1 Edwards, C.R.W., Kitau, M.J., Chard, T., and Besser, G.M., Br. med. J. *3* (1973) 375.
2 Seif, M.S., Zenser, T.V., Chiarochi, F.F., Davis, B.B., and Robinson, A.G., J. clin. Endocr. Metab. *46* (1979) 381.
3 Nilsson, I.M., Mikaelsson, M., and Vilhardt, H., Scand. J. Haemat. *29* (1982) 70.
4 Ramsden, P.D., Hindmarsk, J.R., Price, D.A., Yeates, W.K., and Bowditch, J.D., Br. J. Urol. *54* (1982) 256.
5 Salacinski, P., Hope, J., McLean, C., Clement-Jones, V., Sykes, J., Price, J., and Lowry, P.J., J. Endocr. *81* (1979) P131.
6 Tausch, A., Stegner, H., Leake, R.D., Artman, H.G., and Risher, D.A., J. clin. Endocr. Metab. *57* (1983) 777.
7 Rooke, P., and Baylis, P.H., J. Immunoassay *3* (1982) 115.
8 Robinson, I.C.A.F., J. Immunoassay *1* (1980) 323.
9 Sofroniew, M.V., Madler, M., Müller, D.H., and Scriba, P.C., Fresenius Z. analyt. Chem. *290* (1978) 163.
10 Möhring, B., and Möhring, J., Life Sci. *17* (1975) 1307.

0014-4754/85/070933-03$1.50 + 0.20/0

Oenocyte and prothoracic gland activity in *Manduca sexta* under varying photoperiod and light conditions*

Luo Ma and B.J.R. Philogène[1]

Department of Biology, University of Ottawa, Ottawa (Ontario, Canada K1N 6N5), 5 July 1984

Summary. Manduca sexta larvae were subjected to diapause-inducing and diapause-preventing photoperiods, using two types of fluorescents (Indorsun and Blacklight-blue). The oenocytes, prothoracic glands (PTG) and ecdysone levels were examined in 3-day-old 5th instar larvae, 2-day-old and 10-day-old pupae. Our results indicate that oenocytes and PTG cells tend to be more active under long photoperiods while oenocytes only are active under short photoperiods in pupae in diapause. UV light has a definite effect on oenocytes while PTG cells seem to be unaffected. Ecdysone and ecdysterone levels vary with PTG and oenocyte activity at the pupal stage. The significance of these findings is discussed.
Key words. Manduca sexta larvae; photoperiod; oenocytes; prothoracic gland; ecdysone; diapause conditions.

The use of histochemical and biochemical techniques, and of electron microscopy has contributed extensively to our understanding of the structure and function of oenocytes. It is now known that these cells play a role in hormonal metabolism, converting ecdysone to ecdysterone and, possibly, synthesizing ecdysterone[2,3]. The participation of oenocytes in cuticle formation was demonstrated by ^{14}C incubation in vitro and in vivo[4-6]. In the course of insect development, the ultrastructure of oenocytes has been shown to undergo significant changes[7-14]. Little, if no attention, has been paid to the participation of oenocytes in the phenomenon of diapause. Because the latter is under hormonal control, it would seem reasonable to assume that oenocytes have a role in the induction, maintenance and/or prevention of diapause[15]. By studying the changes taking place in oenocytes under changing photoperiod and light regimes it might be possible to get a better understanding of how diapause is controlled.

In the present study, we present evidence that the oenocytes of *Manduca sexta* undergo changes under diapause-inducing and diapause-preventing conditions and that such changes are synchronous with variations in prothoracic gland activity and ecdysone levels.

Materials and methods. Larvae of the tobacco hornworm, *Manduca sexta,* were reared under diapause-inducing (6L:18D) and diapause-preventing (18L:6D) photoperiod conditions at 26 °C on a synthetic diet[16]. Indorsun fluorescent tubes (Verd-A-Ray Corp.) were used emitting 25 W/m^2 at 20 cm. Larvae were also subjected to UV light conditions provided by Westinghouse blacklight-blue fluorescent tubes (0.4 W/m^2 at 20 cm). There were therefore four rearing conditions: 1) Indorsun short photoperiod: ISP; 2) Indorsun long photoperiod: ILP; 3) UV short photoperiod: UVSP; 4) UV long photoperiod: UVLP.

