

# EFFECTS OF LIPOLYTIC AND ANTILIPOLYTIC AGENTS ON GLYCEROL AND FREE FATTY ACID RELEASE FROM ISOLATED ADIPOCYTES OF NORMAL AND DIABETIC RATS

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## ABSTRACT

Isolated adipocytes from severely diabetic rats exhibited hypersensitivity to epinephrine at low concentrations (0.05–0.1  $\mu$ M) on lipolysis, compared with isolated adipocytes from normal and mildly diabetic rats. Hypersensitivity to dibutyryl cyclic AMP and theophylline at concentrations from 0.05 to 0.50 mM was not observed in adipocytes of severely diabetic rats. Insulin could not exert an inhibitory effect on epinephrine-induced lipolysis in adipocytes of severely diabetic rats. In isolated adipocytes from normal rats, hyperosmolarity due to the combination of 50 mM glucose and 100 mM sodium chloride only had an inhibitory effect on 0.25  $\mu$ M epinephrine-induced lipolysis. Ten mM  $\beta$ -hydroxybutyrate did not inhibit lipolysis caused by epinephrine although any lipolysis stimulated by epinephrine, dibutyryl cyclic AMP and theophylline was inhibited by insulin. Our present findings may partly explain insulin resistance in the severely diabetic state and the pathogenesis of the absence of ketosis in hyperglycemic hyperosmolar conditions.

Key Words:  $\beta$ -Hydroxybutyrate, Hyperosmolarity, Insulin, Isolated adipocytes, Lipolytic and antilipolytic agents

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## INTRODUCTION

It is well established that diabetes mellitus is associated with a state of increased lipolysis.<sup>1,2)</sup> Furthermore, it has also been demonstrated that ketoacidosis is accompanied by a temporary insulin resistance that is possibly due to multiple factors such as increased release of counterregulatory hormones.<sup>3,4)</sup> However, in vitro studies on adipocytes have not established yet whether or not an increased effect of counterregulatory hormones on lipolysis and a decreased antilipolytic effect of insulin actually occur in diabetic adipocytes.<sup>5-8)</sup> This confusion is based mainly on the different conditions of the diabetic state.

Thus, the present study was undertaken in an attempt to clarify the effects of counterregulatory hormones on lipolysis and the antilipolytic action of insulin in diabetic adipocytes. In addition, the effects of insulin on lipolysis induced by epinephrine, dibutyryl cyclic AMP and theophylline in isolated adipocytes from normal rats were studied in comparison with the effects of  $\beta$ -hydroxybutyrate and hyperosmolarity.

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## MATERIALS AND METHODS

Male Wistar rats weighing 200–250 g were used. The experimental groups of rats were divided into the following: (1) normal group, (2) mildly diabetic group, and (3) severely diabetic group. The two different groups of diabetic rats were prepared by the following procedure: mild and severe diabetes were induced by the intravenous injection of 40 mg/kg and 60 mg/kg, respectively, of streptozotocin (The Upjohn Co., Kalamazoo, USA) dissolved in 0.9 % NaCl after 20 hr' fasting. The studies were performed seven days after the administration of streptozotocin and the rats were kept on standard chow (Oriental Kobo Co., Tokyo, Japan) without any insulin injections. In each of these three groups the experiments were performed after 24 hr' fasting.

