There was no significant difference, however, in the PRL surge between animals given 50 µg PEP and animals given 100 µg PEP ($p > 0.05$).

Discussion. Pituitaries extracted at pH 7.6, regardless of the buffer used, did not adequately reflect the differences in PRL concentration when varying doses of estrogen were administered. Extraction at pH 10.6 resulted in almost a 2-fold increases in PRL for OVX control AP over those extracted at pH 7.6 and now a clear elevation in PRL concentration was noted for animals injected with 50 or 100 µg PEP. The PBS buffer at pH 10.6 gave the greatest recovery of AP PRL of the buffers and pHs examined. The more efficient extraction of AP PRL by high pH buffers has been reported before and was attributed to the disruption of PRL storage granules thus making PRL more available for binding to the antibody[10].

The PRL concentration of AP extracted at pH 10.6 more closely corresponds to the magnitude and extent of the estrogen-induced plasma PRL afternoon surge than does extraction at pH 7.6. However, even at pH 10.6 the PRL surge observed in the plasma by the administration of 25 µg PEP was not reflected in AP PRL concentration. Thus, AP PRL concentration, even under optimum extraction conditions, does not reflect the plasma PRL surge level when the estrogen level is low. At higher levels of estrogen, however, AP PRL concentration appears to reflect the afternoon surge level.

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Changes of duodenal spermine-binding activity caused by vitamin D deficiency

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Summary. Duodenal spermine-binding activity declines to a very low level during the development of chicks fed with a rachitogenic diet. A single injection of 1,25-dihydroxycholecalciferol is able to restore the activity shown by chicks fed with a normal diet.

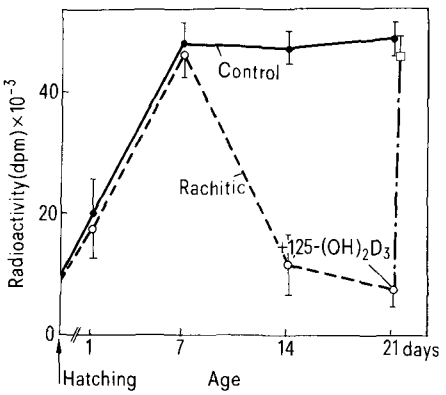
A cytosol protein binding specifically and with high affinity to spermine has been identified in the chick duodenal mucosa[1]. This protein has a molecular weight of about 32,000 Daltons and from competition studies, it appears that it is not a calcium binding protein[2]. Its binding activity is very rapidly induced in rachitic chicks intestine by a single intracardial dose of 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D_3$)[3], generally considered the active form of vitamin D.

Although some possible involvements of the binding protein in some vital processes of the duodenal cell have been speculated[4], until now, the physiological role of the protein remains obscure. Therefore, to contribute to the understanding of the biological function of the protein, it is necessary to define precisely the apparent induction of its binding activity by the active form of vitamin D. In the present work we report the behavior of the binding protein during the post-natal period in chicks fed from hatching with a rachitogenic diet, and the capability of the hormonal form of vitamin D to restore the normal spermine binding activity when injected in rachitic animals.

Materials and methods. Fertilized White Leghorn eggs were incubated in a humidified egg incubator at 38 °C until hatching. The new-born chicks were divided into 2 distinct groups and raised for 3 weeks with a rachitogenic diet[5] and with a standard laboratory diet, respectively. Vitamin D-dosed chicks received 150 ng of $1,25\text{-}(OH)_2D_3$ (Roche, Milano)[6] intracardially in 0.1 ml of ethanol/propylene glycol (1:9 v/v) 5 h before being killed. Further procedures were carried out at 0–2 °C. The duodenum was removed, rinsed in 40 mM Tris HCl, pH 7.5 and the mucosal layer separated from the muscle. After homogenization in 2 vol. of the same buffer, the cytosol fraction was prepared by centrifugation at 105,000 × g for 1 h. The cytosol fraction (300 mg) was then chromatographed on a DEAE cellulose (Whatman DE-52) column (1.5 × 20 cm) as described previously[3]. The fraction eluted between 0.2 M and 0.3 M KCl was used as source of purified spermine-binding protein. The freshly fractionated protein (150 µg) was incubated with 0.2 µCi of 15 µM [^{3}H] spermine (NEN 44.3 Ci/mmole) in 0.35 ml of 40 mM glycine, pH 8.7, at 0 °C for 10 min. The spermine binding activity was then analyzed by gel filtration on Sephadex G-25 as described previously[1]. Protein concentration was measured by the method of Warburg and Christian[7].