On the 3rd day following ecdysis to the 5th instar, five larvae from each rearing condition were frozen rapidly, cut into small sections and fixed in Carnoy's. After paraffin embedding, the material was sectioned at 8 µm and stained with Feulgen's reagent, with 1% light green as counterstain. The same procedure was followed with 2-day- and 10-day-old pupae.

Measurement of ecdysone levels in 3-day-old 5th instar larvae, 2-day- and 10-day-old pupae was carried out by high-performance liquid chromatography (HPLC) using a Beckman model 332 gradient system, with an ODS 5 reverse phase column (4.5 × 250 mm). Ecdysone and ecdysterone standards were supplied by Sigma Chemicals. The hemolymph was collected in vials placed in an ice bath and processed according to the method of Lafont et al[17]. An acetonitrile buffer system and gradient solution (20:80) was used for HPLC analysis of the ecdysones, the detection limit of which is 20 ng.

Results. 1) Oenocytes. Larval oenocytes of *M. sexta* exhibited slight histological differences under diapause-inducing and diapause-preventing conditions. Under ILP conditions the DNA positive material of the nuclei was not homogeneous and a large nucleolus sometimes present. Under ISP conditions, nucleoli were seldom seen.

There were clear-cut differences between oenocytes of larvae reared under UVSP and those reared under ISP conditions (fig. 1). UVSP oenocytes had abundant, dark-stained cytoplasm. The nuclei were smaller in UVSP oenocytes than in their ISP counterparts. UVSP oenocytes looked active as opposed to the 'exhausted' appearance of the irregularly shaped ISP oenocytes. While the oenocytes of 2-day-old developing pupae (ILP) were essentially similar to their larvae counterparts pupae in diapause (ISP) were characterized by irregularly shaped oenocytes with less dense cytoplasmic material.

10-day-old developing pupae (ILP) had two distinct populations of oenocytes (fig. 2): 1) the fading larval oenocytes and 2) the newly formed imaginal oenocytes.

2) Prothoracic glands. There were remarkable differences between larval PTG cells under ILP and ISP conditions (fig. 3). ILP cells were characterized by a smooth surface though they could be irregularly shaped. The nucleus had homogeneously distributed DNA, and prominent nucleoli.

Under ISP conditions PTG cells tended to be vacuolated. DNA positive material was unevenly distributed in the nuclei. Nucleoli were not visible.

There seemed to be no striking difference between PTG cells of larvae reared under UV light and under Indorsun conditions. They all had lightly staining cytoplasm, the peripheral striation of ISP cells being more pronounced. Nucleic material was distributed unevenly but stained strongly for DNA.

2-day-old pupae in diapause (ISP and UVSP) had PTG cells characterized by an absence of layers in the lightly stained homogeneous cytoplasm (fig. 4). In developing pupae (ILP and UVLP), PTG cells had a rough surface and an unlayered cytoplasm. DNA staining was distributed along the nucleus membrane.

PTG cells of 10-day-old pupae in diapause (ISP, UVSP) were elongated, with an irregular outline. The unlayered cytoplasm was striated while the nuclei were circular, with DNA staining less homogeneous than in the 2-day-old pupae, and prominent nucleoli. In developing pupae (ILP, UVLP), PTG cells had highly vacuolated cytoplasm and nuclei (fig. 5).

3) Ecdysone levels. Both ecdysone and ecdysterone were detected with HPLC in the hemolymph of *M. sexta* and only significantly detectable levels are illustrated (fig. 6). In 3-day-old 5th instar larvae both, ecdysone and ecdysterone levels were very low, barely at the detection level. In 2-day-old pupae only ISP and UVLP conditions had high ecdysone levels while ecdysterone levels were low throughout. In 10-day-old pupae, ecdysone levels were generally low while ecdysterone levels were high for ILP, UVSP, and UVLP rearing conditions.