Isolated adipocytes were prepared with collagenase (CLS II: Worthington Biochemical Co., New Jersey, USA) by the method of Rodbell,<sup>9)</sup> with only minor modifications, which are described in detail in our previous publication.<sup>10)</sup> The fat cells obtained were finally suspended in 4 % (W/V) bovine serum albumin (fraction V: Worthington Biochemical Co., New Jersey, USA)–Krebs Ringer bicarbonate buffer containing 5  $\mu$  moles of glucose (Katayama Chemical Co., Tokyo, Japan) per ml. Bovine serum albumin used in the present study was dialyzed against 0.9 % NaCl solution in the cold room after it was defatted with 95 % ethyl alcohol solution (Katayama Chemical Co., Tokyo, Japan). A cell count was performed on an aliquot in a Bürker-Türk chamber and the final volume of the cell suspension was adjusted to  $2-3 \times 10^6$  cells per vial. A 0.25 ml volume of adipocyte suspension was pipetted into a plastic vial and the volume was made up to 2 ml with Krebs Ringer bicarbonate buffer containing 4 % bovine serum albumin and 5  $\mu$  moles of glucose per ml. After a preincubation period of 15 min, the solutions of hormones and drugs were added to the medium, as indicated in the figures and tables, and incubated for 120 min at 37°C in a Dubnoff Metabolic Shaking Incubator (GCA Co., USA), at pH 7.4, with a gas mixture of 95 % O<sub>2</sub> and 5 % CO<sub>2</sub> atmosphere. Experiments were performed in duplicate. At the end of incubation, aliquots of the medium were analyzed in duplicate for glycerol by the enzymatic method of Eggstein and Kuhlmann<sup>11)</sup> and for free fatty acids (FFA) by the colorimetric method of Duncombe.<sup>12)</sup> Blood glucose levels of the rats were measured by the glucose-oxidase method using a Glucostat<sup>R</sup> kit (Worthington Biochemical Co., New Jersey, USA).

Most reagents used in the present study were obtained from Boehringer Mannheim Yamanouchi Co. (Tokyo, Japan) and Sigma Chemicals (St. Louis, USA). Epinephrine was purchased from Daiichi Pharmaceutical Co. (Tokyo, Japan) and bovine insulin was a gift from Shimizu Pharmaceutical Co. (Shimizu, Japan).

The significance was calculated using Student's t test for paired data within groups and for unpaired data between the two groups applying a 5 % confidence limit.

## RESULTS

### *Conditions of experimental animals.*

In Table 1, the mean values of fasting blood glucose, body weight, epididymal fat pad weight and the ratio of epididymal fat pad weight to body weight are shown. For fasting blood glucose,  $259 \pm 49$  mg/dl in mildly diabetic rats and  $440 \pm 40$  mg/dl in severely diabetic rats were significantly high values compared with the level of  $102 \pm 5$  mg/dl in normal rats. All other values went in the order of normal, mildly diabetic and severely diabetic rats.

### *Lipolytic actions of epinephrine, dibutyryl cyclic AMP and theophylline*

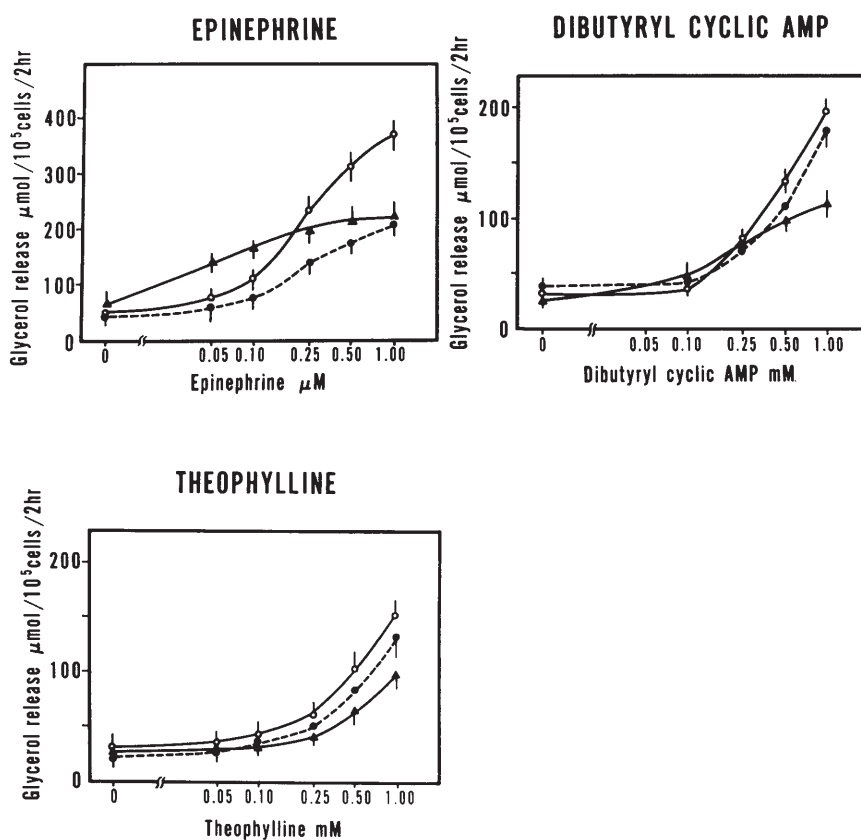
Fig. 1 shows the dose-response curves for epinephrine-, dibutyryl cyclic AMP- and theophylline-induced glycerol release from isolated adipocytes of normal, mildly, and severely diabetic rats.

