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Effects of Feeding and Infusion of Short-Chain Fatty Acids and Glucose on Plasma Insulin and Blood Glucose Levels in Sheep

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Summary

Two experiments were conducted to investigate the regulation of insulin secretion in sheep.

1) The diurnal variations of plasma immunoreactive insulin (IRI) and blood glucose levels were measured with sheep fed once daily. There was no significant changes in the plasma IRI levels, although the levels tend to raise during the 12 hr after feeding. Blood glucose levels were significantly higher at 8 and 12 hrs after feeding as compared with prefeeding levels.

2) The effects of intravenous injections of glucose, lactic, acetic, propionic, butyric, iso-valeric, capronic and caprylic acids on plasma IRI and blood glucose levels were observed.

Glucose had only little effect on the plasma IRI level. All of the short-chain fatty acids evoked a rapid and sustained elevation of plasma IRI, which were accompanied by the elevation of blood glucose. Both insulinogenic and hyperglycemic responses to the fatty acids agreed with increasing chain length, hexanoate being the most effective.

In monogastric animals, glucose is the major source of metabolic energy which is made available to the animal by digestion and absorption. Unlike monogastric animals, ruminants derive most of their energy from short-chain fatty acids, mainly acetate, propionate and butyrate, which are major end products of ruminal microbial fermentation of dietery carbohydrates in the rumen and are absorbed from the rumen. Therefore, the pattern of ruminant nutrition and intermediary metabolism differs in many respects from that of the usual monogastric patterns.

Manns and Boda demonstrated that the intravenous injection of propionate and butyrate result in a greater release of insulin than glucose in adult sheep(1). Horino *et al.* reported that infusion of several short-chain fatty acids stimulate insulin secretion in sheep and cow, but not in monogastric animals such as rats, rabbits and

pigs, and that infusion of glucose was much less effective than short-chain fatty acids on plasma insulin concentration in sheep(2).

These evidences may suggest that control of insulin secretion in ruminant is closely related to the production of short-chain fatty acid during rumen fermentation.

It is generally accepted that propionic acid of short-chain fatty acids have a glycogenic function, whereas acetic acid and butyric acid are ketogenic in function in the ruminant.

However Manns and Boda reported that the intravenous injection of butyrate and propionate produced similar hyperglycemia, and butyrate evoked greater release of insulin than propionate (1). Referring to butyrate-induced hyperglycemic response, several investigators (3, 4, 5) suggested that intravenously administered butyrate caused an increase of blood glucose by inducing glucagon release by the pancreas and subsequent hepatic glycogenolysis.

However the mechanism on the stimulative effect of butyrate on secretions of insulin and glucagon in ruminants remains to be demonstrated.

The present experiment was conducted to study the effects of feeding and infusion of several short-chain fatty acids on plasma insulin and glucose levels, and also to find a clue on the mechanism of insulin secretion in ruminant.

Materials and Methods

The animals used in the present experiment were crossbred ewes weighing 40 to 50 kg.

In the study on the magnitude of diurnal variation of plasma insulin and blood glucose, the animals were housed indoors in individual pens and fed a maintenance ration of hay and concentrates once daily at 9.00 A.M. and had free access to water. The samples of jugular blood were taken by venipuncture at 0, 2, 4, 8, 12 and 24 hours after feeding.

In the infusion experiments, the saline solutions of glucose, lactate, acetate, propionate, butyrate, iso-valerate or capronate nutralized to pH 7.0 with sodium hydroxide at a final concentration of 2.5 M were each given intravenously within 2 minutes at a dose of 1.25 m moles per kg. Caprylate solution was neutralized with sodium hydroxide after dissolving in 0.5% bovine serum albumin solution at a final concentration of 1.25 M, and given in the same way as other solution. Blood samples were taken at 0, 5, 15, 30, 60, 90 and 120 minutes after the infusion. In these experiments, at least 90 minutes before the infusion, a polyethylen tube was inserted in the bilateral jugular vein of animals, to facilitate the administration of the test solution and to aid in the collection of blood samples at precise intervals. Also 20 ml of 0.9% saline solution and 0.5% bovine serum albumin solution were each given as control experiment.

Blood plasma was separated from each sample and was frozen until insulin assay. A protein-free filtrate was prepared and used for glucose analysis.

