adult rats[17]. The trypsin concentration (0.01%) sufficient to prepare islet cells from newborn rats as well as adult rats[18] was not suitable for preparing pancreatic islet cell suspensions from C57BL/6J mice (results not shown). The choice of a particular trypsin concentration and the digestion period which permits the isolation of islet cells in a high yield but with a small degree of damage of the cells depends on the species and the age of the animals used.

Lernmark[10] and Niki et al.[11] have already shown that pancreatic islet cell suspensions of adult rats prepared by mechanical disintegration respond normally to glucose stimulation with an increased (pro)insulin biosynthesis. Therefore it was of interest to investigate whether the trypsin treatment of the pancreatic islets of newborn rats has an influence on the ability of the isolated cells to synthesize (pro)insulin.

We found that cells isolated by trypsin treatment of islets of Langerhans of newborn rats also responded with a significantly increased (pro)insulin biosynthesis to glucose stimulation (fig.). In a series of experiments with different cell numbers per tube, we observed a 4–8-fold stimulation of (pro)insulin biosynthesis when glucose was increased from 1.5 mmoles/l to 15 mmoles/l. A number of 1×10^4 cells per tube was already sufficient to detect the glucose stimulation of (pro)insulin biosynthesis (data not shown). These results clearly demonstrate that the glucose recognition mechanism as a necessary prerequisite for the stimulation of (pro)insulin biosynthesis is not altered by the enzymatic treatment of the cells during the preparation procedure. A portion of $19.8\% \pm 1.58$ ($n=7$) of the total protein synthesized in a 2 h incubation at a glucose concentration of 15 mmoles/l was determined to be (pro)insulin. In comparison the proportion of (pro)insulin synthesized by intact islets of Langerhans of newborn rats amounted to only $11.6\% \pm 0.60$ ($n=9$) of the total protein. This suggests a certain enrichment of β-cells in the islet cell suspension, possibly due to a removal of non-insulin producing cells during the preparation procedure.

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Effect of estrogen administration on the induction of the plasma prolactin afternoon surge and on anterior pituitary prolactin concentration extracted at different pHs[1]

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Summary. Ovariectomized (OVX) rats were injected with various doses of polyestradiol phosphate (PEP); the anterior pituitary (AP) prolactin (PRL) concentration and the plasma afternoon surge of PRL were observed 1 week later by radioimmunoassay. AP PRL was extracted using carbonate and phosphate buffers at either pH 7.6 or 10.6. The AP concentration of PRL was greater when the AP was extracted with buffers at pH 10.6 and the phosphate buffer was the most efficient. The concentration of PRL in the AP more closely reflected the magnitude of the estrogen-induced afternoon surge when the AP was extracted at pH 10.6 and this was especially so when the higher levels of estrogen were administered.

Before the advent of RIA the effect of estrogen on prolactin (PRL) secretion could only be determined by measuring the anterior pituitary (AP) content[4–6]. Once specific RIAs were firmly established to measure plasma PRL levels, the AP was usually overlooked in examining the influence of estrogen on prolactin secretion[7,8]. In some studies where AP and plasma PRL were measured, the correlation between the two was not always good[9]. Part of the reason for this poor correlation may have been due to the method of extracting PRL from the AP for RIA[10,11], while another part may have been due to making comparisons to only basal plasma levels[9]. In this study we examined the influence of various doses of polyestradiol phosphate (PEP), a long acting estradiol, on the induction of the afternoon plasma PRL surge and compared this response with the AP PRL concentration extracted with different buffers at various pHs. It was the intent of this study to put into proper perspective the relationship between AP PRL concentration and plasma levels when stimulated by estrogen.

Influence of polyestradiol phosphate (PEP) on body and anterior pituitary (AP) weights and on plasma prolactin (PRL) levels 1 week after injection

Experimental group	No. animals	Body weight (g)	AP weight (mg/100 g b.wt)	Serum PRL (ng/ml)
Control	4	203 ± 3	3.78 ± 0.26	12.4 ± 2.8
25 µg PEP	5	207 ± 3	3.90 ± 0.30	7.0 ± 1.7
50 µg PEP	5	207 ± 3	4.11 ± 0.09	7.8 ± 1.5
100 µg PEP	4	206 ± 3	4.55 ± 0.32	10.7 ± 1.8

± SEM.

Materials and methods. Sexually mature female Sprague-Dawley rats were obtained from Spartan Research Animals, Inc., Haslett, MI and housed 2/cage in a temperature (24 °C) and light (L14:D10; lights on 06.00-20.00 h) controlled room. All animals were ovariectomized (OVX) 2-3 days after arriving at the laboratory. In the 1st experiment animals were fitted with an atrial catheter at the time of OVX and given a single s.c. injection of either 25, 50 or 100 µg of polyestradiol phosphate (PEP). One week later the animals were attached to a catheter extention at 09.00 h and blood samples (0.4 ml) were obtained at 11.00, 13.00, 15.00, 17.00, 19.00 and 21.00 h. The fluid removed each time was replaced with an equal volume of warm (37 °C), sterile saline[12]. The plasma recovered was frozen and stored at 20 °C until assayed for PRL at 2 dilutions in duplicate using a specific rat PRL RIA[13]. NIAMDD RPI_5 and NIAMDD RP-1 were used for iodination and standard curve, respectively.

