ecdysone and ecdysterone in the larval hemolymph[20]. 3 days after molting ecdysone levels should be low and the cellular activity observed could actually represent remnants of the moulting cycle with its events of cuticular formation.
Pupae in diapause had a higher ecdysone than ecdysterone level 2 days after their formation while 10-day-old pupae had a detectable ecdysone level under ISP and a significant level of ecdysterone under UVSP. This seems to correlate well with the level of activity of PTG cells and oenocytes respectively and raises a question as to the particular significance of higher UV levels for insects.
Under diapause-preventing conditions (ILP and UVLP) ecdysterone levels were consistently higher at 10 days, again highly correlated with secretory activity levels of oenocytes. It is to be noted however that adult, mature oenocytes were also present at this stage.
The results presented here underline 1) the great similarity in structure and activity of PTG cells and oenocytes; 2) the particular influence of photoperiod on the activity of the PTG and oenocytes, a result which requires further probing to grasp its significance in our understanding of the hormonal implications of diapause, particularly with respect to ecdysone and ecdysterone levels; and 3) the particular effect of UV light on insect developmental processes.

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Development of antral gastrin-like immunoreactivity and pituitary CCK8/gastrin-like immunoreactivity in rats

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Summary. Gel filtration of antral extract of adult rats revealed gastrin-17 and gastrin-34. Gel filtration of anterior pituitary extract showed CCK8 and gastrin-17, whereas posterior pituitary extract showed only a CCK8. Antral gastrin-like immunoreactivity (G-LI) increased after milk feeding and especially after weaning was started. Changes in diet may exert a profound influence on the ontogenic development of antral G-LI, but not pituitary CCK8/G-LI.
Key words. Gastrin-like immunoreactivity; CCK8/gastrin-like immunoreactivity; antrum; pituitary; rat.

Antral gastrin levels are low at birth and increase markedly after weaning[1,2]. Recently, there have been reports that gastrin/cholecystokinin (CCK) peptides are present in the pituitary gland, but there are no reports describing the ontogenic profile of these pepides in the pituitary gland[3–5]. The present study was undertaken to evaluate the ontogenic development of gastrin-like immunoreactivity (G-LI) in the antrum and CCK8/gastrin-like immunoreactivity (CCK8/G-LI) in the pituitary gland of rats and the relationship of these developmental changes to changes in diet and age.
Materials and methods. Pregnant Wistar stain rats were decapitated at 20 days of gestation (2 days before birth); the fetuses were removed by hysterotomy and decapitated. Other rats were decapitated immediately after birth and before suckling (day 0), at 2, 5, 10, 15 20 and 25 days postnatally and at 12 weeks of age (250 g adults). 40 rats were examined at 20 days of gestation, 28 rats each on days 0 and 2, 16 rats each on days 5 and 10, eight rats each on days 15 and 20, and four each on the other days. Gastric antrums and pituitary glands were dissected just after decapitation.
Pituitary glands were not separated into anterior and posterior lobes in the ontogenic developmental study.
Samples of both antrums and pituitary glands were pooled from 10 rats at 20 days of gestation, from seven rats each on day 0 and day 2, from four rats each on day 5 and day 10, and from two 15- and two 20-day-old rats for G-LI determination. Each pooled sample was considered as $n = 1$ for statistical purposes. No pooling was required for the other ages.
Extraction and radioimmunoassay. The frozen antrums, and pituitary glands were boiled in distilled water for 20 min. The tissues were then homogenized in a glass homogenizer and centrifuged at 1500 × g for 15 min at 4 °C. A small aliquot was removed for protein estimation, and the aqueous supernatant was lyophilized. The dry extracts for G-LI or CCK8/G-LI radioimmunoassay (RIA) were dissolved in 0.067 M phosphate buffered saline, pH 7.4, containing 0.1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) and centrifuged at 1500 × g for 15 min at 4 °C. The supernatants were assayed for G-LI or CCK8/G-LI.
Radioimmunoassay of tissue extracts was performed according to the method of Yalow and Berson[6]. Gastrin antiserum was obtained by immunizing rabbits with gastrin-17-I bovine serum albumin conjugate prepared by the carbodiimide method[7]. Gastrin was iodinated by the lactoperoxidase method[8], then pu-

rified by Sephadex G-10 gel chromatography (0.8 × 15 cm). The radioimmunoassay was performed in a total volume of 0.5 ml; the antiserum was diluted 1:50,000 with 0.25 ml of 0.067M phosphate buffered saline, pH 7.4, containing 0.1% bovine serum albumin, 0.1 ml of sample or standard, 0.1 ml of [^{125}I]iodogastrin (3000 cpm), and 0.05 ml of 0.1 M EDTA. The RIA tubes were incubated at 4 °C for 24 h.

Increasing amounts of standard (5–1000 pg gastrin-17-I) were assayed in an identical manner in 0.1 ml of assay buffer. After incubation, 'free' [^{125}I]iodogastrin was separated from antibody bound [^{125}I]iodogastrin with 20% polyethyleneglycol, and radioactivity was measured with a gamma counter.

