tracts was determined by the fat pad method[15] as previously described[16].

Insulin was found to be absent from the gut in all species studied but the rat. Gut insulin (IRI and ILA) in the rat was associated almost exclusively with the duodenal mucosa (Table). Only traces of IRI and ILA were found in other parts of the gastro-intestinal tract. The source of the insulin of the rat duodenum is unknown. The possibility was considered that the presence of insulin in the gut was a result of accumulation of insulin from the blood and that it reflected a high local insulin uptake. In order to test this assumption, I^{125}-labelled insulin (specific radioactivity 290 mc/mg; Hoechst AG, Frankfurt) was injected i.v. (10 µc in 0.4 ml 0.9% saline); 10 min later the rats were killed by decapitation and the insulin uptake of the tissues was measured by γ-spectrometry (Packard Auto-Gamma Spectrometer, M 3001). There was no evidence of a specific accumulation of insulin in the duodenal mucosa as compared with other tissues. On the contrary, the uptake of insulin in the midgut (31,000–43,000 cpm/g; instrumental values) was only about 50% of that found in the antrum, fundus and colon (62,000–77,000 cpm/g). The liver had the highest uptake (91,000 cpm/g) while the uptake in skeletal muscle (gastrocnemius) and adipose tissue (epididymal fat pad) was low (8,900 and 4,200 cpm/g, respectively). Also considered was the possibility that gut insulin originates from some endocrine cell system similar to the pancreatic β-cells, and that accordingly the pancreatic and gut insulin stores would be similarly depleted by treatment with alloxan. Alloxan (50 mg/kg) was given i.v. to freely fed rats. One week later, blood samples were taken by orbital puncture[12] for estimation of the glucose level by the method of MARKS[17]. Rats with a blood glucose level below 300 mg/ml after alloxan treatment were discarded. Alloxan-diabetic and untreated control rats were sacrificed by decapitation and the gastrointestinal mucosa was collected for determination of IRI and ILA. Alloxan-induced diabetes was found to coincide with a depletion of the gut insulin stores (Table).

There is no evidence that gut insulin in the rat is of physiological significance; the amount of insulin found in the duodenal mucosa is only a fraction of that of the pancreas. However, the possibility that in some species β-cells or insulin-storing cells similar to β-cells may occur in the gut, would seem to support the view that all endocrine cells which constitute 'the abdominal endocrine organ' have a common origin[18].

Effect of alloxan on mucosal IRI and ILA in various parts of the digestive tract

Tissue source	IRI (µU/100 mg mucosa)		ILA (µU/100 mg mucosa)	
	No treatment	Alloxan	No treatment	Alloxan
Fundus	0	0	8	18
Antrum	16	0	4	6
Duodenum	244	8	270	4
Jejunum	16	0	2	12
Ileum	0	6	6	4
Colon	0	0	10	12

Determinations of IRI and ILA were on aliquots of the same extracts. Results are from 1 typical experiment.

Resumé. Un taux faible d'insuline immunoréactive (IRI) et d'activité semblable à l'insuline (ILA) a été décelé dans la muqueuse duodénale du rat, mais non pas dans celles du lapin, du chat et du chien. Les autres régions du tractus digestif de toutes ces expèces présentaient un taux extrèmement bas d'insuline. Le traitement par l'alloxane entraîne le vidage de l'IRI et l'ILA duodénales.

R. HÅKANSON and I. LUNDQUIST

Department of Pharmacology
University of Lund, S-22362 Lund (Sweden),
23 April 1971.

[15] A. E. RENOLD, D. B. MARTIN, Y. M. DAGENAIS, J. STEINKE, R. J. NICKERSON and M. C. SHEPS, J. clin. Invest. *39*, 1487 (1960).
[16] I. LUNDQUIST, Acta endocrin., Copenh. *58*, 11 (1968).
[17] V. MARKS, Clin. chim. Acta *4*, 395 (1959).
[18] Acknowledgments. Grant support from the Swedish Medical Research Council (Project No. B72-14X-1007-07), the Medical Faculty of Lund and the Albert Påhlsson Foundation.

