Net water absorption $Jv = Vin - Vout - \Delta V_i$

$$\Delta V_i = 2 \frac{\sum M_{i-1}}{PEGin + PEGout_{i-1}} - 2 \frac{\sum M_i}{PEGin + PEGout_i}$$

Net soluble flux $Js = Vin\, Sin - Vout\, Sout - \Delta V_i \left(\dfrac{Sin + Sout}{2}\right)$.

where Vin and Vout are, respectively, the volumes of solution entering and leaving the perfused organ over a given time period, ΔV_i is the variation of the dead space volume, PEGin and PEGout the PEG ^{14}C concentration in the solution flowing in and out, $\sum M_i$ the total PEG ^{14}C cumulated in the dead space from the beginning of the study, and Sin and Sout the solute concentrations. A positive net flux (absorption) denoted a net movement from the mucosal to the serosal compartments and vice versa. The data for Jv and Js were expressed in terms of ml × hr^{-1} and μeq × hr^{-1} per gram of dry mass perfused jejunum ; for example, 1 g of DM jejunum was equivalent to about 12.3 cm and 3.7 g of wet weight tissue.

Results.

As shown in a comparison of the means in table 1, egg-shell calcification did not change the net fluxes of water and minerals. It could be argued that variability bet-

TABLE 1

Experiment 1. — Effect of egg-shell formation on net water and solute fluxes through the upper jejunum

Physiological stage	Water (ml h^{-1} g DM^{-1})	Ca^{++}	K^{+}	Na^{+}	Cl^{-}
			(μeq h^{-1} g DM^{-1})		
Without egg-shell formation (6)	-2.35 ± 0.62 [a]	104 ± 12	191 ± 15	-816 ± 55	-540 ± 50
With egg-shell formation (6)	-1.71 ± 0.56	85 ± 11	169 ± 14	-760 ± 33	-465 ± 46
F (1.6)	0.24	1.71	0.34	0.25	0.56

[a] Mean ± SEM, a positive net flux denotes a net flux from the muscosal to the serosal compartments.

( ) Number of birds. Different groups of birds were used in each trial.

ween birds might hide the process we were studying, but as seen in table 2, water and solute fluxes were clearly unmodified by egg-shell mineral deposition when measured in the same bird a few hours before and after the onset of calcification.

TABLE 2

Experiment 2. — Net water and solute fluxes through the upper jejunum measured on the same bird just before and after the beginning of shell calcification

Physiological stage	Water (ml h^{-1} g DM^{-1})	Ca^{++}	K$^+$	Na$^+$	Cl$^-$
			(μeq h^{-1} g DM^{-1})		
Before egg-shell deposi- tion (8)	— 2.32 ± 0.24 [a]	144.5 ± 13	143 ± 10	— 555 ± 31	— 207 ± 34
During egg-shell depo- sition (8)	— 2.28 ± 0.52	144 ± 11	152 ± 7	— 556 ± 74	— 215 ± 87
F (1.6)	0.003	0.08	2.5	0	0

[a] See legend table 1.

We checked the immaturity of the pullets before the beginning of laying by their total plasma calcium content (120 mg × l^{-1}). Under these conditions, and whatever the calcium concentration in the lumen, calcium absorption was always reduced in immature birds (table 3).

TABLE 3

Net flux of calcium through the upper jejunum in laying and immature birds

Calcium concentration in the perfused solution	Laying bird		Immature bird
	First trial	Second trial	
25 meq l^{-1}	89 ± 10 [a]	98 ± 10 (4)	10 ± 3.3 ** (3)
40 meq l^{-1}	150 ± 22	191 ± 11	57 ± 4.8 **

[a] Mean + SEM in μeq × h^{-1} g^{-1} of DM.
( ) Number of animals.
** Significantly different from laying bird (P < 0.01).

In one bird perfused a few days before ovulation of the first egg (as judged by the maturity of the ovary and its plasma calcium), the calcium flux was 133 ± 8 μeq × h^{-1} × g^{-1} DM for a concentration in the perfused solution equal to 40 meq × l^{-1}.

