Beta Adrenergic Receptor Mechanisms in the Metabolic Effects of Diazoxide in Fasted Rats

Paul G. Walfish, M.D., F.R.C.P. (C), Renato Natale, Hon. B.Sc., and Catherine Chang, M.Sc., Toronto

SUMMARY

The role of beta adrenergic receptor mechanisms in the metabolic effects of diazoxide was assessed in anesthetized fasted or partially refed rats using the beta blocker propranolol. The prior administration of propanolol significantly inhibited the expected diazoxide-induced increases in blood sugar, plasma lactate and glycerol. Also, propranolol blockade of diazoxide hyperglycemia was more effective in fasted than partially refed rats. It was concluded that diazoxide hyperglycemia in fasted rats is accompanied by increases in plasma lactate and glycerol as a consequence of beta adrenergic receptor stimulation. These observations indicate that extrapancreatic beta receptor catecholamine factors are important in diazoxide-induced hyperglycemia and support the theory for at least a partial role of catecholamine mechanisms in the metabolic effects of diazoxide. DIABETES *19*:228-33, April, 1970.

Diazoxide, a member of the benzothiadiazine family of compounds, with nonsaluretic,[1] antihypertensive effects[1,2] has also been recognized for its potent hyperglycemic properties.[3] Although the mechanisms of hyperglycemia are not completely understood, diazoxide therapy has been a frequently useful adjunct in the clinical management of a number of hypoglycemic disorders.[3]

Current observations appear to support both pancreatic and extrapancreatic mechanisms in the diabetogenic activity of diazoxide. Inhibition of pancreatic insulin release in response to glucose stimulation has been reported both in vitro[4-6] and in vivo.[4,7-9] Since there is no demonstrable increase in the rate of removal of I-131 labeled insulin,[10] reduction in plasma insulin levels in both insulinoma patients and experimental subjects treated with diazoxide appears also to indicate a direct inhibition of pancreatic insulin secretion by diazoxide. However, the induction by diazoxide of further elevations of blood sugar in surgically depancreatized dogs,[11] alloxanized mice,[11] and in patients with noninsulogenic hypoglycemia such as glycogen storage disease[12] and nonpancreatic tumors,[13] supports possible concomitant extrapancreatic mechanisms.

Studies have established that both hemodynamic[14,7,8] and metabolic effects[7,8] of the acute administration of diazoxide are remarkably similar to those of epinephrine. Following the initial observation of Porte and coworkers[15] there has been in vivo[16] and in vitro[17] confirmation that epinephrine is also a potent inhibitor of pancreatic beta cell secretion. These observations are in accord with the theory that the pancreatic and extrapancreatic mechanisms of diazoxide may not be exclusive of each other and indeed both may be mediated by catecholamines.

With the increased awareness of the role of the adrenergic receptors as defined by Ahlquist[18] and of cyclic adenosine monophosphate in the mediation of the metabolic responses of tissue to hormones and drugs,[19,20] it was considered of interest to investigate the influence of these mechanisms in the metabolic effects of diazoxide. The present study attempts to elucidate the role played by beta adrenergic receptors in the metabolic effects of diazoxide in both fasted and partially refed rats, and appears to add further support to the theory for at least a partial role of catecholamine mechanisms in the diabetogenic action of diazoxide.

METHODS

Experiments were conducted on male albino Wistar rats weighing between 200 and 275 gm. All food, but not water, was removed from rats commencing the morning of the day preceding the experiment.

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From the Department of Medicine, Division of Endocrinology and Metabolism, Mount Sinai Hospital and University of Toronto, Toronto 101, Ontario, Canada.

The following day, beginning at 7.30 a.m. (after twenty-four hours of fasting), the animals were divided into either fasted or partially refed groups. The partially refed animals received one pellet of Ralston Purina Rat Chow (5.4 gm.) which was consumed within fifteen minutes by all animals within this group, while the fasting group was continued on water only.