Effects of Ovariectomy and of Oestrogen Administration on the Decrease in Pituitary Prolactin Content which Occurs on the Afternoon of Pro-Oestrus in the Rat

In a previous paper[1], we demonstrated that prolactin (PL) content and concentration of the anterior pituitary gland in the rat decreased on the afternoon of pro-oestrus (PE), and that this decrease was blocked by the i.p. injection of sodium pentobarbitone at 13.30 h of PE. Similar findings were recently reported by several workers by the use of radioimmunoassay[2–5]. These results strongly suggest that a surge of prolactin occurs on the afternoon of PE around the 'critical period' for LH release, and that the central nervous system could participate in the release.

Many workers have reported that the surge of LH is triggered by an increase in circulating level of oestrogen between the 2nd day of dioestrus (D_2, day before PE) and the morning of PE in the rat[6–9], although a possible role of progesterone in triggering the surge of LH cannot be ruled out in certain circumstances[10–13]. Both the pituitary content and the serum level of PL were reported to be increased by the exogenous administration of estrogen[14–16]. Therefore, an attempt was made to elucidate the role of ovarian oestrogen secretion in the release of PL observed on the afternoon of PE.

Material and Methods. Virgin female rats of the Wistar-Imamichi strain weighing 250–290 g were used. Animals were housed in a light-controlled (on at 05.00 h and off at 19.00 h) animal room with controlled temperature (24 ± 2 °C). They were maintained on a stock diet (CA-1, Nihon CLEA Ltd., Tokyo) and water ad libitum. Vaginal smears were examined every morning between 09.30–10.00 h. Only animals showing at least 2 consecutive 4-day-cycles were used.

Ovaries were removed under ether anaesthesia on the afternoon (around 16.30 h) of D_2 or on the morning (around 10.30 h) of PE. In sham-operated animals, ovaries were exposed but not removed around 16.30 h of D_2. 1 µg of oestradiol benzoate (EB) in 0.1 ml of soy bean oil was injected s.c. in some animals ovariectomized on the afternoon of D_2 at the time of operation. Ovariectomized ani-

Prolactin content and concentration of the anterior pituitary of ovariectomized rats killed at pro-oestrus 17.30 h

Operation and treatment	Time of operation and/or treatment	No. of rats	Anterior pituitary (AP) weight (mg)	Prolactin content[a] (μg/AP)	Prolactin concentration[a] (μg/mg AP)
Ovariectomy	Dioestrus 2[b] 16.30 h	5	10.04 ± 0.46[c,1]	106.50 ± 19.78[2]	10.59 ± 1.97[5]
Ovariectomy + 1 μg oestradiol benzoate	Dioestrus 2 16.30 h	5	10.66 ± 0.34[1]	35.81 ± 3.82[4]	3.34 ± 0.29[7]
Ovariectomy + 0.1 ml oil	Dioestrus 2 16.30 h	5	9.68 ± 0.46[1]	64.11 ± 4.49[3]	6.63 ± 0.81[6]
Ovariectomy	Pro-oestrus 10.30 h	5	10.38 ± 0.52[1]	39.99 ± 2.01[4]	3.85 ± 0.05[7]
Sham ovariectomy	Dioestrus 2 16.30 h	5	10.36 ± 0.71[1]	45.17 ± 4.67[4]	4.35 ± 0.33[7]

[a] Prolactin content and concentration are expressed as μg of NIH-P-B 3. [b] Second day of dioestrus (day before pro-oestrus). [c] Mean ± SEM. [1–7] Means which have the same superscripts are not significantly different from each other.

mals injected with 0.1 ml of soy bean oil s.c. served as the oil-injected control. Each group consisted of 5 animals. All animals were killed by decapitation under light ether anaesthesia between 17.30–18.00 h on PE.

Anterior pituitaries were removed, weighed and frozen at -20 °C until assayed. PL contents were determined by disc electrophoresis on polyacrylamide gel and measured by a microdensitometer (Chromoscan, MK II, Joyce Loebl & Co. Ltd., England). A linear relationship between the graded doses of the standard preparation of PL and their optical densities, reported by YANAI and NAGASAWA[17] and NICOLL, PARSONS, FIORINDO and NICHOLS[18], was confirmed in our laboratory by using NIH-P-B 3. The results are, therefore, expressed as μg of NIH-P-B 3. Significance of differences between groups was determined by DUNCAN's multiple range test[19].

Results and discussion. The weight, PL content and concentration of the pituitary gland in each group are shown in the Table.