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Table 1. Effect of diabetes with treatment of different doses of streptozotocin after 1 week

Rats	Fasting blood glucose (mg/dl)	Body weight (g)	Epididymal fat pad weight (mg)	EFPW(mg)/BW(g)
Normal (n = 58)	102 ± 5	248 ± 5	1820 ± 156	706 ± 35
Mild diabetes (n = 82)	259 ± 49 <sup>b</sup>	224 ± 4 <sup>a</sup>	1062 ± 122 <sup>a</sup>	449 ± 33 <sup>a</sup>
Severe diabetes (n = 138)	440 ± 40 <sup>a,d</sup>	201 ± 2 <sup>a,c</sup>	528 ± 55 <sup>a,c</sup>	242 ± 14 <sup>a,c</sup>

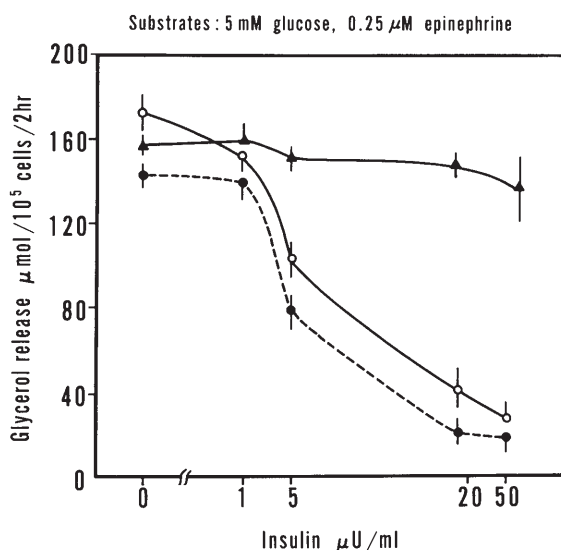
Mean values ± SEM

<sup>a,b</sup>P < 0.001, 0.02 versus normal; <sup>c,d</sup>P < 0.001, 0.02 versus mild diabetesFig. 1 Effects of epinephrine, dibutyryl cyclic AMP, and theophylline on lipolysis in isolated adipocytes from normal ( $\circ$ ), mildly diabetic ( $\bullet$ ) and severely diabetic ( $\blacktriangle$ ) rats. Mean values  $\pm$ SEM from 4-6 experiments.

The effect of epinephrine on the lipolytic action at low concentrations (0.05–0.1  $\mu\text{M}$ ) was stronger in the adipocytes of severely diabetic rats than in those of either normal or mildly diabetic rats. However, at high concentrations of epinephrine (0.25–1.0  $\mu\text{M}$ ), the isolated adipocytes from normal rats were more sensitive to epinephrine than those of both mildly and severely diabetic rats. The adipocytes from mildly diabetic rats had a low sensitivity to epinephrine-induced glycerol release at concentrations ranging from 0.05 to 0.50  $\mu\text{M}$  compared with the adipocytes from severely diabetic rats. On the other hand, as to dibutyryl cyclic AMP- and theophylline-induced glycerol release, there were no significant differences at the concentration range 0.05 to 0.5 mM among the three groups, but the isolated adipocytes from severely diabetic rats were less sensitive to 1.0 mM dibutyryl cyclic AMP compared with the other two groups.