Plasma insulin was measured by the double antibody method using a radioimmunoassay kit (Kaken Kagaku). The kit consists of a) anti-insulin serum which was obtained from guinea pig immunized with bovine insulin, b) the second precipitating antibody which was obtained from rabbits immunized with guinea pig gamma-globulin, c) highly purified crystallin human insulin which was used as the standard and d) insulin-I^{125}.

Plasma samples or the standard insulin were reacted with antibodies for 6 hrs. at 4°C before addition of I^{125} insulin. The tubes were standed for another 18 hrs. at 4°C. After immunoreaction, the precipitate of plasma insulin or standard insulin to antibodies were filtered by membrane filter under light vacuo, and were counted in a well-type crystal-scintillation counter (Kobe KOGYO CORP.). All plasma smaples and standard were analized in duplicate for each assay.

Blood glucose concentrations were determined by the glucose oxidase method described by Huggett and Nixon (6).

Results and Discussion

Diurnal Variation of Plasma Insulin and Blood Glucose:

Figure 1 shows diurnal variations of concentrations of plasma insulin and blood glucose in sheep fed once daily.

Plasma insulin level at immediately befor feeding was $14.75\pm2.50\,\mu$U/ml and altered to $18.50\pm4.43\,\mu$U/ml during the first 2 hours after feeding. There were no significant changes of plasma insulin concentration during 12 hours after feeding, although the concentrations tend to upward. Twentyfour hours after feeding, plasma insulin returned to approximately prefeeding values ($14.00\pm1.41\,\mu$U/ml).

In contrast, there were significant increases in blood glucose concentrations following the feeding, that is significantly higher at 8 hours (48.8 ± 3.30 mg/dl) and at 12 hours (54.8 ± 4.03 mg/dl) after feeding as compared with concentrations (33.50 ± 2.65 mg/dl) before feeding.

Trenkle observed that plasma insulin level in sheep increased about 23% at 4 hours after feeding, and then returned to below the prefeeding level with in 12 hours after feeding(7). He also reported blood glucose concentrations were slightly higher at 4 hours after feeding as compared with concentrations before feeding. Shambye who studied blood glucose concentrations of sheep fed every 12 hours reported that hourly blood sampling gave almost constant concentrations of glucose in portal vein and carotid artery blood for 0 until 12 hours after feeding(8). Annison *et al.* fed sheep once daily and observed a tendency for blood glucose to increase only slightly beginning at 6–8 hours after feeding(9). The changes was from an initial concentration of about 45 mg/dl to 55 mg/dl and occured over a period of 2 to 3 hours. The data presented in Fig. 1 are considerably similar to results observed by Annison and Trenkle.