The antiserum did not cross-react with secretin, insulin, glucagon, or substance P, but showed 50% cross-reactivity with CCK8 and 8% with caerulein. Intra- and interassay coefficients of variation were 8.5% and 15.8%, respectively, and the sensitivity of the assay was 5 pg/tube. All samples for G-LI or CCK8/G-LI were assayed in duplicate and together in the same assay.

Chromatographic procedure. Gel chromatography of antral extract of one adult rat and of pooled anterior and of pooled posterior pituitary extracts of 30 adult rats was performed at 4 °C on a Sephadex G-50 superfine column (1.0 × 90 cm). An 0.25-ml sample was applied to the column and eluted with phosphate buffered saline, pH 7.4, containing 0.1% bovine serum albumin and 0.1% sodium azide (Katayama Chemical Co., Osaka, Japan). The elution patterns of G-LI in antrum and pituitary extracts were compared with the elution of blue dextran (void volume, V_0) (Pharmacia Fine Chemicals, Uppsala, Sweden), human gastrin-17 (G17) and gastrin-34 (G34) of antral extracts of adult human, CCK8 (Protein Research Foundation, Osaka, Japan), and Na[^{125}I] (included volume, V_t) (Amersham Corp., Arlington Heights, IL). Fractions of 1.0 ml were collected and 0.2 ml of each fraction was assayed for its G-LI content. Protein was determined by the Lowry method[9].

Statistical analysis was performed by Student's t-test.

Results. Gel filtration of extract of antrum revealed gastrin-17 and gastrin-34 peaks. Anterior pituitary gel filtration showed CCK8 and gastrin-17 peaks, whereas posterior pituitary extract showed only a CCK8 peak (fig. 1).

Ontogenic development of antral G-LI and pituitary CCK8/G-LI is shown in figure 2. The mean concentration of antral G-LI in fetuses and just after birth was very low. After milk feeding, the G-LI concentration increased suddenly, and at 5 days of age it was 20 times higher than in the fetus or on day 0 ($p < 0.01$); thereafter mean values remained constant until 15 days of age, when weaning was started, after which antral G-LI again increased abruptly. At 25 days of age the mean level was three times higher than at 15 days ($p < 0.02$). Pituitary CCK8/G-LI gradually decreased from 2 days before birth to 2 days of age (the change was not significant). It increased from 2–5 days (but not significantly), and remained constant thereafter except at 20 days. There was a significant difference between the levels at day 2 as compared to the levels at day 20 ($p < 0.02$).

Discussion. Rehfeld reported that pig pituitary extract contains peptides which coeluted with gastrin but not chelecystokinin[5]. Beinfeld et al. observed that rat posterior pituitary contains CCK8 sulfate-like peptides but lacks gastrin, and porcine pituitary contains gastrin[3,4]. These authors also reported that the rat anterior lobe lacks CCK8, but did not investigate the pituitary gastrin content[3,4]. In our study, we observed that the rat posterior pituitary contained only CCK8, which is in accord with the observation of Beinfeld et al.; the anterior lobe contained both CCK8 and gastrin. Those findings indicate that specific differences exist in the CCK8/gastrin peptide and that the anterior and posterior pituitary may have different CCK8/G-LI patterns. The results of our ontogenic development study agree with previously reported observations that the onset of milk feeding and especially weaning play major roles in the development of antral gastrin concentration[1,2,10]. Lichtenberger et al. observed that the antral gastrin content of rats increased from birth to day 3 of life after milk feeding began, remained fairly constant during milk feeding alone, and then increased dramatically after weaning[1]. Prolongation of suckling did not delay the appearance of gastrin receptors or the increase in tissue gastrin but did prevent receptor and hormone levels from increasing at the same rate as in weaned rats[2]. Those observations suggested that both the appearence of gastrin receptors and the increase in antral gastrin levels are enhanced but not triggered by the shift from liquid to solid food[2].

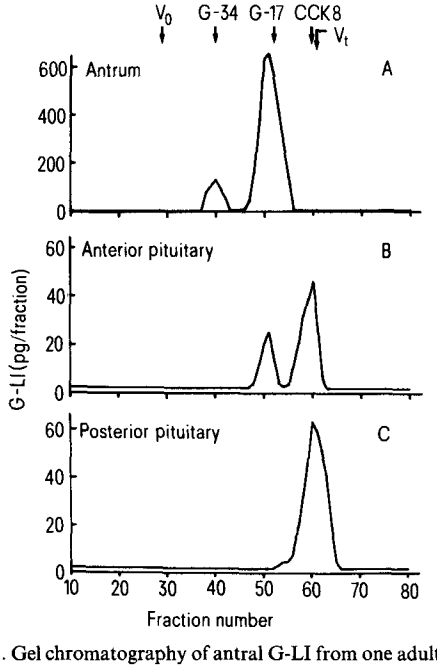

Figure 1. Gel chromatography of antral G-LI from one adult rat (A), and pooled anterior (B) and pooled posterior (C) pituitary gland G-LI from 30 adult rats.