At 9.30 a.m. the water was removed from both groups and the animals were anesthetized with sodium pentothal (40 mg./kg.) intraperitoneally. Subsequently the fasting and partially refed groups were further subdivided into four groups which consisted of the following: (1) saline + alkali diluent, (2) saline + diazoxide, (3) propranolol + alkali diluent, and (4) propanolol + diazoxide. These agents were administered in the following manner:

At time zero, immediately after the anesthesia, all animals received saline (control) or propranolol 0.1 mg./kg. (beta blocker) subcutaneously. At time thirty minutes, either diazoxide (100 mg./kg.) or an equivalent volume of its alkali diluent (pH 10.5 to 11.5) was administered intraperitoneally; at time ninety minutes, saline or propranolol was repeated; and at time 180 minutes (i.e., 150 minutes following diazoxide) the animals were sacrificed by decapitation and mixed venous and arterial blood samples were collected in chilled sodium oxalate and fluoride tubes with added heparin and were placed on ice.

Duplicate samples were analyzed immediately for blood sugar using a modified Nelson-Somogyi micromethod.[21] The remaining blood was separated by centrifugation and plasma lactate[22] and glycerol[23] were determined by standard enzymatic technics. The lactate analysis was done the same day; the glycerol, however, was usually done the following day in which case the plasma was stored at—20° C.

Statistical significance of the difference in means among the subgroups of each major group (fasting and partially refed), was determined for each variable using the Student's *t* test. Although a p value less than 0.05 is conventionally judged to be statistically significant, the analysis of data in this report permitted the consigning of a p value less than 0.02 to the term "significant."

RESULTS

Fasted rat studies (see figure 1)

1. *Blood sugar responses*

Rats receiving diazoxide without propranolol pretreatment (S+D) had the expected significant increase in blood sugar level as compared to its saline control group (S+A). The propranolol control group (P+A) had a small but significant rise in blood sugar over the saline control group (S+A). However, the prior administration of propranolol to animals receiving diazoxide (P+D) resulted in a marked inhibition of the expected hyperglycemia. In fact (P+A) versus (P+D) group was not statistically significantly different and as

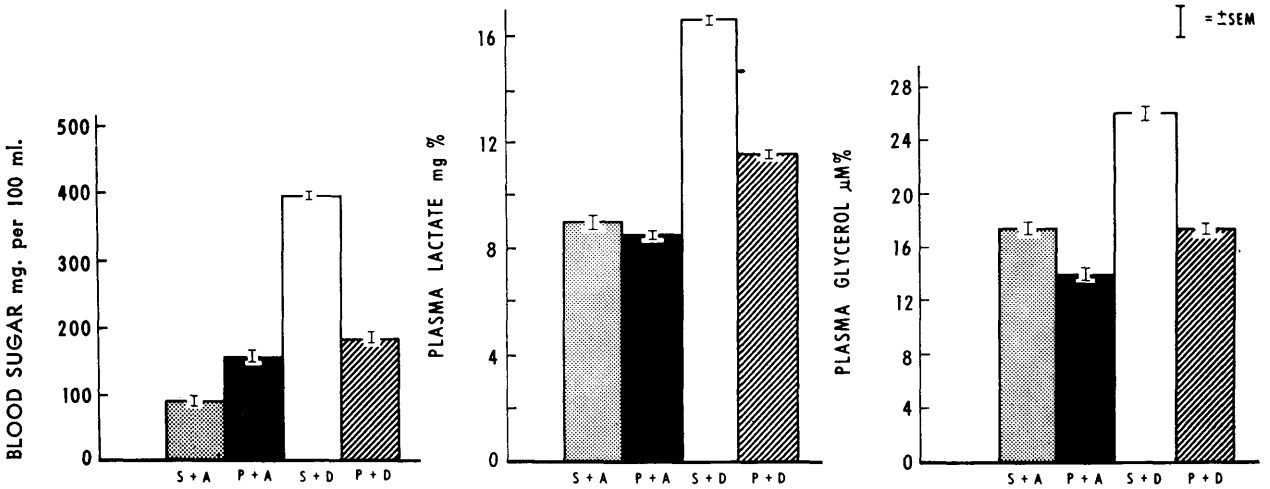

FIG. 1. Fasted rat studies—The effect of propranolol on diazoxide-induced changes in blood sugar, plasma lactate and glycerol. The number of animals in each group was ten, with each animal represented by the mean of duplicate determinations. The height of each bar represents the mean ± standard error of the mean (S.E.M.) values for each group. None of the p values was > 0.02 and most were < 0.01. S = saline (no blockade). P = propranolol (beta adrenergic blockade). D = diazoxide. A = alkali diluent (control).

shown in table 1, the calculated* per cent propranolol inhibition of the expected diazoxide response was 91 per cent.