Figure 1 shows also the results of the diurnal variation on serum insulin and

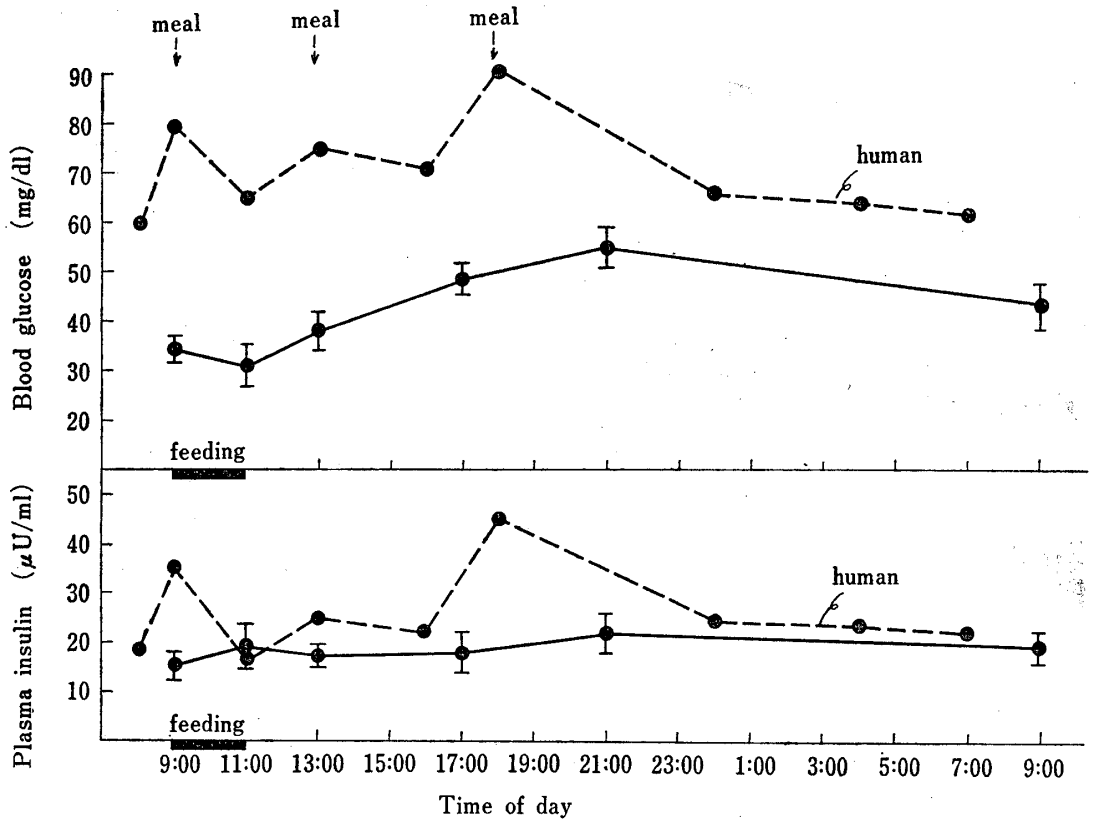

FIG. 1. Diurnal changes of plasma insulin and blood glucose levels in sheep fed once daily. The data are the mean values from 4 sheep. The data on human, presented here, was from a study reported by Toyota (10).

blood glucose concentrations in human reported by Toyota(10). There are remarkable difference in both concentrations of blood glucose and plasma insulin between human and sheep. In human, both blood glucose and plasma insulin concentrations rose accordance with each food intake at the hours of 9, 12 and 18.

These different patterns will be realized by the fact that in sheep, dietary carbohydrates are converted rapidly to short-chain fatty acids during rumen fermentation and little glucose is absorbed from digestive tract into circulation system.

Although blood glucose in sheep during pre and post feeding had low concentrations and much less fluctuations compared with patterns in human, it is certain that slight increase in blood glucose concentration occured at 12 hours after feeding. Since it is generally considered that concentrations of short-chain fatty acids in rumen contents increased to a maximum level at 3 to 6 hours after feeding, this increase in blood glucose levels would seem to be caused by gluconeogenesis from propionate and other glucogenic substances produced in and absorbed from rumen.

Changes of Blood Glucose and Plasma Insulin Concentrations in Response to Short-Chain Fatty Acids, Lactic Acid and Glucose Injection:

Typical alteration of blood glucose and plasma insulin concentrations in sheep before and after intravenous administration of glucose, lactic acid and several short-chain fatty acids are shown in Fig. 2 and 3. No significant changes were observed in blood glucose after injections of lactate, acetate, saline and 0.5% albumin solutions. The injections of short-chain fatty acids, except acetate, caused rapid increases in blood glucose concentration with varied degrees of the magnitude. In the injection of propionate, butyrate or caprylate, blood glucose concentration rose from approximately 40 mg/dl to 70 mg/dl respectively within 20 minutes, then started to decline at 30 minutes and remained somewhat higher levels than pre-injection level at 120 minutes after injection. Iso-valerate and capronate had more potent effect on the hyperglycemic response than propionate and others; the highest glucose level, 90 to 95 mg/dl, was attained within 15 minutes after injection.