In the 2nd experiment a group of OVX animals were given a single injection of either 25, 50 or 100 µg PEP and sacrificed 1 week later by decapitation between 11.00 and 12.00 h. The serum recovered was frozen for PRL RIA. The AP was weighed and divided into 4 equal sections. A section from each pituitary was placed into one of the following buffers: 0.05 M $Na_2CO_3 - NaHCO_3$-0.1 M NaCl-1% bovine serum albumin, (CBS) pH 7.6; 0.01 M $NaH_2PO_4 - Na_2HPO_4$-0.14 M NaCl-1% bovine serum albumin, (PBS) pH 7.6; CBS, pH 10.6; and PBS, pH 10.6. The pH of the buffers was adjusted to the appropriate level using either 5 N NaOH or 2 N HCl. The AP were homogenized, centrifuged and the supernatants were stored frozen until assayed for PRL.

Differences in plasma PRL for the afternoon surge were analyzed statistically using a 2-way analysis of variance. Anterior pituitary and body weights, basal serum PRL levels and AP PRL concentrations were analyzed using the analysis of variance, and when a statistical difference was observed the means were analyzed using Duncan's New Multiple range test. Analyses of PRL concentration for AP homogenized at the same pH but in a different buffer system were performed by the paired t-test. A statistical difference of $p < 0.05$ was considered significant.

Results. There was no significant difference ($p > 0.05$) in either body weight, AP weight, or basal serum PRL levels for animals injected with varying doses of PEP when compared to that of controls (table). The AP PRL concentration of animals injected with estrogen was not significantly different ($p > 0.05$) from that of control AP when the AP were extracted at pH 7.6 except for those AP obtained from 100 µg PEP injected animals extracted with PBS buffer ($p < 0.05$). Pituitaries extracted at pH 10.6 had a significantly higher PRL concentration when compared with the same buffers at pH 7.6 ($p < 0.05$ or 0.01; see fig. 1). In addition, when AP were obtained from animals injected with either 50 or 100 µg PEP and extracted with buffers at pH 10.6, there was a significantly greater ($p < 0.05$) PRL concentration when compared to OVX control AP (fig. 1). Also the pituitary PRL concentration from animals given 100 µg PEP and extracted with PBS buffer at either pH 7.6 or 10.6 was significantly greater ($p < 0.01$, paired t-test) than that observed for CBS buffer at the same, respective pH (fig. 1). The afternoon PRL surge of animals injected with either 50 or 100 µg of PEP was significantly greater ($p < 0.01$) than that of animals given 25 µg PEP (fig. 2).

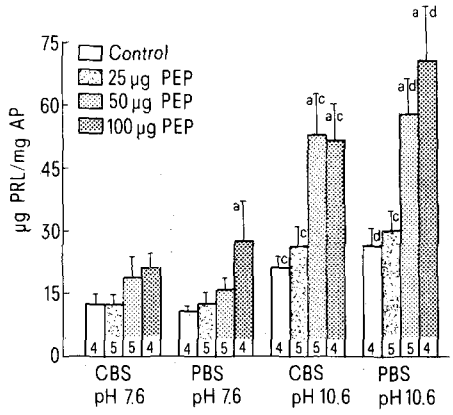

Figure 1. Anterior pituitary prolactin (PRL) concentration for animals given a single s.c. injection of polyestradiol phosphate (PEP) 1 week prior to sacrifice. For composition of CBS and PBS buffers see text. Numbers in bars are number of animals per group. Vertical lines above bars are the SEM. Letters to the left of the SE represent statistical comparisons to control values; a, $p < 0.05$. Letters to the right of SE represent statistical comparison of buffer systems at different pH; c, $p < 0.05$; d, $p < 0.01$.

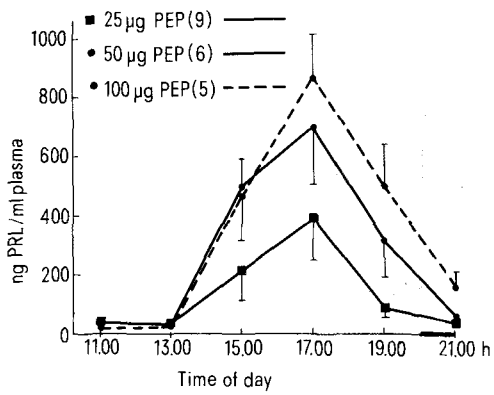

Figure 2. Plasma prolactin (PRL) levels for animals given a single s.c. injection of polyestradiol phosphate (PEP) and sampled 1 week later. Numbers in parentheses represent number of animals per group. Vertical lines associated with points represent the SEM. The dark bar on the horizontal axis represents the dark period of the light-dark cycle.

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