2. *Plasma lactate responses*

As shown in the (S+D) group, diazoxide hyperglycemia is accompanied by a concomitant rise in plasma lactate. The propranolol control group (P+A) had values not significantly different from the saline control group (S+A). Prior treatment with propranolol to animals receiving diazoxide (P+D), however, resulted in a significant reduction in the expected diazoxide response. As seen in table 1, the calculated per cent propranolol inhibition of the expected diazoxide effect on plasma lactate was 57 per cent.

TABLE 1

Calculated per cent propranolol inhibition of expected diazoxide response

	Glucose	Lactate	Glycerol
Fasted	91	57	65
Refed*	55	134	58

*Fasted and partially-refed with 5.4 gm. of Purina Rat Chow.

3. *Plasma glycerol responses*

Diazoxide administration (S+D) is also accompanied by a marked increase in plasma glycerol. Pretreatment with beta blocker (P+D) again significantly inhibited the expected metabolic effects of diazoxide on this parameter. The calculated per cent inhibition by propranolol as summarized in table 1 was 66 per cent.

Partially-refed rat studies (see figure 2)

1. *Blood sugar responses*

Similar to the results in fasted rats, diazoxide induced a marked rise in blood sugar (S+D). Propranolol pretreatment (P+D) reduced the expected diazoxide response. However, in these studies it will be noted that the propranolol control group had no significant increase in blood sugar over the saline control and the per cent propranolol inhibition on the refed group was 55 per cent as compared to 91 per cent for the fasted rat studies (table 1).

2. *Plasma lactate responses*

A rise in plasma lactate again was noted following diazoxide administration (S+D). However, in contrast to the studies in fasted animals, propranolol was effective in completely inhibiting the expected diazoxide response. The calculated per cent inhibition (table 1) was 134 per cent for the refed group compared to 57 per cent in the rats maintained in the fasted state.

3. *Plasma glycerol responses*

While the absolute values in the partially refed animals were less than those for fasted rat studies, a similar pattern of plasma glycerol response was obtained. Diazoxide again produced a significant rise in plasma glycerol while propranolol pretreatment achieved a 58 per cent inhibition of the expected diazoxide response as compared to a value of 66 per cent in the fasted group (table 1).

DISCUSSION

While the alpha and beta receptor theory of Ahlquist[18] proposed for catecholamines was based upon skeletal, smooth and cardiac muscle studies, it has now been amply confirmed that a similar classification may be applied to their metabolic effects on tissue.[24] Free fatty acid and glycerol mobilization from adipose tissue lipolysis and lactic acid production from muscle glycogenolysis appear to be increased by stimulation of beta adrenergic receptors,[25,26] whereas liver glycogenolysis and inhibition of pancreatic insulin release in rat and man appear to be mediated by alpha adrenergic receptor stimulation.[15,27]

Evidence from the present study is in agreement with the observations of others that diazoxide-induced hyperglycemia may be accompanied by increases in plasma glycerol and free fatty acids,[28-30] as well as lactic acid.[11,31] Propranolol was observed to inhibit both diazoxide hyperglycemia and its expected beta receptor mediated increases in plasma glycerol and lactate. Blockade of diazoxide hyperglycemia in rats using the beta blocker MJ1999,[32] as well as the inhibition of plasma free fatty acid increases in dogs using propranolol,[28] has been previously documented and, similarly, support the theory for diazoxide stimulation of beta adrenergic receptors.

Using the alpha blocker phenoxybenzamine in rats[29] and phentolamine in dogs,[28] the diabetogenic-induced glucose tolerance response and the suppression of insulin

*In order to assess the inhibitory effects of beta adrenergic blockade on the expected response in both the fasted and the partially refed groups, the following formula was used:

$$(S+D) - (S+A) = D(S) \text{ Response} \quad (1)$$

$$(P+D) - (P+A) = D(P) \text{ Response} \quad (2)$$

$$\%\ P\ \text{Inhibition} = \frac{D(S) - D(P)}{D(S)} \times 100 \quad (3)$$

S = saline (no blockade). P = propranolol (beta adrenergic blockade). D = diazoxide administration. A = alkali diluent.