Discussion. Photoperiod and light conditions have an effect on the activity of oenocytes and PTG cells in *M. sexta*. Active PTG cells are characterized by large and more irregular nuclei, increased cytoplasmic volume and vacuolation, and maximum peripheral striations in the cytoplasm[18,19]. This was found under diapause-preventing conditions in 3-day-old 5th instar larvae. Oenocytes also exhibited characteristics strikingly similar to PTG cells. Both types of cells were more active uner ILP than under ISP conditions. When light quality rather than photoperiod is considered, larval PTG cells do not seem to be particularly affected by UV conditions while oenocytes show greater secretory activity. The apparent higher activity under ILP or UV conditions is however not translated into significant levels of

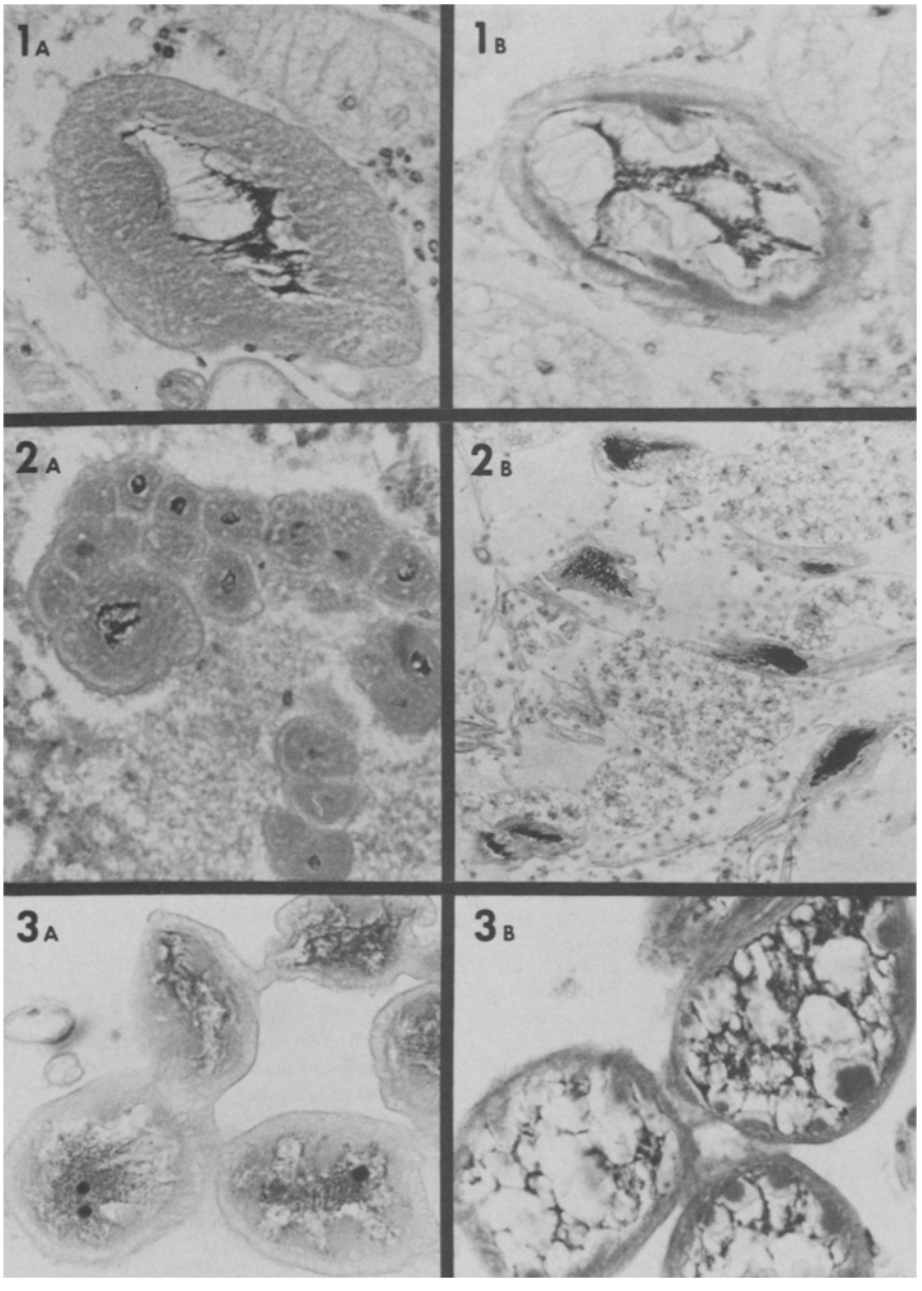

Figure 1. Oenocyte of 3-day-old larvae, 5th instar larvae of *M. sexta*. *A* UVSP; *B* ISP. Note disintegrating aspect of ISP cells. ×650.

Figure 2. Oenocytes of 10-day-old pupa, under ILP conditions. *A* New adult oenocytes; *B* disintegrating larval oenocytes. ×165.

