PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 286–287 (1978)

Relation of Vitamin D-Dependent Intestinal Calcium-Binding Protein to Calcium Absorption during the Ovulatory Cycle in Japanese Quail (40333)

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The concentration of the vitamin D-dependent calcium-binding protein (CaBP) in the small intestine correlates with the degree of vitamin D-mediated intestinal absorption of calcium with few exceptions (1). One presumed exception was from studies with the laying hen and laying Japanese quail. In both of these species, it was noted that the absorption of calcium was greater during the period when the eggshell was undergoing calcification than when no eggshell was being formed; however, the amount of CaBP in the intestinal mucosa did not change correspondingly (2–4). It was proposed by Bar, Hurwitz, and colleagues (2–4) that there exists in the laying bird a rapidly modulating calcium transport mechanism not associated with CaBP.

In evaluating the relation between CaBP concentrations and calcium absorption, critical consideration must be given to the method by which calcium absorption is measured. In the Bar–Hurwitz experiments, use was made of a nonabsorbable indicator method that measures *net* calcium absorption. Distinct from this are procedures that measure the unidirectional movement of calcium from intestine to blood using a radiotracer of calcium. The latter gives an estimate of the *efficiency* of the calcium absorptive mechanisms, which better correlates with CaBP concentrations than would net calcium absorption.

The present experiment was undertaken to determine if there is, in fact, a change in the efficiency of calcium absorption during the egg-laying cycle in Japanese quail. The results indicate no significant difference in calcium translocation across the intestine as a function of eggshell formation.

Methods. Japanese quail in the egg-laying stage were individually housed and periodicity of oviposition was recorded for each bird. Calcium absorption was measured in quail forming an eggshell (12–17 hr after oviposition verified by intrauterine presence of an egg) and in quail not forming an eggshell (1–2 hr after oviposition). For the measurement of the absorption of calcium, quail were anesthetized with ether, a laparotomy was performed, and a 0.5-ml dose of ^{47}Ca (1 m*M* $CaCl_2$, 150 m*M* NaCl, pH 7.4, 0.1 μCi ^{47}Ca) was injected into the lumen of the ligated loop of duodenum. The loop was replaced into the peritoneal cavity and the incision was closed with wound clips. After 15 min, the quail were bled by heart puncture and then they were killed with an overdose of nembutal. The duodenal loop was excised and counted immediately for residual ^{47}Ca activity using a gamma scintillation detector with a single-channel analyzer set to eliminate any contribution from the ^{47}Sc daughter. After the gut loop was counted, the residual contents in the lumen were removed by rinsing, the loop was cut open and scraped, and the concentration of CaBP in the intestinal mucosa was determined by a radial immunoassay, as previously described (5). The tibiae were also excised and counted for ^{47}Ca.

The calcium content of the plasma was determined by atomic absorption spectrometry, and plasma phosphorus by the Fiske–Subbarow method (6).

Calcium absorption is expressed as a percentage of administered dose, and CaBP as micrograms per milligram of total soluble protein. Protein was determined by the Lowry procedure (7).

Results. The data in Table I indicate that there were no significant differences ($P > 0.05$) in any of the measured parameters between those Japanese quails in which eggshells were being calcified and in those quail in the noncalcifying stage. The only exception was body weight ($P < 0.025$) which undoubtedly reflects the presence or absence of the forming egg in the body cavity.