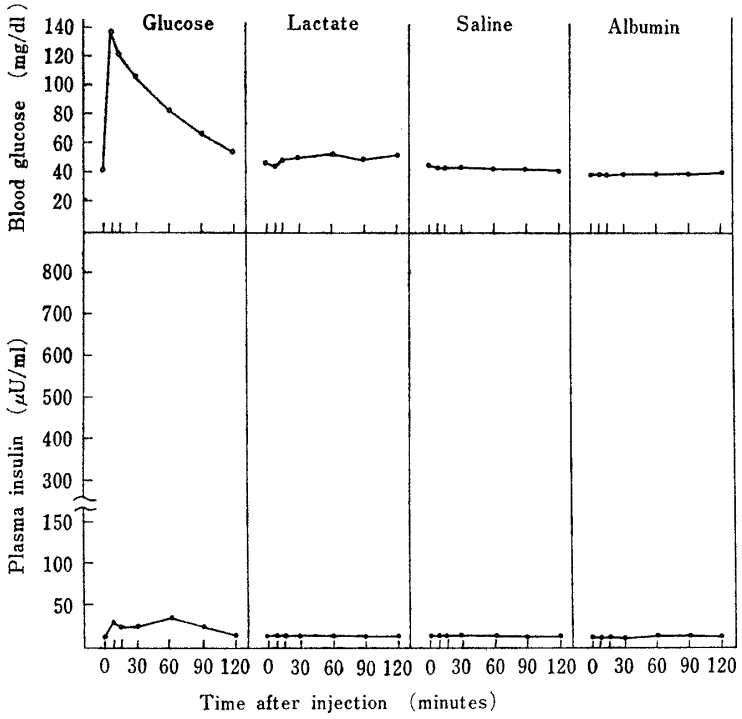

FIG. 2. The effect of intravenous injections of glucose (1.25 m moles/kg), lactate (1.25 m moles/kg), saline and albumin on plasma insulin and blood glucose in sheep.

Plasma insulin concentrations changed with similar pattern to that in blood glucose concentration after administration of each substance with exceptions of glucose and acetate. The injection of equimolar quantities of glucose produced significantly less elevation in plasma insulin concentration despite blood glucose

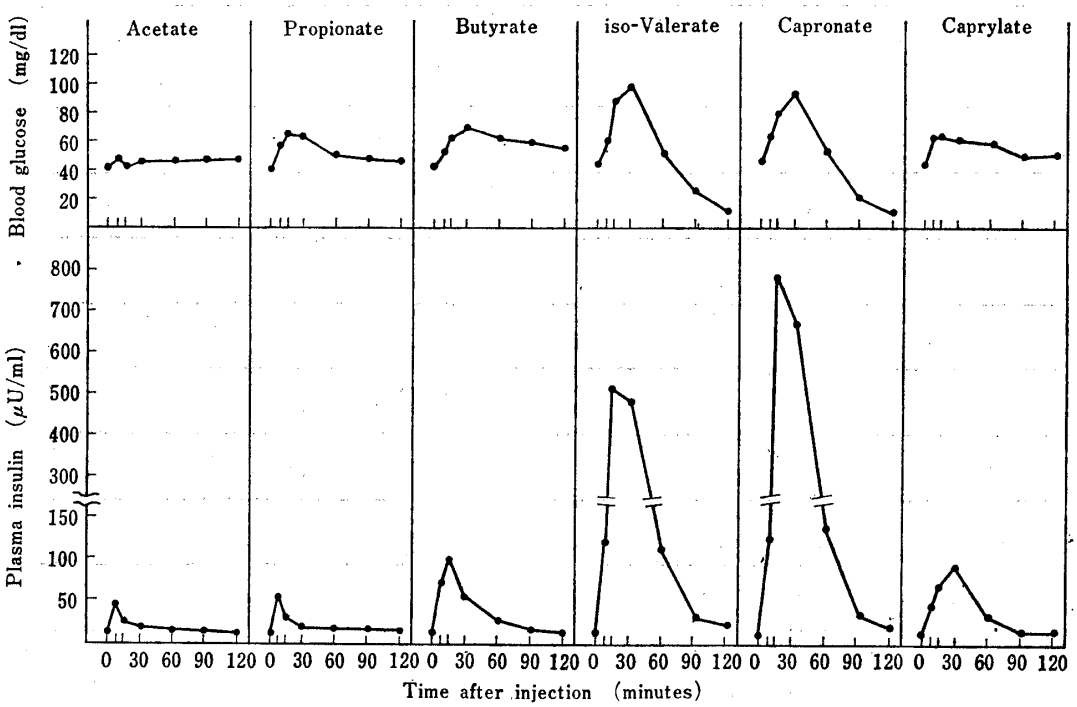

FIG. 3. The effect of intravenous injection of equimolar amounts (1.25 m moles/kg) of short-chain fatty acids on plasma insulin and blood glucose in sheep.

level was several times greater than those seen following injections of the short-chain fatty acids. Acetate injection produced a small but substantial increase in the plasma insulin concentration although acetate did not cause any changes in the blood glucose concentration. The injections of saline and albumin solution did not affect plasma insulin concentration.

The injection of short-chain fatty acids produced apparent increases in plasma insulin concentration within 15 minutes in all cases. This elevation was particularly striking in the case of iso-valerate or capronate injection; 520 and 780 μU/ml respectively.