However, insulin did not inhibit 0.25  $\mu\text{M}$  epinephrine-induced lipolysis in isolated adipocytes from severely diabetic rats, as it did in adipocytes from normal and mildly diabetic rats (Fig. 2).

Fig. 2 Interrelation between epinephrine and insulin on lipolysis in isolated adipocytes from normal ( $\circ$ ), mildly diabetic ( $\bullet$ ) and severely diabetic ( $\blacktriangle$ ) rats. Mean values  $\pm$ SEM from 5–6 experiments.



#### *Antilipolytic effects of insulin, hyperosmolarity and $\beta$ -hydroxybutyrate*

In Table 2, the inhibitory effects of insulin, hyperosmolarity and  $\beta$ -hydroxybutyrate on epinephrine-, theophylline- and dibutyryl cyclic AMP-induced lipolysis in isolated adipocytes from normal rats are shown, with the values of the percent changes versus the results of each control (the values of FFA or glycerol release caused by 0.25  $\mu\text{M}$  epinephrine, 0.5 mM theophylline or 0.5 mM dibutyryl cyclic AMP alone). Insulin (50  $\mu\text{U/ml}$ ) had a marked inhibitory effect on the lipolysis caused by all three agents (Table 2). The antilipolytic action of hyperosmolarity, induced by the combination of 50 mM glucose and 100 mM NaCl, was observed only in epinephrine-induced lipolysis. On the other hand, 10 mM  $\beta$ -hydroxybutyrate inhibited theophylline- and dibutyryl cyclic AMP-induced lipolysis. Although marked inhibition was observed in theophylline-induced lipolysis, there was no inhibitory effect of  $\beta$ -hydroxybutyrate on epinephrine-induced lipolysis, as observed with insulin or hyperosmolarity.

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Table 2. Effects of insulin, hyperosmolarity and  $\beta$ -hydroxybutyrate on epinephrine-, dibutyryl cyclic AMP- or theophylline-induced lipolysis in isolated adipocytes from normal rats

Additions	% Changes of the rate with hormones and drugs	
	FFA	Glycerol
Epinephrine 0.25 $\mu$ M (Control)	100	100
+Insulin (50 $\mu$ U/ml)	15 $\pm$ 7 <sup>a</sup>	39 $\pm$ 3 <sup>a</sup>
+Glucose (50mM) and NaCl(100mM)	55 $\pm$ 7 <sup>a</sup>	58 $\pm$ 7 <sup>a</sup>
+ $\beta$ -Hydroxybutyrate (10mM)	101 $\pm$ 4	100 $\pm$ 1
	$p < 0.001$	$p < 0.001$
	$p < 0.001$	$p < 0.02$
	$p < 0.001$	$p < 0.001$
Dibutyryl cyclic AMP 0.50mM (Control)	100	100
+Insulin (50 $\mu$ U/ml)	23 $\pm$ 10 <sup>a</sup>	57 $\pm$ 3 <sup>a</sup>
+Glucose (50mM) and NaCl(100mM)	100 $\pm$ 2	121 $\pm$ 28
+ $\beta$ -Hydroxybutyrate (10mM)	81 $\pm$ 14 <sup>b</sup>	85 $\pm$ 2 <sup>a</sup>
	$p < 0.001$	$p < 0.001$
	$p < 0.01$	$p < 0.01$
	$p < 0.01$	$p < 0.05$
Theophylline 0.50 mM (Control)	100	100
+Insulin (50 $\mu$ U/ml)	24 $\pm$ 12 <sup>a</sup>	50 $\pm$ 9 <sup>a</sup>
+Glucose (50mM) and NaCl(100mM)	104 $\pm$ 13	118 $\pm$ 22
+ $\beta$ -Hydroxybutyrate (10mM)	51 $\pm$ 10 <sup>a</sup>	36 $\pm$ 6 <sup>a</sup>
	$p < 0.001$	$p < 0.01$
	$p < 0.01$	$p < 0.01$
	NS	NS

Substrate : 5 mM glucose ; mean values  $\pm$  SEM from 4 ~ 8 experiments

Epinephrine-induced glycerol and FFA releases were 252  $\pm$  8 and 455  $\pm$  40 n mol/10<sup>5</sup> cells/2hr ;  
 Dibutyryl cyclic AMP-induced glycerol and FFA releases were 129  $\pm$  8 and 301  $\pm$  46 n mol/10<sup>5</sup> cells/2hr ;  
 Theophylline-induced glycerol and FFA releases were 125  $\pm$  12 and 271  $\pm$  36 n mol/10<sup>5</sup> cells/2hr.