Discussion. The present finding that the

0037-9727/78/1592-0286$01.00/0

TABLE I. RELATION OF INTESTINAL CaBP AND DUODENAL CALCIUM ABSORPTION TO THE EGG-LAYING CYCLE OF JAPANESE QUAIL.[a,b]

Group	Body weight (g)	Duodenal absorption of ^{47}Ca (% dose)	^{47}Ca in tibia (% dose)	Intestinal CaBP (μg/mg of protein)	Plasma	
					Ca (mg/100 ml)	P_i (mg/100 ml)
Noncalcifying (9)	128 ± 3	52.1 ± 6.8	0.99 ± 0.08	32.8 ± 3.2	14.2 ± 1.4	5.2 ± 0.7
Calcifying (9)	139 ± 3	46.1 ± 3.0	1.00 ± 0.04	32.5 ± 2.3	17.4 ± 0.9	6.8 ± 0.8

[a] The values represent the means ± standard errors of the mean of nine birds per group.
[b] None of the values for any parameters were significantly different from one another at $p > 0.05$ except body weight ($p < 0.025$).

absorption of calcium does not change as a function of eggshell formation is in *apparent* disagreement with the information previously reported by Bar, Hurwitz, and colleagues (2–4). However, the disparity is more likely to be conceptual than real. The significant difference between the two studies is the manner by which calcium absorption was measured. As alluded to previously, the Bar–Hurwitz technique measures net calcium absorption and, by this procedure, the net absorption of calcium was observed to be greater in the quail during the period of eggshell formation than during the period when no eggshell was being formed. This is reasonable since the forming eggshell constitutes a significant calcium "sink" into which absorbed calcium is deposited and, thus, less calcium is available for return to the intestinal tract. When no eggshell is in the formative stage, more of the absorbed calcium can be endogenously secreted into the intestinal lumen, yielding a decrease in net calcium absorption.

By the procedure used in the present study, the measurement of calcium absorption is independent of the subsequent fate of the absorbed cation. Over the 15-min absorption period, the amount of absorbed ^{47}Ca that returns to the intestinal tract is negligible and, under these conditions, no difference in ^{47}Ca absorption was detected between the different phases of the egg-laying cycle.

The conclusion is offered that the efficiency of calcium absorption does not change between the calcifying stage and the noncalcifying stage of the egg in Japanese quail. This finding is consistent with the observation that the kidney 25-hydroxycholecalciferol-1-hydroxylase activity does not differ during shell formation and in the noncalcifying period, i.e., up to 4 hr after ovulation (4, 8). Thus, there appears to be a reasonable correlation between the intestinal transport of calcium, intestinal CaBP levels, and the activity of the kidney-1-hydroxylase enzyme system in this physiological state, and the proposal that the laying quail contains a rapidly modulating, non-CaBP-dependent, calcium-absorptive mechanism appears to be unwarranted.

Summary. Duodenal CaBP levels and the efficiency of ^{47}Ca absorption by the duodenal segment of ovulating Japanese quail were determined as a function of eggshell formation. No differences in these parameters were noted in quail in which eggs were being calcified and in quail with no egg in the calcification stage. Thus, there is a correlation between the efficiency of Ca absorption and the level of vitamin D-dependent intestinal calcium-binding protein in this physiological state in Japanese quail.

Technical assistance and advice by M. Brindak, Dr. C. S. Fullmer, F. Davis, Dr. R. A. Corradino, and N. Jayne are gratefully acknowledged. Supported in part by National Institutes of Health Grant AM-04652.

1. Wasserman, R. H., and Corradino, R. A., *in* "Vitamins and Hormones," Vol. 31, pp. 43–103. Academic Press, New York (1973).
2. Hurwitz, S., Bar, A., and Cohen, I., Amer. J. Physiol. **225,** 150 (1973).
3. Bar, A., Eisner, U., Montecuccoli, G., and Hurwitz, S., J. Nutr. **106,** 1336 (1976).
4. Montecuccoli, G., Hurwitz, S., Cohen, A., and Bar, A., Comp. Biochem. Biophys. **57A,** 335 (1977).
5. Corradino, R. A., J. Cell Biol. **58,** 64 (1973).
6. Fiske, G. H., and Subbarow, Y., J. Biol. Chem. **66,** 375 (1925).
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
8. Kenny, A. D., Amer. J. Physiol. **230,** 1609 (1976).

Received April 10, 1978. P.S.E.B.M. 1978, Vol. 159.