under a phase contrast microscope with 100–400-fold magnification. The physiological stage of meiotic maturation was classified as intact germinal vesicle (dictyate) and metaphase I (M-1) to metaphase II with extrusion of the first polar body (1-P.B.). Oocyte abnormalities suggesting degeneration consisted of a) obvious deformation of oocytes described as a helmet, a kidney, an amoeba or an ovoid shape, b) fragmentation of the oocyte and c) others such as oocytes with cytoplasmic granulation or pigmentation[6].

Degeneration ratio is defined as percent ratio of degenerating oocytes to the total oocytes exmained. As was shown in the table, the degeneration ratio of the 10-mg group increased significantly in comparison with that of the control group; the ratio $72 \pm 5.6\%$ (mean $\pm$SD) was reached on day 11 ($p < 0.01$), and this ratio was maintained on day 14. A similar remarkable increase in the degeneration ratio of the 1-mg group was observed on day 14, when it reached $70.3 \pm 4.5\%$.

Strangely enough, in spite of the uniform symptom of anovulation, little observation has been focussed on the oocytes of experimental animals or patients with the P.C.O. syndrome. Knudsen et al.[7] reported that in rats it was possible to induce ovulation using pregnant mare serum gonadotropin (PMSG) and/or human chorionic gonadotropin (HCG) after DHA administration, but the recovery of ovulated ova from the Fallopian tubes by flushing was as low as 2–4 ova per animal. The increase in the degeneration ratio of ovarian oocytes seen in the present study cannot be the full explanation for this poor recovery of ova from the Fallopian tubes, but may be an important factor, because a degenerated oocyte probably does not contribute to ovulation. Mrinal et al.[8] categorized human ovarian oocytes into 4 basic types, and reported that large numbers of oocytes from P.C.O. (77%) were degenerating and frequently contained massive clumps of chromatin material associated with the nucleolus. Our results show that oocyte degeneration occurs in the animal model as well, and the degeneration ratio finally exceeded 70%. In this respect, the experimentally induced P.C.O. in rats, and human P.C.O., seem to have a common feature. As for the mechanism which gives rise to degeneration of ovarian oocyte and the relationship with cystic change of ovarian follicles, future work is awaited.

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2 V.B. Mahesh and R.B. Greenblatt, Nature *191*, 888 (1961).
3 R.C. Ward, A. Costoff and V.B. Mahesh, Biol. Reprod. *18*, 614 (1978).
4 R.C. Ward, A. Costoff and V.B. Mahesh, Anat. Rec. *184*, 556 (1976).
5 R.P. Donahue, J. exp. Zool. *169*, 237 (1968).
6 E.S.E. Hafez, in: Methods in mammalian embryology, p. 120. W.H. Freeman, 1971.
7 J.F. Knudsen, A. Costoff and V.B. Mahesh, Fert. Steril. *26*, 807 (1975).
8 K.S. Mrinal, L.T. Melvin and J.B. Merle, Fert. Steril. *27*, 501 (1976).

Hypercalcitonemia in pernicious anemia

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Summary. Gastrin has been suggested as a natural secretogogue of the hormone calcitonin. We have found hypercalcitonemia in 55% of patients with pernicious anemia, and the gastrin levels, although usually increased, did not correlate.

Several investigators have demonstrated that gastrin may be a secretogogue for the hormone calcitonin. In vitro, pentagastrin releases calcitonin from slices of normal human thyroid[1]. In vivo, the injection of pentagastrin increases both serum and urinary calcitonin[2,3]. In the pig, an increase of endogenous gastrin was thought to mediate secretion of calcitonin from the thyroid gland[4]. Patients with pernicious anemia are known to have high levels of endogenous gastrin, presumably as a result of their achlorhydria[5–7]. Accordingly, a study was undertaken of serum calcitonin levels of such patients.

Materials and methods. A morning fasting serum specimen was obtained from 11 patients with pernicious anemia on replacement with parenteral vitamin B_{12}. Calcitonin was determined by radioimmunoassay by the procedure we have described, using Ab-I, an antibody with recognition for several regions of the calcitonin molecule[8,9]. Serum gastrin was determined by a modification of the method of McGuigan[10]. Serum total calcium was determined by atomic absorption spectrometry, and serum ionized calcium was determined by the Orion ion flowthrough technique[11].