PL content and concentration on the afternoon of PE in the sham-operated animals were as low as those previously obtained in intact cyclic rats killed on the PE afternoon[1]. In animals ovariectomized at PE 10.30 h and killed on the afternoon of the same day, PL content and concentration were also low and the values were not significantly different from those obtained in the sham-operated animal.

When ovariectomy was performed at D_2 16.30 h, PL content and concentration of pituitary gland remained high at the time of autopsy (17.30 h of PE). The level was about two times higher than that of the sham-operated animals and of animals ovariectomized at PE, and the level was very similar to that obtained in the animal killed on the morning of PE reported in the previous paper[1].

Subcutaneous administration of 1 μg of EB at the time of operation to animals ovariectomized at D_2 16.30 h resulted in a decrease in PL content and concentration compared with those of ovariectomized animals treated with oil ($P<0.05$). The level of PL in EB-treated animals was not significantly different from that obtained in sham-operated animals and in animals ovariectomized on PE.

No significant differences in the weight of the pituitary gland were observed among the groups described in the present paper.

These results indicate that ovarian oestrogen secreted between D_2 afternoon and PE morning is responsible for the surge of PL observed on the afternoon of PE. The central nervous system may normally be a target of endogenous oestrogen, since blockade of PL surge by pentobarbitone anaesthesia has been reported previously[1,4].

Résumé. L'oestrogène secrété entre l'après-midi du D_2 et le matin du PE est responsable de l'apparition de PL dans l'après-midi du PE.

A. YOKOYAMA, H. TOMOGANE and K. ÔTA[20]

Faculty of Agriculture, Nagoya University, Chikusa-ku, Nagoya, 464 (Japan), 9 May 1971.

[1] A. YOKOYAMA, H. TOMOGANE and K. ÔTA, Experientia *27*, 578 (1971).
[2] J. D. NEILL, Endocrinology *87*, 503 (1970).
[3] J. D. NEILL and L. E. REICHERT JR., Endocrinology *88*, 548 (1971).
[4] W. WUTTKE and J. MEITES, Proc. Soc. exp. Biol. Med. *135*, 648 (1970).
[5] W. WUTTKE, E. CASSELL and J. MEITES, Endocrinology *88*, 737 (1971).
[6] N. B. SCHWARTZ, Am. J. Physiol. *207*, 1251 (1964).
[7] F. KOBAYASHI, K. HARA and T. MIYAKE, Endocr. jap. *15*, 313 (1968).
[8] F. KOBAYASHI, K. HARA and T. MIYAKE, Endocr. jap. *16*, 261 (1969).
[9] N. B. SCHWARTZ, Rec. Progr. Horm. Res. *25*, 1 (1969).
[10] J. W. EVERETT, *Sex and Internal Secretion* (Ed. W. C. YOUNG; The Williams & Wilkins Co., Baltimore 1961), vol. 1, p. 497.
[11] S. Y. YING and R. K. MEYER, Endocrinology *84*, 1466 (1969).
[12] F. KOBAYASHI, K. HARA and T. MIYAKE, Endocr. jap. *17*, 149 (1970).
[13] R. K. MEYER, H. J. KARAVOLAS, M. KLAUSING and D. W. NORGARD, Endocrinology *88*, 983 (1971).
[14] J. L. VOOGT, C. L. CHEN and J. MEITES, Am. J. Physiol. *218*, 369 (1970).
[15] C. L. CHEN and J. MEITES, Endocrinology *86*, 503 (1970).
[16] Y. AMENOMORI, C. L. CHEN and J. MEITES, Endocrinology *86*, 506 (1970).
[17] R. YANAI and H. NAGASAWA, Proc. Soc. exp. Biol. Med. *131*, 167 (1969).
[18] C. S. NICOLL, J. A. PARSONS, R. P. FIORINDO and C. W. NICHOLS JR., J. Endocr. *45*, 183 (1969).
[19] D. B. DUNCAN, Biometrics *11*, 1 (1955).
[20] We wish to thank Dr. R. W. BATES, Hormone Distribution Officer, National Institute of Arthritis and Metabolic Diseases, for supplying NIH-Prolactin B-3. Our thanks are also due to Drs. J. S. TINDAL and G. S. KNAGGS, National Institute for Research in Dairying, England, for reading the manuscript. Part of these data was presented at the 59th Annual Meeting, Japanese Society of Zootechnical Science, Nagoya, 7–8 April 1971.