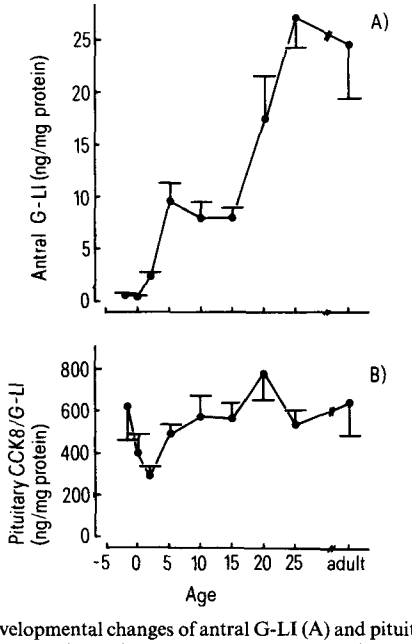

Figure 2. Developmental changes of antral G-LI (A) and pituitary CCK8/G-LI (B) concentrations of rats. Values are represented as mean ± SE of four determinations.

Other factors such as the pH in the stomach, certain gut hormones, mechanical distension of the stomach due to transition from liquid to solid food, autonomic nervous system activity, neurotransmitters, or corticosterone may also contribute to antral gastrin production[2, 11, 12].

In the ontogenic development study in the pituitary gland, during the first 3 or 4 weeks there may actually be a change in pituitary gastrin concentration masked by a similar change in CCK8 concentration in the opposite direction. Furthermore, there were no statistically significant difference between CCK8/G-LI levels except the levels between day 2 and day 20. However, the degree of variation seems to exclude statistical significance. In conclusion, G-LI was detected in the gastric antrum and CCK8/G-LI in the pituitary glands of rats. Antral G-LI increased after milk feeding and especially after weaning was started. Changes in diet may exert a profound influence on the ontogenic development of antral G-LI, but not pituitary CCK8/G-LI.

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Anticomplementary fraction from the poisonous secretion of the paratoid gland of the toad (*Bufo marinus paracnemis* Lutz)[1]

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Summary. Fractionation of the poisonous secretion of the toad *Bufo marinus paracnemis* Lutz, by dialysis and chromatography on QAE-Sephadex, led to the isolation of a fraction which was adsorbed to the ion exchanger. This fraction, when incubated with human serum, yielded an anticomplementary effect that was evaluated by measuring the kinetics of lytic activity on sensitized sheep red cells (classical pathway) and unsensitized rabbit cells (alternative pathway).
Key words. Anticomplementary activity; toad venom.

The study of substances having anticomplementary activity from the venom of several species of snakes has provided important evidence for the understanding of the complexity of the complement system and of its mobilization[2]. The property of interaction with complement has been studied comparatively in venoms from vertebrate and invertebrate animals using guinea pig serum complement[3]. By applying a kinetic method for the evaluation of anticomplementary activity, we observed that the poisonous secretion of the paratoid gland of *Bufo marinus paracnemis* Lutz interacts with the complement system. In the present paper we report the inhibitory action of this venom on the hemolytic activity mediated by the classic and alternative pathways of human serum complement.

Material and methods. Venom. Venom was obtained by applying pressure to the paratoid glands of male and female toads of the subspecies *Bufo marinus paracnemis* Lutz, collected in the region of Ribeirão Preto, Brazil, and stored frozen at −20 °C until the time for use.

Isolation of active material. 30 mg of crude venom protein was dialyzed against 0.01 M Tris-HCl buffer, pH 7.4 at 4 °C, with three changes over a period of 24 h. The dialyzed material was chromatographed on QAE-Sephadex A-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with the same buffer. The components adsorbed to the resin were eluted with a linear gradient of NaCl and monitored by recording absorbance at 280 nm with a LKB Uvicord. Experimental details are given in the legend to figure 1.

Protein measurement. Proteins were measured by the method of Lowry modified by Hartree[4], using bovine serum albumin (Calbiochem, La Jolla, USA) as reference.

Determination of anticomplementary activity. Classical pathway. The anticomplementary effect was evaluated by a kinetic method for determining the hemolytic activity of human serum on hemolysin-coated sheep red cells, as follows: 2.0 ml human serum containing 10 CH_{50} complement units diluted in borated

Relationship between the concentration of the active *B. marinus paracnemis* Lutz venom fraction and the inhibition of the lytic activity of the classical pathway of complement*

AVF (μg protein in the reaction medium)	$t_{½}$ (s)**	% inhibition
—	383	—
0.42	405	5
0.84	427	10
1.69	440	13
3.37	470	19
6.75	555	31
13.50	655	42
27.00	1065	64

* Human serum (2.0 ml) diluted to contain 10 CH_{50} units, was incubated for 1 h at 37 °C with 0.2 ml borated saline (control) or with 0.2 ml borated saline containing the amounts of AVF (fraction VI) shown above. The lytic reaction was started by addition of 0.4 ml of a 2.5% suspension of sensitized sheep red cells. ** Time needed for lysis of 50% of the red cells.