Figure 3. The prothoracic gland of 3-day-old, 5th instar larvae. *A* Under ILP conditions; *B* under ISP conditions. ×615.

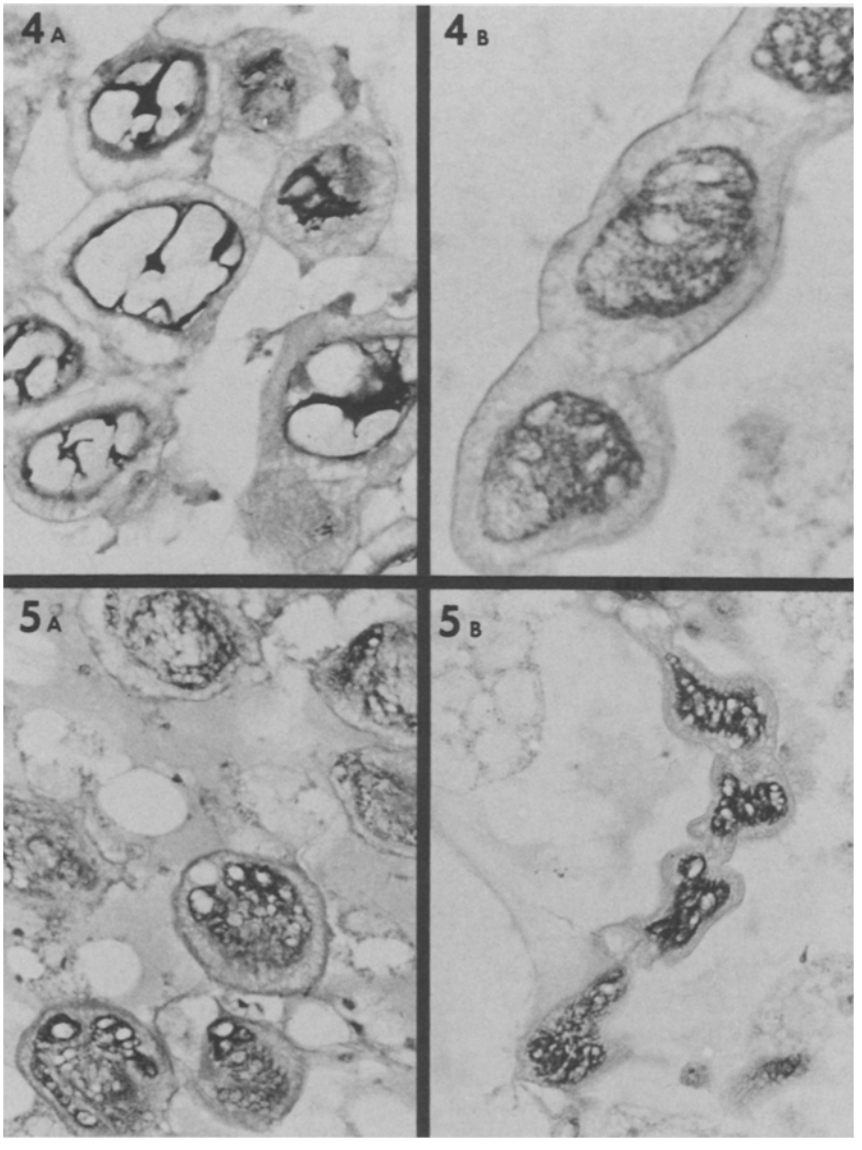

Figure 4. The prothoracic gland of 2-day-old pupae. *A* Developing pupae; *B* pupae in diapause. ×615.

Figure 5. The prothorathic gland of 10-day-old pupae. *A* developing pupae; *B* pupae in diapause. ×615.