<sup>a, b</sup>  $p < 0.001, 0.01$  versus control (epinephrine, dibutyryl cyclic AMP or theophylline alone)

## DISCUSSION

In the poorly controlled diabetic state, there are high levels of counterregulatory hormones like epinephrine and growth hormone,<sup>3,4)</sup> resulting in multiple abnormalities of metabolism such as increased lipolysis together with a low serum insulin level.<sup>4)</sup> It is reported that this increased lipolysis in diabetics is based on the hypersensitivity of adipocytes to epinephrine<sup>5,7)</sup> and growth hormone.<sup>8)</sup> However, other reports showed no alteration of the sensitivity to lipolytic stimuli in diabetic adipocytes.<sup>6)</sup>

From our present study, it is clear that the presence or the absence of hypersensitivity in diabetic adipocytes depends on whether there is a mild or severe diabetic state. Zapf et al.<sup>5)</sup> have reported that adipose tissue from severely diabetic rats (diabetes induced by injection of streptozotocin 70 mg/kg) exhibited half-maximal lipolytic responses to epinephrine at concentrations 5 to 10 times lower) 0.0027 to 5.4  $\mu$ M than those required in adipose tissue from normal rats. This observation was supported by our present study of low concentrations of epinephrine. However, we did not observe the hypersensitivity of adipocytes of severely diabetic rats to epinephrine at high concentrations (over 0.25  $\mu$ M) which they also reported. There are several reasons for the differing results of the two studies: (1) we used isolated adipocytes in contrast with their use of intact adipose tissue;<sup>5)</sup> (2) the numbers of cells and the triglyceride content in the tissue per vial were about 5 times higher in their study than in ours, following from the previous study<sup>10)</sup> compared with those by Zapf et al.<sup>5)</sup>

We did not observe hypersensitivity of adipocytes from severely diabetic rats to dibutyryl cyclic AMP and theophylline as observed with epinephrine. A similar phenomenon is reported for ACTH and glucagon, that diabetic adipose tissue is less responsive to these two hormones than normal

adipose tissue.<sup>5)</sup> The finding of the marked contrast between epinephrine and the other two hormones was explained by the concept of different hormone discriminators on the adipocyte membranes. However, this explanation is not sufficient in our case. The control of lipolysis is a complex process involving receptor binding of the lipolytic agent and post-receptor events including the cyclic AMP cascade. At the fat cell membrane level, increased hormone-receptor binding<sup>13)</sup> and/or increased stimulus generation via the adenylyl cyclase system<sup>13,14)</sup> result in increased cyclic AMP formation. Inside the fat cell (beyond hormone-receptor interaction) cyclic AMP-degradation by phosphodiesterase may be decreased or protein kinase and/or triglyceride lipase may be more sensitive to activation.<sup>16,17)</sup> Our data, showing no significant differences of dibutyryl cyclic AMP- and theophylline-induced lipolysis among mildly diabetic, severely diabetic, and normal rats, strongly indicate that there is no intracellular difference in the sensitivity of the lipolytic response among the three groups, since theophylline is a potent inhibitor of phosphodiesterase.<sup>18)</sup> Moreover, in addition to our report and that of Zapf *et al.*,<sup>5)</sup> it was also reported that adenosine deaminase greatly enhanced lipolysis in isolated adipocytes from diabetic rats compared with controls.<sup>8)</sup> Thus, the hypersensitivity of adipocytes in severe diabetes to epinephrine, and the lessened sensitivity to insulin effects on epinephrine-induced lipolysis may be related to the enhanced sensitivity to adenosine suppression of lipolysis observed in the diabetic state. In our study, the lipolytic and antilipolytic responses in mild diabetes were similar to those in normal rat adipocytes. This might be strongly related to the relative preservation of pancreatic  $\beta$ -cell function in mildly diabetic rats.