Results. The mean serum calcitonin was 412 ± 335 pg/ml (SD), and the range was 50–1,100 pg/ml (95% upper confidence limits for normal: 260 pg/ml). 6 patients, or 55%, had increased serum calcitonin. The mean serum gastrin was 1490 ± 1160 pg/ml, and the range was 126–3206 pg/ml (95% upper confidence limits for normal: 200 pg/ml). 81% of patients had increased serum gastrin. Serum total and ionic calciums were all within the normal range. There was no significant correlation between the level of serum calcitonin and gastrin, nor between either of these hormones and the serum total or ionized calcium.

Discussion. It has been demonstrated that the gastrointestinal hormone, gastrin, may influence calcitonin secretion. Hypercalcitonemia has been found in patients with increased serum gastrin due to Zollinger-Ellsion syndrome, and this was interpreted as suggesting a possible interhormonal relation[12]. In the present study, while hypercalcitonemia occurred in over half of the pernicious anemia patients, it did not correlate with serum gastrin levels. Fahrenkrug et al.[6] have measured serum calcitonin levels in pernicious anemia, and found no difference from controls, although all of their patients had increased serum gastrin. In contrast, Franchimont and Heynen[7] found increased serum calcitonin in 25% of their patients, and also noted no correlation with levels of serum gastrin. Serum calcitonin exists in multiple heterogeneous forms, and different antisera detect these various forms with different avidity[9]. This phenomenon may explain why all investigators are not in accord that hypercalcitonemia may occur in pernicious

anemia. However, it is apparent that these 2 aforementioned studies are in agreement with the present report that endogenous hypergastrinemia in pernicious anemia does not directly control endogenous calcitonin levels. Nevertheless, the interrelationship may be complex since calcitonin is known to decrease both gastric acid and serum gastrin[13-15]. It is apparent that calcitonin is not under the unique influence of gastrin. Is the main regulator of calcitonin secretion some other gastrointestinal factor, serum calcium, some as yet undescribed chemical intermediary or a combination of these factors? As yet, the in vivo physiology of calcitonin secretion remains to be elucidated.

1 H.P. Selawry, K.L. Becker, L.E. Bivins, R.H. Snider and O.L. Silva, Horm. Metab. Res. *7*, 432 (1975).
2 C.W. Cooper, W.H. Schwesinger, A.M. Mahgoub and D.A. Ontjes, Science *172*, 1238 (1971).
3 O.L. Silva, R.H. Snider, Jr, C.F. Moore and K.L. Becker, Ann. Surg. *189*, 269 (1979).
4 C.W. Cooper, J.E. McGuigan, W.H. Schwesinger, R.L. Brubaker and P.L. Munson, Endocrinology *95*, 302 (1974).
5 J.E. McGuigan and W.L. Trudeau, N. Engl. J. Med. *282*, 358 (1970).
6 J. Fahrenkrug, O.B. Schaffalitzky de Muckadell and J.F. Rehfeld, Acta endocr. *86*, 140 (1977).
7 P. Franchimont and G. Heynen, in: Parathormone and Calcitonin Radioimmunoassay in Various Medical and Osteoarticular Disorders, p.115. Masson Inc., Paris 1976.
8 O.L. Silva, R.H. Snider and K.L. Becker, Clin. Chem. *20*, 337 (1974).
9 R.H. Snider, O.L. Silva, C.F. Moore and K.L. Becker, Clin. chim. Acta *76*, 1 (1977).
10 J.E. McGuigan, Gastroenterology *54*, 1005 (1968).
11 T.K. Li and J.T. Piechocki, Clin. Chem. *17*, 411 (1971).
12 G.W. Sizemore, V.L.W. Go, E.L. Kaplan, L.J. Sanzenbacher, K.L. Holtermuller and C.D. Arnaud, N. Engl. J. Med. *288*, 641 (1973).
13 R.D. Hesch, M. Hufner, B. Hasenjager and W. Creutzfeld, Horm. Metab. Res. *3*, 140, 1971.
14 R.M. Bolman III, C.W. Cooper, S.C. Garner, P.L. Munson and S.A. Wells, Jr, Endocrinology *100*, 1014 (1977).
15 H.D. Becker, D.D. Reeder, M.T. Scurry and J.C. Thompson, Am. J. Surg. *127*, 71 (1974).