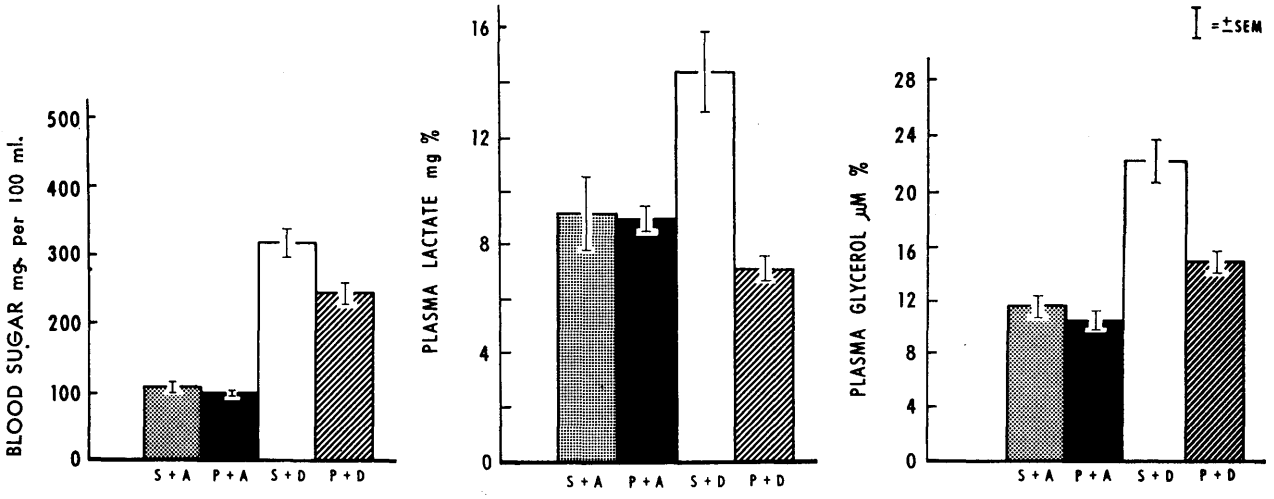

FIG. 2. Partially-refed rat studies—The effect of propranolol on diazoxide-induced changes in blood sugar, plasma lactate and glycerol. Experimental details, numbers of animals, statistical significance and symbols as in figure 1.

release to glucose stimulation following diazoxide administration could be reversed. Thus the available evidence would clearly support stimulation of both alpha and beta adrenergic receptors in the action of diazoxide and at least a partial role in its hyperglycemic mechanisms.

Fleming and Kenny[33] have shown that isoproterenol, a beta receptor stimulator, produces hyperglycemia more effectively in fasted rats; in contrast, norepinephrine, an alpha stimulator, is more effective in the fed state. However, our observation of the ability of diazoxide to induce an almost comparable degree of hyperglycemia in both fasted and partially refed rats more closely resembles the alpha and beta-stimulating effects of epinephrine.[33] It is therefore likely that diazoxide stimulates both alpha and beta receptors regardless of the prandial status. However, in accord with the observations of others on the effectiveness of propranolol in epinephrine-induced hyperglycemia in fasted ketotic human subjects[26] and the influence of nutritional status on catecholamine responses in rats,[33] propranolol was also demonstrated in our studies to inhibit diazoxide hyperglycemia more effectively in rats fasted more than twenty-four hours, while partial refeeding appeared to reduce the effectiveness of the beta blocker (table 1). These observations confirm the importance of prandial status in the interpretation of catecholamine and adrenergic blocking mechanisms.

The induction of diazoxide hyperglycemia in rats fasted more than twenty-four hours, when basal liver glycogen and circulating plasma insulin levels are reduced, suggests that increased glucose output from the liver and decreased peripheral glucose utilization were more important mechanisms accounting for the hyperglycemia. In fact, propranolol administration has been shown to further reduce insulin levels during epinephrine[27] and diazoxide[28] administration. Thus, in spite of the probable reduction in plasma insulin levels, propranolol significantly reduced diazoxide hyperglycemia, indicating the importance of extrapancreatic beta receptor catecholamine factors in the metabolic effects of diazoxide, especially during the fasted state.

