Decrease in amylase (*EC* 3.4.21.4) synthesis in lactating rats

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1. The amylase (*EC* 3.4.21.4) and trypsin (*EC* 3.2.1.1) activities in the pancreas in rats during pregnancy, lactation and after the weaning period, and the secretory responses to a secretagogue (caerulein) in the exocrine pancreas of lactating rats were measured.

2. Trypsin activity increased as lactation progressed and reached twice that of unmated rats in the second half of the lactation period. The amylase activity fell before parturition and failed to recover even after the start of lactation and was significantly decreased throughout the lactation period.

3. The total amount of pancreatic juice produced in the lactating rats was significantly greater than that of unmated rats; the amylase output was significantly less than that of unmated rats.

4. When the pups were removed, amylase activity in the pancreas returned to the value in unmated rats. Furthermore, the amylase activity in lactating rats receiving a daily injection of insulin significantly exceeded that of normal lactating rats.

5 These results indicate that the decrease in amylase activity in lactating rats is due to the reduction of amylase synthesis and there is a possibility that insulin is required for normal or elevated rates of amylase synthesis in lactating rats.

The food intake of pregnant rats exceeds that of unmated rats by no more than 50%. However, following parturition, the amount of food intake increases suddenly and reaches three to four times that of unmated rats in the second half of the lactation period (Wang, 1926; Coli & Hart, 1938; Lichtenberger & Trier, 1979; Shirley, 1984).

In the late stage of pregnancy and during the lactation period, hypertrophy and hyperplasia of the gastrointestinal organs occur, such as an increase in the weight of the stomach, increase in the intestinal mucosal surface area, hyperplasia of the small intestinal epithelium and increase in crypt length and villus height (Crean & Rumsey, 1971; Cripps & Williams, 1975; Rolls, 1975; Jolicoeur *et al.* 1980; Remesar *et al.* 1981). These changes are induced by increases in the plasma level of gastrointestinal hormones such as gastrin, secretin and cholecystokinin (CCK), which have been shown to have significant trophic effects on the gastrointestinal organs (Rolls *et al.* 1979). Gastrin and CCK stimulate not only the secretion of exocrine pancreatic enzymes but also their synthesis in the acinar cells (Mainz *et al.* 1973; Barrowman & Mayston, 1974). Therefore, pancreatic proteolytic enzyme activity increases during the lactation period (Barrowman & Mayston, 1973; Rolls *et al.* 1979; Jolicoeur *et al.* 1980). In addition to the exocrine function, the endocrine function of the pancreas in the maternal rat also changes during the lactation period. Recently, a number of reports have indicated that plasma insulin levels in lactating rats are significantly lower than those in unmated rats (Burnol *et al.* 1983; Grigor & Gain, 1983; Grigor *et al.* 1984). In a previous paper, we showed that the decrease in the circulating concentration of insulin during lactation is induced by decreased pancreatic secretion of insulin (Mizoguchi & Imamichi, 1986).

It is generally assumed that pancreatic exocrine enzyme activities are controlled mainly

by gastrointestinal hormones and nutritional status. On the other hand, it has been reported that insulin plays a permissive role in pancreatic amylase (*EC* 3.4.21.4) synthesis (Kramer & Tan, 1968; Henderson, 1969; Owerbach *et al.* 1981; Sofrankova & Dockray, 1983). The present study concerned the changes in amylase concentration and responsiveness to the secretagogue caerulein in the exocrine pancreas of lactating rats.

MATERIALS AND METHODS

Animals

Female Crj:CD(SD) rats, 9 weeks old and weighing 230–250 g (Charles River Japan Inc.) or Wistar-Imamichi rats (*in situ* experiment only), 12 weeks old and weighing 260–300 g (Imamichi Institute for Animal Reproduction) were used. All animals were allowed free access to a commercial diet (CLEA Japan, Tokyo) and water, and daily food intake was measured. Animals were mated after confirmation of vaginal pro-oestrus. The presence of spermatozoa in the vaginal smear the next morning was regarded as evidence of impregnation. In pregnant animals, pregnancy was confirmed by inspection of the uterus at the end of the experiment. The day of parturition was considered as day 0 of lactation. After parturition, the number of pups per litter was adjusted to ten or four or all pups were removed according to the purpose of the experiment. The lactation period studied was 21 d following delivery.