It is also interesting that hyperosmolarity, prepared with the combination of 50 mM glucose and 100 mM sodium chloride, strongly inhibited epinephrine-induced lipolysis and that insulin,  $\beta$ -hydroxybutyrate, and hyperosmolarity, respectively, exerted different antilipolytic actions on epinephrine-induced lipolysis in isolated adipocytes from normal rats. Our findings suggest that one of the possible factors for the absence of ketosis that is sometimes seen under hyperglycemic hyperosmolar conditions may be the combination of hyperglycemia and hypernatremia observed during the clinical course.<sup>19)</sup> Hyperosmolarity inhibited only epinephrine-induced lipolysis, while  $\beta$ -hydroxybutyrate, one of the ketone bodies, markedly inhibited theophylline-induced lipolysis and mildly inhibited dibutyryl cyclic AMP-stimulated lipolysis, although lipolysis caused by all of these three agents was markedly inhibited by insulin. It is well known that the inhibitory action of insulin on lipolysis is related to many steps of the mechanism of the lipolytic response.<sup>20-22)</sup> The antilipolytic effect of hyperosmolarity in isolated adipocytes might be due to the following mechanisms: (1) direct inhibitory action on the fat cell membrane at the level of the hormone-receptor and/or the adenylyl cyclase system; (2) inhibition of the passage of the products of lipolysis through the cell membrane; and (3) interference with the  $\text{Na}^+/\text{K}^+$ -ATPase mechanism linked with the active transport of FFA as mentioned by Shimmel and Goodman.<sup>21)</sup> In contrast, there is the possibility that the antilipolytic action of  $\beta$ -hydroxybutyrate may occur via the post-receptor mechanism, especially via increased degradation of cyclic AMP.

## CONCLUSION

Our present findings indicate that the high concentrations of FFA and glycerol in the serum of diabetics with poorly controlled blood glucose are possibly related to the hypersensitivity to catecholamines seen in adipocytes from severe diabetics, and this hypersensitivity to catecholamines may also be linked with insulin resistance. However, none of these phenomena are observed in isolated adipocytes from mildly diabetic rats, which may be related to their relative preservation of  $\beta$ -cell function. Since the absence of ketosis under hyperglycemic hyperosmolar conditions is occasionally observed clinically, hyperosmolarity may act through inhibition of FFA release from adipocytes via a different mechanism from the antilipolytic action of insulin. From our data and

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those of others it would appear that the lipolytic and antilipolytic processes are complicated by the availability and actions of a variety of hormones and reagents.