Sokal,[34] using an isolated rat liver perfusion system, failed to demonstrate a direct effect of diazoxide on the stimulation of phosphorylase activity, glycogenolysis or gluconeogenesis. It is therefore possible that the increases in hepatic glucose production are mediated by peripheral changes in hormonal and metabolic factors which could include the effects of catecholamines and their subsequent influence on the metabolic substrates presented to the liver. The observed increases in plasma lactate and glycerol could provide the liver with the necessary substrates for enhancing the increased gluconeogenesis which is provoked by prolonged fasting. Moreover, increases in plasma free fatty acids and their rates of oxidation by the liver have been demonstrated to enhance hepatic gluconeogenesis.[38] A rise in blood urea nitrogen following diazoxide therapy[11] is also in keeping with enhanced gluconeogenesis. Thus, the peripheral substrates released by diazoxide stimulation of beta receptors could enhance hepatic glucose production, particularly in the fasting state, and contribute to the hyperglycemia.

However, it is also possible that the increased availa-

bility of free fatty acids as a peripheral tissue energy substrate could spare glucose utilization in a similar manner to the previous demonstrated effects of diazoxide in vivo on decreasing glucose-C-14 incorporation into fasted rat hemidiaphragm glycogen[35] and the reduction of plasma glucose C-14 disappearance rates in dogs.[30] Studies by some investigators have also indicated diazoxide inhibition of basal glucose uptake in vitro[36] while others have shown no effects.[37]

On the basis of the available evidence, it is concluded that the diazoxide-induced hyperglycemia obtained in rats fasted more than twenty-four hours is likely mediated at least partially by extrapancreatic beta adrenergic receptor stimulation of peripheral substrates which could result in a decrease in peripheral glucose utilization and an enhancement of endogenous hepatic glucose production by gluconeogenesis.

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REFERENCES

[1] Rubin, A. A., Taylor, R. M., and Rosenkilde, A.: Pharmacology of diazoxide, an antihypertensive, nondiuretic benzothiadiazine. J. Pharmacol. Exp. Ther. *136*:344, 1962.

[2] Rubin, A. A., Taylor, R. M., and Roth, F. E.: A brief review of the development of diazoxide as an antihypertensive agent. Ann. N.Y. Acad. Sci. *150*:457, 1968.

[3] Weyer, E. W., Ed.: Symposium on diazoxide and the treatment of hypoglycemia. Ann. NY Acad. Sci. *150*, Art 2, 191-467, 1968.

[4] Loubatieres, A., Mariani, M. M., and Arlic, R.: The action of diazoxide on insulin secretion, medullo-adrenal secretion, and the liberation of catecholamines. Ann. N.Y. Acad. Sci. *150*:226, 1968.

[5] Howel, S. L., and Taylor, K. W.: Effects of diazoxide on insulin secretion in vitro. Lancet *1*:128, 1966.

[6] Frerichs, H., and Creutzfeldt, W.: Insulin release from pancreas of rat, rabbit and miniature pig in vitro (Abstract). Diabetologia *1*:80, 1965.

[7] Graber, A. L., Porte, D., Jr., and Williams, R. H.: Clinical uses of diazoxide and mechanisms for its hyperglycemic effects. Diabetes *15*:143, 1966.

[8] Fajans, S. S., Floyd, J. C., Jr., Knopf, R. F., Rull, J., Guntsche, E. M., and Conn, J. W.: Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man. J. Clin. Invest. *45*:481, 1966.

[9] Marks, V., Clifford, R. F., and Samols, E.: Hyperinsulinism due to metastasizing insulinoma treatment with diazoxide. Proc. Roy. Soc. Med. *58*:577, 1965.

[10] Walfish, P. G.: Insulin I-131 disappearance rates following diazoxide, epinephrine and glucose administration. Ann. NY Acad. Sci. *150*:54, 1968.

[11] Tabachnick, I. I. A., Gulbenkian, A., and Seidman, F.: The effects of a benzothiadiazine, diazoxide on carbohydrate metabolism. Diabetes *13*:408, 1964.

[12] Bleicher, S. J., Chowdhury, F., Podolsky, F., Fleishman, P., and Goldner, M. G.: Studies on diazoxide-induced hyperglycemia: An extrapancreatic mechanism. Ann. NY Acad. Sci. *150*:395, 1968.

[13] Tucker, W. R., Ryan, W. G., Martin, B. F., and Schwartz, T. B.: Studies in a patient with retroperitoneal sarcoma associated with severe hypoglycemia. Ann. NY Acad. Sci. *150*:395, 1968.