At weekly intervals after impregnation, rats were removed without previous starvation between 11.00 and 13.00 hours to avoid circadian variation of enzyme secretion from the pancreas (Kanno *et al.* 1979). The pancreas was quickly removed under light diethyl ether anaesthesia, freed from fat and connective tissue, and weighed immediately. The isolated pancreas was homogenized in physiological saline solution (9 g sodium chloride/l) in an ice-cold bath and centrifuged at 6000 ***g*** for 15 min at 0°. Activities of amylase and trypsin (*EC* 3.2.1.1) and the concentration of protein in the supernatant fraction were measured.

In situ *preparation of the pancreas*

The *in situ* experiment was carried out on anaesthetized rats, as described by Kanno *et al.* (1973). Under initial anaesthesia with diethyl ether and subsequent anaesthesia with sodium pentobarbital (Somnopentyl; Pitman-Moore Inc., Washington), the abdomen was opened and a polyethylene tube was inserted into the hepatic bile duct toward the hilum of the liver for removal of the bile. The bile duct was then ligated below this point before entry of the bile duct into the pancreatic tissue. A stainless-steel cannula (about 0·5 mm outside diameter) was also inserted into the duodenal end of the common duct to collect pure pancreatic juice, uncontaminated with bile. In all experiments, the pylorus was ligated and vagi were cut right below the diaphragm to eliminate neural influences. A cannula was inserted into the femoral vein to inject caerulein (Ceosunin; Kyowa Hakko Kogyo Co. Ltd, Tokyo) as a secretagogue, and sodium pentobarbital. The animal was placed on a cork board and maintained at a body temperature of about 37° by the heat of an electric lamp. Every 10 min, the tube was replaced and the rate of pancreatic juice flow (μl/min) was calculated by the amount of juice obtained. After two basal collections, 200 ng caerulein/kg body-weight were quickly injected into the femoral vein, and stimulated secretion was collected for eight periods. The collected juice sample was diluted with physiological saline solution, and amylase activity and protein content were measured.

Measurement of enzyme activity

Amylase activity in an appropriately diluted sample was measured by a modification of the method of Bernfeld (1955) as described by Kanno *et al.* (1976). One unit of amylase