To know the relation between plasma insulin secretory response and hyperglycemic responses to the injection of the substances mentioned above, the maximum concentrations of plasma insulin and b ood glucose, and the times required to reach maximum concentration after injection were presented in Fig. 4. It is suggested that all fatty acids containing 3 to 8 carbon atomes evoked greater release of insulin than glucose and that the increase of plasma insulin levels were associated with the hyperglycemic responses to the short-chain fatty acids and both responses increased with increasing chain length of the short-chain fatty acids. Both hyperglycemic and insulinogenic responses were maximum after injection of hexanoate and were much less by octanoate than hexanoate.

Concerning the effect of short-chain fatty acids on blood glucose concentration, Ash *et al.*(11) reported that acetate had a very small effect on blood glucose con-

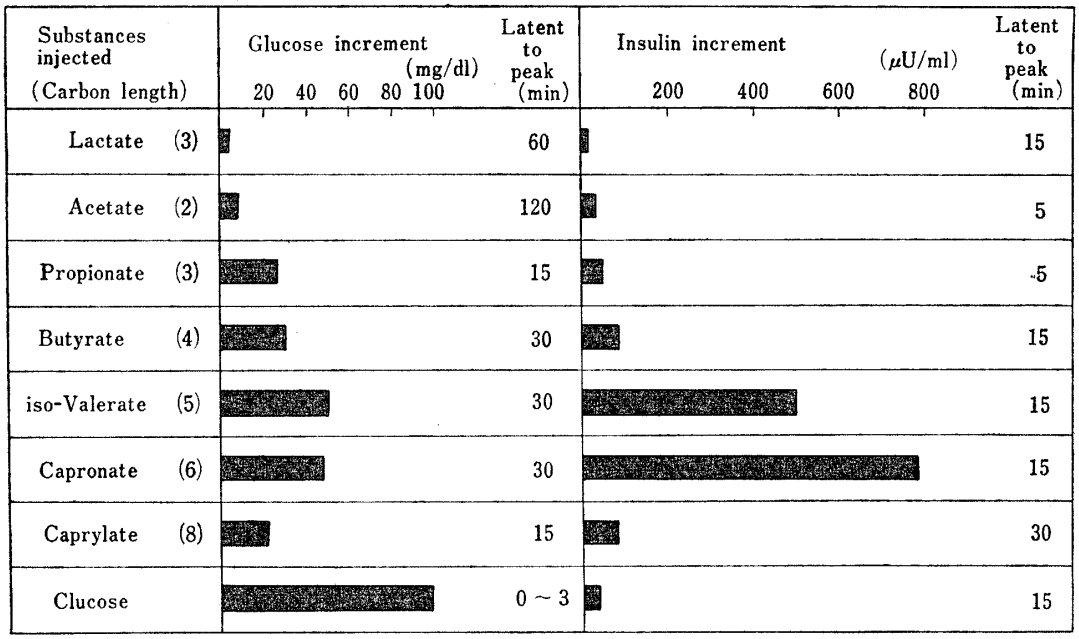

FIG. 4. The relation of insulinogenic and hyperglycemic responses to the intravenous injection of equimolar amounts (1.25 m moles/kg) of short-chain fatty acids in sheep.

centration, butyrate was more effective than equimolar amount of propionate, and capronate appeared to be more potent than butyrate. Phillips *et al.* injected several fatty acids (C2 to C10) into sheep and indicated that a maximal glycemic response was induced by hexanoate and octanoate, and that decanoate was much less than octanoate(4). The changes in blood glucose concentration presented here are similar to results reported by Ash *et al.* and Phillips *et al.*

In this study we found that the degree of plasma insulin response was also proportional to the chain length of fatty acids. In view of the increasing glycemic response with increase in chain length of the acids, Ash studied the possibility that their hyperglycemic effect may be due to the surface activity of the fatty acids(11). To test this possibility, they injected intravenously sodium dodecyl sulfate with sufficient amounts to cause slight haemolysis, and observed that little or no effect upon the blood glucose concentration occured. Several workers have investigated the changes of blood glucose concentration in sheep and goats given short-chain fatty acids particularly butyrate. It was earlier considered that the butyrate-induced hyperglycemia was due to excitement which resulted in release of epinephrine and, subsequently, in increased hepatic glycogenolysis. However Phillips *et al.* demonstrated, using adrenalectomized sheep, that butyrate -induced hyperglycemic response was not due to epinephrine. Phillips and Black reported that butyrate administered intravenously to sheep was not converted to glucose and resulted in increased hepatic phosphorylase activity and decreased hepatic glycogen content (3). Furthermore, Phillips *et al.* (4) and Jones *et al.* (5) demonstrated that sodium butyrate administered intravenously to pancreatectom-