Discussion.

Kenny (1976) studying the Japanese quail, suggested that the increased calcium absorption occurring during egg formation could be due to enhanced production of

1-25 dihydroxycholecalciferol (1-25 DHCC). In fact, he observed a peak of 25-hydroxy-cholocalciferol-1 hydroxylase activity in kidney homogenates at the time of ovulation and a reduction in activity when no egg was present in the oviduct.

More recent results have indicated that the temporal pattern of this enzymatic activity and that of calcium absorption are not in phase. A reduction of 1-hydroxylase activity is only observed 20 hrs after the oviposition of the last egg of the clutch, while already at 2 hrs after oviposition calcium absorption is one-third of that prevailing during shell calcification (Montecuccoli et al., 1977). Furthermore, there is ninefold more 1-25 DHCC in the laying hen than in the non-laying control (Spanos et al., 1976), but in birds having a pause of one day only, 1-25 DHCC remains the main vitamin D metabolite, whereas 24-25-DHCC prevails in the non-laying hen (Sedrani and Taylor, 1977).

Finally, the lack of variation of CaBP concentration in the duodenum and jejunum during egg-shell formation (Bar and Hurwitz, 1975) is also consistent with the idea that vitamin D is not involved in cycle calcium absorption during shell calcification.

The present results show that when calcium concentration is imposed in the intestinal lumen, a diurnal change in net calcium absorption is not observed. It can therefore be concluded that an enhanced gut capacity for calcium absorption at the time of shell mineralization is not the cause of the increased calcium absorption, and hence that the idea of a modification in the passive Ca permeability of the jejunal mucosa must be discarded.

An alternative way to explain these changes in net calcium absorption is by variations in the driving forces involved. Calcium concentration gradient is one such force, as shown in table 3, and apparently a main one (Nys and Mongin, unpublished data). In fact, modifications in the composition of the liquid content of the upper intestine have been observed previously (Mongin, 1976a, b). The mean calcium concentration in the duodenum and upper jejunum ranges between 10 to 15 meq/l without egg-shell deposition, and increases generally up to 50-60 meq/l during egg-shell formation (Mongin, unpublished data).

Furthermore, if the ability of the laying bird to ingest more calcium just before egg calcification is considered (Mongin and Sauveur, 1974) together with the alteration of digestive tract motility (Roche and Decerpit, 1977), such an hypothesis seems more plausible.

Recent observations of Bar et al. (1976) are consistent with our data. They reported that Japanese quails fed synthetic 1α-hydroxycholecalciferol (1α-HCC) absorbed calcium better than those fed a vitamin D3 diet. The changes during the laying cycle were not only maintained, but amplified, by 1α-HCC. This enhanced daily variation in net absorption was the result of long-term improvement of the calcium absorption capacity induced by 1α-HCC. However, this mechanism was fully operative only when there was a sufficient amount of solubilized calcium. Here, it is worthwhile to note that not only calcium, but potassium, absorption shows daily variations (Taylor and Kirley, 1967 ; Nys, 1979).

It is concluded that the hourly regulation of calcium absorption in laying hens is governed by the action of several factors in the upper digestive tract modifying the

solubility of calcium carbonate. This does not exclude a possible modification of endo-genous Ca secretion, as proposed by Wasserman and Combs (1978), but it would take place in the segments distal to the upper jejunum.

Résumé. Nous avons mesuré sur des poulettes immatures et sur des poules pondeuses, juste avant et pendant la calcification de l'œuf, la perméabilité de la muqueuse jejunale à l'eau, au calcium, potassium, sodium et chlore par une méthode de perfusion *in vivo*. La perméabilité jejunale, notamment calcique, ne varie pas au cours du cycle de formation de l'œuf. L'augmentation de l'absorption nette de Ca observée pendant la calcification de l'œuf ne semble donc pas due à une modification de la capacité de transport de la muqueuse intestinale. Elle résulterait plutôt d'une meilleure solubilisation du carbonate de Ca dans l'intestin proximal. Par contre, cette capacité de transfert chez la poule mature est plus importante que celle de la poulette immature.

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