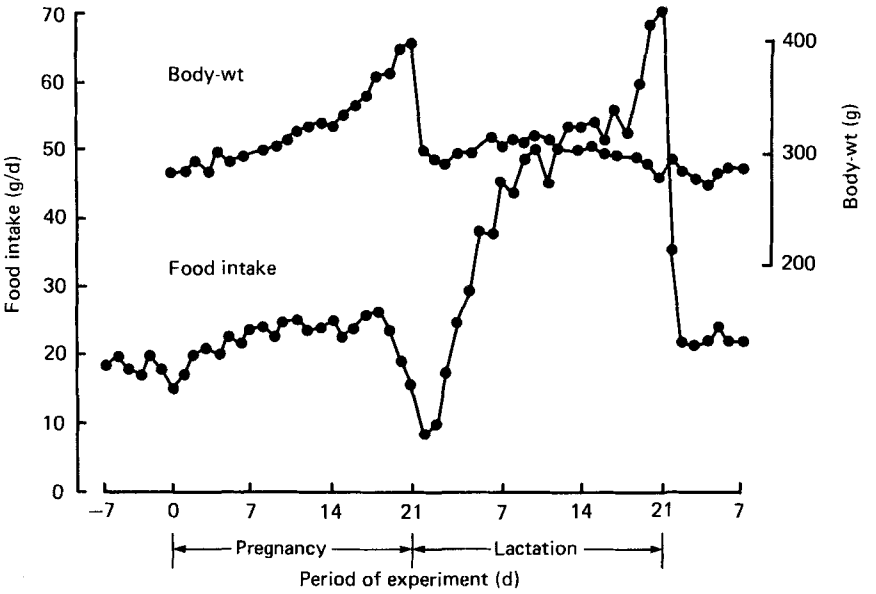

Fig. 1. Mean daily food consumption and body-weight during pregnancy and lactation in rats. Measurements were performed on seven rats per group. For all values, standard errors did not exceed 10% of means. Food intakes were significantly increased above unmated levels from days 5–19 of pregnancy and days 4–21 of lactation ($P < 0.05$).

activity was defined as the amount of enzyme which catalysed the production of 1 mg maltose from soluble starch (E. Merck, Darmstadt, W. Germany) during 5 min incubation with the diluted sample at 37°. A portion of the sample was incubated at 37° with enterokinase (Sigma, St Louis, MO) and the activity of trypsin was assayed colorimetrically according to the Bratton–Marshall reaction using α-N-benzoyl-arginine-*p*-nitroanilide (Sigma, St Louis, MO) as substrate (Erlanger *et al.* 1961). Total proteins in the pancreatic juice and pancreatic tissue were assayed by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

All results were expressed as means with standard errors. Significant differences in mean values between groups were determined by unpaired Student's *t* test; $P < 0.05$ was considered significant.

RESULTS

Food intake and body-weight during pregnancy and lactation

Changes of mean daily food intake and body-weight of female rats during pregnancy and lactation are shown in Fig. 1. The mean body-weight of female rats increased as pregnancy progressed, but there was a sharp decrease after parturition, and no significant change was observed during lactation. The food intake of the female rats increased slightly with the progress of pregnancy, and the value of 28·6 (SE 1·0) g/d on day 18 of pregnancy was significantly higher than that of 18·7 (SE 0·6) g/d in unmated rats. From 3 d before parturition, food intake suddenly declined. A rapid increase in food intake was observed from day 2 to day 17 of lactation and reached 56·6 (SE 2·2) g/d on day 17 of lactation. Thereafter a slight fall in food intake was observed, but it increased again and became 70·1 (SE 1·3) g/d on day 21 of lactation. After weaning, the food intake declined sharply on days

Table 1. *Effects of pregnancy and lactation on body-weight, pancreas weight, protein content and activities of amylase* (EC *3.4.21.1*) *and trypsin* (EC *3.2.1.1*) *of rats*

(Values are means with their standard errors for seven rats, except for 7 d lactation when six rats were used)

			Pancreas wt				Protein content				Amylase activity				Trypsin activity			
	Body-wt (BW) (g)		Absolute (mg)		Relative (mg/kg BW)		Total (mg)		Relative (mg/g pancreas)		Total (U)		Relative (U/g pancreas)		Total (mU)		Relative (mU/g pancreas)	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Unmated control	265	7	547·8	9·9	20·73	0·65	63·7	1·5	1·16	0·03	24005	1750	439·5	33·8	9200	695	167·9	12·2
Pregnant:																		
7 d	282	11	588·3	26·5	20·83	0·63	65·2	4·0	1·31	0·05	24427	2181	390·7	22·0	9705	1214	154·7	31·2
14 d	326**	14	599·8	8·8	19·36	0·71	65·4	3·1	1·05	0·04	21202	1221	343·6*	23·9	9873	1702	160·9	22·8
21 d	369**	11	542·1	35·0	14·78**	1·17	52·4	4·0	0·96**	0·03	9502**	1945	169·2**	23·5	6650*	731	121·5**	9·3
Lactation:																		
7 d	282	6	595·3	40·4	21·02	1·04	73·6	4·9	1·24	0.05	19669	3366	261·5**	60·3	12566	1921	214·2*	28·7
14 d	292	11	596·8	22·1	20·56	8·8	70·9	3·8	1·31	0·06	18153*	1018	304·4**	14·6	16928**	1663	291·0**	40·2
21 d	273	7	582·0	22·8	21·38	1·00	77·3	4·8	1·33	0·08	24493	2881	407·9	43·6	15778**	1408	263·8**	19·8
Weaning:																		
7 d	267	6	523·8	27·3	19·71	1·23	71·9	4·3	1·37*	0·05	23927	1885	459·7	19·5	11909	1286	223·8	33·1
14 d	292*	5	571·4	27·0	19·53	0·82	71·6	4·1	1·20	0·03	25410	1819	456·6	37·5	10871	924	195·6	19·6
21 d	311**	8	577·4	17·4	18·78*	0·26	75·4	3·2	1·29	0·26	23994	2174	457·1	24·7	12579	1493	220·2	30·2

