sues[13–15] has been found to inhibit aggregation. Insulin, also tested, inhibited aggregation only at high concentration (40–80 U/ml).

These studies, taken in light of the work of others, suggest that thrombin may act by producing change in the platelet plasma membrane. Thrombin is known to alter platelet membrane charge[12] as well as the permeability of the membrane to K^+[16]. Recently thrombin has been shown to alter platelet cAMP levels[4,5] and platelet ATPase[2] and AChE[3] activities. One or more proteins of the platelet plasma membrane are changed following exposure to thrombin[17,18]. Others have reported that intracellular Ca^{++} and cAMP levels can be influenced by AChE and ATPase activities of the cell[19–21]. Thus thrombin may alter platelet cAMP levels not only by action on the adenyl cyclase-phosphodiesterase system which does not appear to be located in the plasma membrane but also by influencing membrane associated ATPase and AChE activities. These findings illustrate that the actions of thrombin on the platelet plasma membrane are diverse, a fact supported by our present data with inhibitors of thrombin-induced aggregation[22].

Zusammenfassung. Substanzen, welche die thrombininduzierte Plättchenaggregation hemmen, lassen sich in drei Hauptgruppen aufteilen: 1. membranstabilisierende Steroide, 2. Substanzen, welche die elektrischen Eigenschaften der Membran oder die Permeabilität oder beide beeinflussen, und 3. Substanzen, die eine Erhöhung des intrazellulären cAMP-Spiegels bewirken sollen. Eine Hypothese über die Hemmung der Plättchenaggregation durch Thrombin wird vorgeschlagen.

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Occurrence of Insulin in Rat Duodenum and its Depletion with Alloxan

The pyloric antrum, duodenum and pancreas have been referred to by the collective name of 'the abdominal endocrine organ'[1]. All the endocrine cells of this region are believed to arise from a limited area of the primitive foregut, and their hormones are without exception concerned with the regulation of digestion, absorption and utilization of food[1–3]. The hormones of 'the abdominal endocrine organ' are polypeptides such as gastrin, secretin, cholecystokinin-pancreozymin and enteroglucagon in the antro-duodenal mucosa, and insulin and glucagon (possibly also gastrin[4]) in the pancreatic islets. The chemical similarity between several of these polypeptide hormones (gastrin and cholecystokinin[5], secretin, glucagon and insulin[5,6]) supports the concept of a closely integrated hormone-producing locus, comprising several endocrine glands, which conceivably are developed from the same primordial origin. Moreover, the possible existence of an 'enteroinsular axis', whereby insulin-releasing agents from the gut control the insulin release, emphasize the close functional coordination of this particular region[7–12]. There is also some evidence that endocrine cells of this region may have a dual distribution. Thus, glucagon or glucagon-like agents (enteroglucagon) have been demonstrated in the intestinal mucosa[7–12], and gastrin-storing cells can be recognized both in the pyloric gland area of the gastric mucosa[13] and in the pancreatic islets[4]. We decided to investigate the possibility that insulin might have a similar dual distribution.

The musosa was scraped off various regions of the bowel of freely fed rats (male Wistar rats weighing 200–300 g; material from 4–5 rats was pooled), rabbits, cats and dogs. Care was taken in dissection and in the collection of mucosa to avoid contamination with pancreatic tissue. The mucosa was homogenized in acidified ethanol (96 vol. ethanol, 2.4 vol. conc. H_2SO_4, 18 vol. water) in a concentration of 50 mg tissue (wet weight) per ml. The homogenate was allowed to stand overnight at +4 °C. The homogenate was then neutralized with 5 *N* ammonium hydroxide and centrifuged at $1500 \times g$. The supernatant was dialyzed overnight at +4 °C against 2 changes of acetate buffer (pH 4.5), 0.1 and 0.02 *M* respectively, and subsequently assayed for immunoreactive insulin (IRI)[14]. After further dialysis overnight against Ca^{++}-free Krebs-Ringer bicarbonate solution, the insulin-like activity (ILA) of the ex-

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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.

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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-