ized sheep did not cause significant increases in blood glucose concentration. These authors suggested the possibility that butyrate administration cause an increase of blood glucose by inducing glucagon release by the pancreas and subsequent hepatic glycogenolysis. Yoshioka demonstrated using adrenalectmized and alloxan treated dog that stimulation of vagus accelerated both insulin and glucagon secretion (12). Obara *et al.* observed in sheep that vagotomy completely abolishes the hyperglycemic response following intraruminal butyrate administration (13). The result obtained by Obara *et al.* indicate the possibility that butyrate accerelates glucagon release by inducing the stimulation of vagus control to the pancreas.

On the other hand, there are very few reports about the mechanism on stimulation of insulin secretion by the short-chain fatty acids in sheep. It has been suggested by several investigators (14, 15, 16, 17) that epinephrine inhibits the insulinogenic effects of glucose under *in vivo* and *in vitro* conditions. Hertelendy *et al.* recently reported that epinephrine inhibits the insulin secretory response to propionate, butyrate and arginine in sheep and that this inhibitory effect of epinephrine was blocked by alpha adrenergic receptor blockade but not by beta adrenergic receptor blockade (18). Turtle and Kipnis (19) demonstrated on the basis of studies of the effects of theophylline on insulin secretion of rat pancreatic islet, that alpha receptor activity inhibits insulin secretion by inducing the decrease of cyclic AMP concentration in the cell and that beta receptor activity stimulates insulin secretion by inducing the increase of cyclic AMP. The results obtained in sheep by Hertelendy *et al.* are consistent with the observation reported by Turtle.

Although a mechanism of insulinogenic effect caused by the administration of short-chain fatty acids is left unsolved, it is significant in that the ruminant nutrition that short-chain fatty acids which represent the major energy source for the ruminant has a much greater effect on insulin release than glucose.

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References

1) Manns, J.G. and Boda, J.M., *Amer. J. Physiol.*, **221**, 747 (1966)
2) Horino, M., Machlin, L.J., Hertelendy, F. and Kipnis, D.M., *Endocrinol.*, **83**, 118 (1983)
3) Phillips, R.W. and Black, A.L., *Comp. Biochem. Physiol.*, **18**, 527 (1966)
4) Phillips, R.W., House, W.A., Miller, R.A., Mott, J.L. and Sooby, D.L., *Amer. J. Physiol.*, **217**, 1265 (1969)

5) Jones, K.L., Bell, R.L., Oyler, J.M. and Goetsch, D.D., *Amer. J. Vet. Res.*, **31**, 81 (1970)
6) Huggett, A.G. and Nixon, D.A., *Lancet*, II, 368 (1957)
7) Trenkle, A., *J. Nutrition*, **100**, 1323 (1970)
8) Shambye, P., *Nord. Veterinar. Med.*, **3**, 555 (1951)
9) Annison, E.F., Hill, K.J. and Lewis, D., *Biochem. J.*, **66**, 592 (1957)
10) Toyota, T., *Tohoku J. exp. Med.*, **98**, 345 (1969)
11) Ash, R.W., Pennington, R.J. and Reid, R.S., *Biochem. J.*, **90**, 353 (1964)
12) Yoshioka, H., *Naibunpitsu*, **3**, 97 (1956) (in Japanese, with English summary)
13) Obara, Y., Sasaki, Y., Watanabe, S. and Tsuda, T., *Tohoku J. Agr. Res.*, **22**, 156 (1971)
14) Coore, H.G. and Randle, P.J., *Biochem. J.*, **93**, 66 (1964)
15) Porte, D., Jr., Graber, A.L., Kuzuya, T. and Williams, R.H., *J. Clin. Invest.*, **45**, 228 (1966)
16) Hertelendy, F., Machlin, L.J., Gordon, R.S., Horino, M. and Kipnis, D.M., *Proc. Soc. Exp. Bio. Med.*, **121**, 675 (1966)
17) Kris, A.O., Miller, R.E., Wherry, F.E. and Nason, J.W., *Endocrinol.*, **78**, 87 (1966)
18) Hertelendy, F., Machinlin, L. and Kipnis, D.M., *Endocrinol.*, **84**, 192 (1969)
19) Turtle, J.R. and Kipnis, D.M., *Biochem. Biophys. Res. Commun.*, **28**, 797 (1967)