Statistical significance of difference between experimental values and those of unmated control: * $P < 0·05$, ** $P < 0·01$.

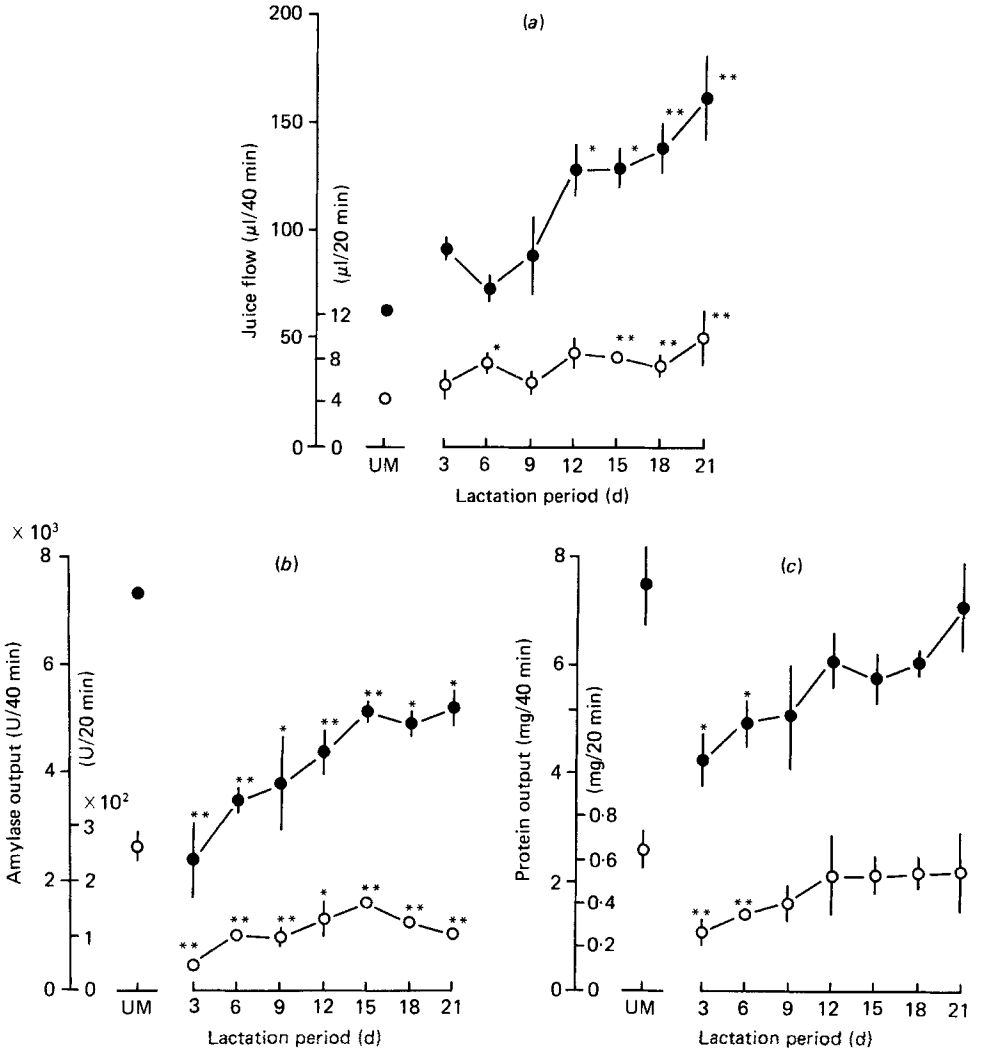

Fig. 2. (*a*) Total pancreatic juice flow, (*b*) amylase (*EC* 3.4.21.4) output and (*c*) protein output induced by caerulein (●, 40 min) and previous spontaneous secretion (○, 20 min) in *in situ* pancreatic preparations. Points are mean values, with their standard errors represented by vertical bars, for five to six observations. Statistical significance of difference between unmated (UM) rats and others: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

1 and 2, and by day 3 the food intake had decreased to a level near to that recorded at the beginning of pregnancy.

Enzyme activity and protein contents of the pancreas

No significant difference was observed in pancreas weight between the unmated and pregnant or lactating rats, excluding day 21 of pregnancy. The pancreatic protein content per g tissue on day 21 of pregnancy was significantly less than that of unmated rats. However, during lactation there was a tendency for the total content and the content per g tissue to increase.

During pregnancy, the amylase activity fell; by day 21 of pregnancy, the total activity

Table 2. *Effect of reduction of litter size on maternal pancreas weight, protein contents and activities of amylase* (EC *3.4.21.4*) *and trypsin* (EC *3.2.1.1*) *of rats*

(No. of pups per litter was adjusted to ten (normal) or four (restricted) or all pups were removed (removed) on day 0 of lactation and enzyme activities were determined on day 14. Results are expressed as mean values with their standard errors for six rats)

	Pancreas wt (mg)		Protein content (mg)		Total activity				Activity (/g pancreas)			