Results and discussion. As reported in the figure, spermine-binding activity from chicks raised on a normal diet showed a remarkable increase during the 1st week of life, reaching a plateau at day 7. The plateau value was constant up to the 3rd week of life and it was about 4 time higher than at hatching. The binding activity extracted from chicks raised since hatching on a vitamin D-free diet showed a parallel stimulation over the 1st week of life, although it sharply declined, reaching thereafter at day 21 a value even lower

than that found at hatching. The fact that the 2 groups raised either on normal or on rachitogenic diets showed about the same qualitative and quantitative behavior during the 1st week is probably due to the presence in both groups of animals of endogenous $1,25(OH)_2D_3$ sufficient to determine the response observed. In fact, it has been reported that chicks raised on the same rachitogenic diet become vitamin D deficient after 3 weeks of life when endogenous active metabolites of vitamin D_3 are exhausted[8]. In the experiments reported, 3 weeks old chicks showed only 15% residual spermine-binding activity of their corresponding controls. However, in several experiments, the values varied from 11 to 20%, the control values being almost constant in all experiments. The figure also reports the effect of administration of $1,25\text{-}(OH)_2D_3$ to the 3-week-old vitamin D-deficient chicks on duodenal spermine-binding activity. 5 h after a single intracardial injection of the active form of vitamin D, the spermine-binding activity increased by 560% above the values obtained from the rachitic chicks reaching 92% of the control animals.



Duodenal spermine-binding activity during normal and rachitic development. Groups of 8 chicks fed with normal and vitamin D-free diet were killed at the age indicated. Duodenal binding activity of each group purified from the pooled tissues was estimated as described in the Methods section. The data reported are expressed as dpm/150 µg of fractionated cytosol protein. Results are means ± SEM for 3–5 separate experiments.

The almost complete recovery in binding activity shown was a constant and very reproducible phenomenon in all groups of animals tested; however different experiments showed values ranging from 85 to 100%.

Although no evidence is available at the moment supporting a specific physiological role of the binding protein, it is noteworthy that this protein strikingly responds to the $1,25(OH)_2D_3$ status of the animal by modifying its spermine-binding activity as a consequence. This finding strongly suggests that the increase of spermine-binding activity at hatching is hormonally-regulated and reflects changes in vitamin D metabolism and/or action. Further studies on the effects of vitamin D analogs and metabolites on the binding activity from duodenum and from other vitamin D sensitive tissues will help to establish the physiological relevance of this protein.

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Brain dopamine variations in gonadotropin-treated immature rat[1]

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Summary. The cortex, cerebellum, caudate nucleus, and hypothalamus of immature rats treated with pregnant mare's serum gonadotropin (PMS) were isolated and analyzed for dopamine (DA) content at 6-h intervals for 72 h. Results showed that PMS injection caused significant elevation in DA levels in all brain regions studied.

There is substantial evidence that central catecholamines, specifically dopamine (DA), mediate the hypothalamic mechanisms governing the release of pituitary LH and FSH[3]. In the hypothalamus, steroid hormones influence DA content[4]. It has also been shown that DA concentration[5,6] and the rate at which it is synthesized[7] change physiologically during the course of estrus cycle in female rats and mice. Meanwhile, in immature PMS-treated rats, alteration in DA metabolism will prevent ovulation[8,9] while inhibition of the conversion of DA to norepinephrine has no effect[10].

Although many attempts have been made to correlate dopamine with the ovarian activity in mature animals[11,12] few, if any, investigators have observed actual levels of the compound during the period leading up to ovulation. It was of interest, therefore, to examine DA levels in the brain at different intervals during the initial ovulatory period in the immature rats.

Materials and methods. Animals. 36 female rats (21 days of age) of the Sprague-Dawley strain (Southern Animal Farm, Prattville, Alabama), weighing 50–70 g each were housed in groups of 4 per cage and maintained under controlled lighting (from 09.00 to 21.00 h daily) and temperature (23 ± 1 °C) till the time of sacrificing. The animals were provided with standard Purina Lab Chow and water ad libitum.

Induction of ovulation. 25 IU pregnant mare serum gonadotropin (PMS, Sigma Chemical Co.) were injected s.c. in a saline solution on day 25. Simultaneously, control animals were injected with an equal volume of saline. The occur-