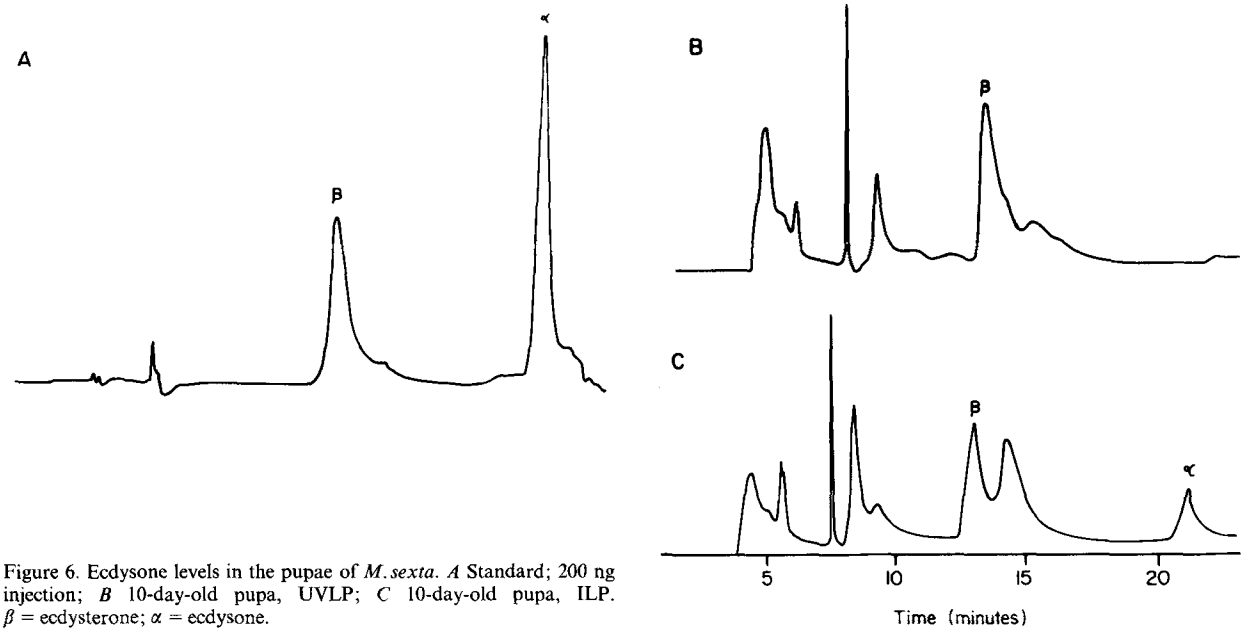

Figure 6. Ecdysone levels in the pupae of *M. sexta*. *A* Standard; 200 ng injection; *B* 10-day-old pupa, UVLP; *C* 10-day-old pupa, ILP. β = ecdysterone; α = ecdysone.

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.

* This work was supported by a research associateship to L.M. by the People's Republic of China and a grant from NSERC to B.J.R.P.
1 Reprint requests to B.J.R.P., Department of Biology, University of Ottawa, Ottawa, Canada K1N 6N5.
2 Romer, F., Experientia *29* (1973) 1417.
3 Studinger, G., and Willig, A., J. Insect Physiol. *21* (1975) 1793.
4 Diehl, P.A., Nature *243* (1973) 468.
5 Diehl, P.A., J. Insect Physiol. *21* (1975) 1237.
6 Romer, F., Histochemistry *69* (1980) 69.
7 Evans, J.T., Z. Zellforsch. *81* (1962) 49.
8 Locke, M., Tissue Cell *1* (1969) 103.
9 Cassier, P., and Fain-Maurel, M.A., Archs Anat. microsc. *61* (1972) 357.
10 Romer, F., Cytobiologie *6* (1972) 195.
11 Romer, F., Z. Morph. Tiere *80* (1975) 1.
12 Sohal, R.L., Z. Zellforsch. *140* (1973) 169.
13 Tobe, S.S., Davey, K.G., and Huebner, E., Tissue Cell *5* (1973) 633.
14 Hintze-Podufal, C., Wilhelm Roux Arch. *178* (1975) 51.
15 Philogène, B.J.R., Bull. Soc. ent. Fr. *88* (1983) 300.
16 Stewart, J.G., and Philogène, B.J.R., Can. Ent. *115* (1983) 295.
17 Lafont, R., J. Chromat. *236* (1983) 137.
18 Novak, V.J.A., Mala, J., Balazs, A., and Blaszek, I., Acta biol. *25* (1974) 107.
19 Yin, C.M., and Chippendale, G.M., Can. J. Zool. *53* (1975) 124.
20 Romer, F., Emmerich, H., and Norvock, J., J. Insect Physiol. *20* (1974) 1975.

0014-4754/85/070935-04$1.50 + 0.20/0

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

H. Okahata, Y. Nishi, K. Muraki, K. Sumii, K. Tanaka, Y. Miyachi and T. Usui

Department of Pediatrics and First Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734 (Japan), and Department of Internal Medicine, Shizuoka General Hospital, Shizuoka 420 (Japan), 15 May 1984.

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-