					Amylase (U)		Trypsin (mU)		Amylase (U)		Trypsin (mU)	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Normal	667	38	82·4	4·0	23195	2533	14537	1467	343·0	22·6	230·9	14·6
Restricted	612	23	79·3	5·0	25388	1746	15158	1549	413·6*	18·6	245·6	18·8
Removed	606	32	79·7	4·4	26528	1375	13023	711	441·9*	26·2	216·0	10·8

Statistical significance of difference between experimental values and normal values: * $P < 0{\cdot}05$.

as well as the activity per g tissue were significantly lower than the corresponding values for the unmated rats. Amylase activity failed to recover even after the start of lactation. The activities per g tissue were about 60 and 70% that of unmated rats on days 7 and 14 of lactation respectively. On day 21 of lactation, at which time food intake had reached the maximum value, amylase activity had not increased beyond that of unmated rats. Trypsin activity on day 21 of pregnancy was also observed to fall. After parturition it started to increase. On day 14 of lactation, total trypsin activity as well as that per g tissue reached the highest values, 1·8 and 1·7 times respectively, of those of unmated rats.

Secretory response of the exocrine pancreas

Fig. 2 shows the time-course of pancreatic juice flow, and amylase and protein outputs induced by caerulein in unmated and lactating rats. The rapid injection of caerulein induced an increase in pancreatic juice flow, and amylase and protein outputs both in unmated and lactating rats. At 10 and 20 min following the injection these responses reached a maximum but after 30 and 40 min returned to the pre-injection values. The caerulein-induced juice flow in the lactating groups was 1·5 to 2·7-fold greater than that of the unmated group. However, in the lactating animals, caerulein-induced amylase output was significantly lower than that of the unmated group.