## REFERENCES

- 1) Östman, J.: Studies in vitro on fatty acid metabolism of human subcutaneous adipose tissue in diabetes mellitus. *Acta Med. Scand.*, **177**, 639–655 (1965).
- 2) Björntorp, P. and Hood, B.: Studies on adipose tissue from obese patients with or without diabetes mellitus. 1. Release of glycerol and free fatty acids. *Acta Med. Scand.*, **179**, 221–227 (1966).
- 3) Waldhäusl, W., Kleinberger, G., Korn, A., Dudczak, R., Bratusch-Marrain, P. and Nowotny, P.: Severe hyperglycemia: Effects of rehydration on endocrine derangements and blood glucose concentration. *Diabetes*, **28**, 577–584 (1979).
- 4) Schade, D.S., Santiago, J.V., Skyler, J.S. and Rizza, R.A.: Intensive Insulin Therapy, (1983), Excerpta Medica, Amsterdam.
- 5) Zapf, J., Feuerlein, D., Waldvogel, M. and Froesch, E.R.: Increased sensitivity of diabetic rat adipose tissue towards the lipolytic action of epinephrine. *Diabetologia*, **11**, 509–516 (1975).
- 6) Lacasa, D., Agli, B. and Giudicelli, Y.: Effects of experimental insulin-dependent diabetes on the  $\beta$ -adrenergic-receptor-coupled adenylate-cyclase system and lipolysis in fat cells of the rat. *Eur. J. Biochem.*, **130**, 457–464 (1983).
- 7) Solomon, S.S., Heckemeyer, C.M., Barker, J.A. and Duckworth, W.C.: Hormonal control of lipolysis in perfused adipocytes from diabetic rats. *Endocrinology*, **117**, 1350–1354 (1985).
- 8) Solomon, S.S., Schwartz, Y. and Rawlinson, T.: Lipolysis in diabetic adipocytes: Differences in response to growth hormone and adenosine. *Endocrinology*, **121**, 1056–1060 (1987).
- 9) Rodbell, M.: Metabolism of isolated fat cells. 1. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.*, **239**, 375–380 (1964).
- 10) Hotta, N., Sirek, O.V. and Sirek, A.: Studies on the interaction between growth hormone and dihydroergotamine in adipose cells. 1. The lipolytic effect of dihydroergotamine. *Horm. Metab. Res.*, **3**, 161–166 (1971).
- 11) Eggstein, M. and Kuhlmann, E.: Triglycerides and glycerol. Determination after alkaline hydrolysis. In *Methods of Enzymatic Analysis*, edited by Bergmeyer, H.U., **4**, 1825–1831 (1974), Verlag Chemie Weinheim, Academic Press, New York, London.
- 12) Duncombe, W.G.: The colorimetric micro-determination of long-chain fatty acids. *Biochem. J.*, **88**, 7–10 (1963).
- 13) Birnbaumer, L. and Rodbell, M.: Adenyl cyclase in fat cells. II. Hormone receptors. *J. Biol. Chem.*, **244**, 3477–3482 (1969).
- 14) Braun, T. and Hechter, O.: Glucocorticoid regulation of ACTH sensitivity of adenyl cyclase in rat fat cell membranes. *Proc. Nat. Acad. Sci. (Wash.)*, **66**, 995–1001 (1970).
- 15) Butcher, R.W.: Cyclic 3', 5'-AMP and the lipolytic effects of hormones on adipose tissue. *Pharmacol. Rev.*, **18**, 237–241 (1966).
- 16) Huttunen, J.K. and Steinberg, D.: Activation and phosphorylation of purified adipose tissue hormone-sensitive lipase by cyclic AMP-dependent protein kinase. *Biochim. Biophys. Acta.*, **239**, 411–427 (1971).
- 17) Soderling, T.R., Corbin, J.D. and Park, C.R.: Regulation of adenosine 3', 5'-monophosphate-dependent protein kinase. II. Hormonal regulation of the adipose tissue enzyme. *J. Biol. Chem.*, **248**, 1822–1829 (1973).
- 18) Fain, J.: Biochemical aspects of drug and hormone action on adipose tissue. *Pharmacol. Rev.*, **25**, 67–118 (1973).
- 19) Matz, R.: Coma in the nonketotic diabetic (Hyperosmolar nonketotic coma (HNKC) in the diabetic), In *Diabetes Mellitus-Theory and Practice*, edited by Ellenberg, M. and Rifkin, H. 655–666 (1983), Medical Examination Publishing Co., New York.
- 20) Senft, G., Schultz, G., Munske, K. and Hoffmann, M.: Influence of insulin on cyclic 3', 5'-AMP phosphodiesterase activity in liver, skeletal muscle, adipose tissue, and kidney. *Diabetologia*, **4**, 322–329 (1968).
- 21) Loten, E.G. and Sneyd, J.G.T.: An effect of insulin on adipose-tissue adenosine 3': 5'-cyclic monophosphate phosphodiesterase. *Biochem. J.*, **120**, 187–193 (1970).
- 22) Desai, K.S., Li, K.C. and Angel, A.: Bimodal effect of insulin on hormone-stimulated lipolysis: Relation to intracellular 3', 5'-cyclic adenylic acid and free fatty acid levels. *J. Lipid. Res.*, **14**, 647–655 (1973).
- 23) Schimmel, R.J. and Goodman, H.M.: Efflux of free fatty acids from adipose tissue. *Am. J. Physiol.*, **221**, 118–122 (1971).