Visual input to rat pineal

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Summary. Electrophysiological recordings from freely behaving rats, previously implanted stereotaxically with permanent electrodes in the pineal, ventromedial hypothalamus, caudate nucleus, lateral geniculate body and medial geniculate body were obtained. The pineal photic responses revealed 5 sequential components. Injection of a neuronal blocker at the level of the superior cervical ganglion did not alter the earlier photic responses, but did eliminate the late components (N_2-P_3) for 60–90 min after the injection. All of the other responses were unchanged during the experiment. The present experiments demonstrated that photic input travels to the pineal through two pathways.

In previous studies reported from this laboratory[2-9], single cell activity as well as bipolar and monopolar evoked responses following photic, acoustic, olfactory bulb, ventromedial hypothalamus (VMH) and amygdaloid complex (Amyg) stimulation were recorded from the pineal and other structures simultaneously. The monopolar and bipolar recordings following photic stimulation were identical[4]; moreover, the initial photic response obtained demonstrated short latency. This short latency response could not be easily explained by the commonly accepted neuronal pathway i.e., it originates in the retina, which projects via the retinohypothalamic tract to suprachiasmatic nucleus, medial forebrain bundle, reticular formation, the upper thoracic intermediolateral cell column (the origin of preganglionic sympathetic fibres) and superior cervical ganglion (scg). The post synaptic sympathetic process, i.e. nervi conarii, from the scg travels along the tentorium cerebelli and enters the pineal with blood vessels[10-14]. The present study was initiated to examine the possibility that photic inputs also reach the pineal via the central nervous system.

6 Sprague-Dawley male rats, weighing 250–350 g, were anesthetized with pentobarbital (50 mg/kg i.p.) and 5 nichrome electrodes (60 µm in diameter) were implanted stereotaxically in the pineal, ventromedial hypothalamus (VMH), caudate nucleus (CN), lateral geniculate body (Lgb) and medial geniculate body (Mgb) using the stereotaxic atlas of Konig and Klippel[15] for coordinates. 4–6 days after electrode implantation, the animals were placed in a plastic cage within an electrophysiological testing chamber. The electrodes were connected to conventional electrophysiological instruments[5]. 32 (1 set) photic or acoustic evoked responses (1/2.5 Hz) were averaged on-line using the NIC 1070 signal averaging computer. 4 sets (each set consisting of the average of 32 consecutive responses) following each modality (photic or acoustic) were recorded prior to bilateral injection of local anesthesia (0.3 ml of 1% xylocaine) in the region of the scg. Recordings were resumed every 5 min until recovery from the xylocaine effects was observed. At the conclusion of each experiment, the animals were sacrificed for histological verification of electrode placement[2-6].

The average photic and acoustic evoked responses recorded from the pineal, VMH, CN, Lgb and Mgb exhibited in general 5 components. The components are: initial positive peak (P_1) followed by negative (N_1) and positive-negative-positive (P_2-N_2 and P_3) deflections. Similar observations were observed previously in monopolar and bipolar recording[4,16]. However, differences in amplitudes and latencies to the different peaks were apparent (figure). Bilateral xylocaine (1%, 0.3 ml in each side) injection eliminated only the N_2 and P_3 deflection of the pineal averaged photic evoked responses for 70–90 min without affecting the evoked responses (photic) recorded from the VMH, CN and Lgb (figure). Moreover, the acoustic responses in all the four structures (pineal, VMH, CN and Mgb) were not altered during the experimental period (figure). All 6 animals exhibited similarity in the control recordings as well as after the xylocaine injection, as demonstrated in the figure, which represents 1 animal.