The total amount of pancreatic juice secreted for 20 min before the caerulein injection in the lactating groups was significantly greater than that of the unmated group. The total amount of juice produced for 40 min following the caerulein injection increased in the lactating groups as lactation progressed. On day 21 of lactation, spontaneous and caerulein-induced juice flows were 2·3- and 2·6-fold respectively of that of the unmated group. The amylase output in the lactating groups, whether spontaneous or induced by caerulein, was significantly less than that of the unmated group. On day 21 of lactation the total amount of amylase output secreted spontaneously and induced by caerulein remained at 39 and 68% of that of the unmated group. The protein output on days 3 and 6 of lactation, both spontaneous and induced by caerulein injection, fell significantly below that of the unmated group. However, following day 6 of lactation, protein output gradually increased, and the difference from that of the unmated group clearly became less.

Table 3. *Effect of insulin treatment on maternal pancreas weight, protein content and activities of amylase* (EC *3.4.21.4*) *and trypsin* (EC *3.2.1.1*) *of rats*

(No. of pups per litter was adjusted to ten on day 0 of lactation and the animals were injected with either saline (9 g sodium chloride/l) or insulin (1 IU/rat per d) from day 0 of lactation and enzyme activities were determined on day 14 of lactation. Values are means with their standard errors)

		Pancreas wt (mg)		Protein content (mg)		Total activity				Activity (/g pancreas)			
						Amylase (U)		Trypsin (mU)		Amylase (U)		Trypsin (mU)	
Group	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Normal	7	596	22	70·9	3·8	18153	1018	15508	1024	304·4	14·6	255·7	22·4
Insulin	6	625	14	77·1	2·1	25020**	1687	12307*	567	406·6**	25·5	200·5*	9·6

Statistical significance of difference between insulin values and normal values: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

Effect of litter size and insulin on amylase activity of the pancreas

When the pups were removed immediately after parturition, the amylase activity of the pancreas returned to the value for the unmated rat on day 14 after delivery. In lactating rats for which the litter size was reduced to four pups immediately after parturition, the amylase activity was intermediate between that of rats for which the litter size was adjusted to ten and that of rats with all the pups removed (Table 2).

The amylase activity on day 14 of lactation of rats receiving a daily subcutaneous injection of insulin following parturition significantly exceeded that of normal lactating rats. The activity of trypsin showed a reverse tendency, being significantly less than that of normal lactating rats (Table 3).

DISCUSSION

Although the daily food intake of the female rats during pregnancy exceeded that of the unmated rats by only 40%, it reached about fourfold the value during the second half of lactation.

There are reports indicating that pancreatic hypertrophy occurs in the second half of pregnancy and hyperplasia in the second half of lactation (Rolls *et al.* 1979; Jolicoeur *et al.* 1980). In the present study, no increase in the weight of the pancreas could be detected during the course of either pregnancy or lactation. Nevertheless, during lactation a slight increase was noted in pancreatic protein content. Trypsin activity in pancreatic tissue decreased in the second half of pregnancy. However, it started to increase after parturition and the rise in the activity paralleled the amount of food intake. It has been reported that plasma gastrin levels and increase in food intake are in parallel with remarkable consistency (Lichtenberger *et al.* 1976; Lichtenberger & Trier, 1979). It is generally accepted that gastrointestinal hormones such as gastrin and CCK not only regulate the pancreatic exocrine secretion of enzymes but increase their synthesis in acinar cells (Rothman & Wells, 1967; Mainz *et al.* 1973; Barrowman & Mayston, 1974; Ihsh *et al.* 1976; Morisset, 1980). As Rolls *et al.* (1979) reported, the rise in trypsin activity observed during lactation may be induced by the trophic effect of gastrointestinal hormones, the levels of which rise with increases in food intake.

During lactation, there was an increase in trypsin activity in the pancreatic tissue but amylase activity was significantly less than that of unmated rats. The reduction in the

activity of amylase in the pancreatic tissue may be a result of a decrease in amylase synthesis in acinar cells or an increase in the secretion of the enzyme. The pancreatic secretory responses of amylase in lactating rats, both basal and induced by caerulein, were significantly less than that of unmated rats. In consideration of these results, the decreaed amylase activity in pancreatic tissue observed in lactating rats appears not to be due to increased amylase secretion, but seems to be caused by reduced amylase synthesis.

In the lactating rats, trypsin activity rose markedly and amylase activity decreased, consequently, the amylase:trypsin ratio markedly decreased compared with that of both pregnant and unmated rats. In acinar cells, zymogen granules are stored at a constant ratio. The so-called parallel secretion theory states that secretion of digestive enzymes is non-selective and, accordingly, the ratio is constant at all times (Palade, 1975). Many papers have been published in support of this theory (Scheele & Palade, 1975; Steer & Glazer, 1976) but there are also numerous papers indicating that the pancreatic enzyme ratio changes under various conditions. In rats given trypsin inhibitor orally, the pancreatic trypsin content increased more than that of amylase (Rothman & Wells, 1967; Ihsh *et al.* 1976). In alloxan-diabetic rats, a drastic reduction in amylase and a concomitant increase in trypsin activities were observed (Palla *et al.* 1968; Soling & Unger, 1972; Sofrankova & Dockray, 1983). However, the enzyme activities in the diabetic rats could be returned to normal levels by treatment with insulin.

As shown in Table 2, when lactation stopped the pancreatic amylase activity in lactating rats returned to the same level as that in unmated rats. Restoration of amylase activity was observed when the litter size was decreased. Mizoguchi & Imamichi (1986) reported that plasma insulin concentrations in lactating rats decreased in a dose-dependent manner with increasing litter size, returning to the same values as in the unmated rats when the litter sizes were decreased and the lactation stopped. From these findings, it seems that decreased pancreatic amylase content in lactating rats was affected by the decreased plasma insulin levels. It has been suggested that the morphological arrangements reflect a regulatory role of the islet hormones in the function of the exocrine pancreas (Lifson *et al.* 1980). In the normal rat, the peri-insular cells have large nuclei and zymogen granules in contrast to the common acinar cells. This phenomenon, the so-called halo phenomenon, has long been observed to disappear in alloxan-diabetic rats (Kramer & Tan, 1968). Insulin potentiated the secretory response of amylase by CCK in the perfused rat pancreas (Saito *et al.* 1980) and also stimulated glucose uptake by pancreatic acini in vitro (Palla *et al.* 1968). Recently, specific insulin receptors have been reported to be present on the surface of acinar cells (Korc *et al.* 1978; Sankaran *et al.* 1981).

In rats, change in pancreatic amylase also seems to be related to some hormones, such as adrenaline, glucagon, thyroxine and cortisol. Harada & Katho (1981) showed that the amylase activity in adult rats was decreased by administration of adrenaline, glucagon or thyroxine. On the other hand, dexamethasone, adrenocorticotropic hormones and thyroxine increased the amylase activity in infant rats (Takeuchi *et al.* 1977) but not in adult rats (Kumegawa *et al.* 1980). Thus these hormones play a permissive role in changing pancreatic amylase but do not have a direct effect on enzyme synthesis. We assumed that the decreased plasma insulin level presumably reduced amylase synthesis in acinar cells. This is clear from our previous experimental results. As shown in Table 3, the amylase activity in lactating rats was restored by the administration of insulin.

The mechanism of insulin in controlling amylase and trypsin activity in the pancreas was not clear from the present study. However, it seems that insulin has an inverse effect on amylase and trypsin synthesis in the pancreas (Table 3). The administration of insulin in lactating rat leads to an increase in amylase activity but a decrease in trypsin activity. Recently, it has been reported that in the diabetic rat, pancreatic messenger-RNA

progressively decreased and along with this decrease, a slight increase in chymotrypsin messenger-RNA was detected (Owerbach *et al.* 1981; Sofrankova & Dockray, 1983).

The results of the present study indicate that the fall in amylase activity observed in lactating rats is due to the reduction of amylase synthesis. Furthermore, there is a possibility that insulin is required for normal or elevated rates of amylase production in lactating rats.

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