[14] Wilson, W. R., and Okun, R.: The acute hemodynamic effects of diazoxide in man. Circulation *28*:89, 1963.

[15] Porte, D., Jr., Graber, A. L., Kuzuya, T., and Williams, R. H.: The effect of epinephrine on immunoreactive insulin levels in man. J. Clin. Invest. *45*:228, 1966.

[16] Seltzer, H. S., and Crout, J. R.: Insulin secretory blockade by benzothiadiazine and catecholamines: Reversal by sulfonylureas. Ann. NY Acad. Sci. *150*:309, 1968.

[17] Coore, H. G., and Randle, P. J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. *93*:66, 1964.

[18] Ahlquist, R. P.: A study of the adrenotropic receptors. Amer. J. Phys. *153*:586, 1948.

[19] Butcher, R. W.: Role of cyclic AMP in hormone actions. New Eng. J. Med. *279*:1378, 1968.

[20] Weyer, E. W., Ed.: Symposium on new adrenergic blocking drugs: Their pharmacological, biochemical and clinical actions. Ann. NY Acad. Sci. *139*:Art 3, 1967.

[21] Campbell, J., and Green, G. R.: Free fatty acid metabolism in Chinese hamsters. Canad. J. Physiol. Pharmacol. *44*: 47, 1966.

[22] Scholz, R., Schmitz, R. H., Buche, T. H., and Lampen, J. O.: Enzymatic determination of L-lactate concentration in blood or serum. Biochem. Z. *331*:71, 1959.

[23] Vaughan, M.: The production and release of glycerol by adipose tissue incubated in vitro. J. Biol. Chem. *237*:3354.

[24] Ellis, S., Kennedy, B. L., Eusebi, A. J., and Vincent, N.H.: Autonomic control of metabolism. Ann. N.Y. Acad. Sci. *139*: 826, 1967.

[25] Burns, J. J., Salvador, R. A., and Lemberger, L.: Metabolic blockade by methoxamine and its analogs. Ann. N.Y. Acad. Sci. *139*:883, 1967.

[26] Antonis, A., Clark, M. L., Hodge, R. L., Moloney, M.,

and Pilkington, T. R. E.: Receptor mechanisms in the hyperglycemic response to adrenaline in man. Lancet *1*:1135, 1967.

[27] Porte, D., Jr.: A receptor mechanism for the inhibition of insulin release by epinephrine in man. J. Clin. Invest. *46*:86, 1967.

[28] Blackard, W. G., and Aprill, C. N.: Mechanism of action of diazoxide. J. Lab. Clin. Med. *69*:960, 1967.

[29] Tabachnick, I. I. A., and Gulbenkian, A.: Mechanisms of diazoxide hyperglycemia in animals. Ann. N.Y. Acad. Sci. *150*:204, 1968.

[30] Sanbar, S. S.: Metabolism of plasma glucose and lipids following diazoxide administration in dogs. Metabolism *16*: 259, 1966.

[31] Schultz, G., Senft, G., Losert, W., and Sitt, R.: Biochemische grundlagen der diazoxide hyperglykamie. Arch. Exp. Path. Pharmak. *253*:372, 1966.

[32] Kvam, D. C., Riggilo, D. A., and Lish, P. M.: Effect of some new B-adrenergic blocking agents on certain metabolic responses to catecholamines. J. Pharmacol. Exp. Ther. *149*:183.

[33] Fleming, W. W., and Kenny, A. D.: The effect of fasting on the hyperglycemic responses to catecholamines in rats. Brit. J. Pharmacol. *22*:267, 1964.

[34] Sokal, J.: Failure of diazoxide to affect carbohydrate metabolism of the isolated liver. Diabetes *17*:256, 1968.

[35] Walfish, P. G.: The acute effect of diazoxide on basal and post-insulin in vivo incorporation of glucose C.14 (U.L.) into rat diaphragm glycogen and epididymal fat pad. Ann. N.Y. Acad. Sci. *150*:254, 1968.

[36] Barnett, C. A., and Whitney, J. E.: The effect of diazoxide and chlorthiazide on glucose uptake in vitro. Metabolism *15*:88, 1966.

[37] Field, J. B., and Mandel, S.: The effects of thiazides on glucose uptake and oxidation of rat muscle and adipose tissue. Metabolism *13*:959, 1964.

[38] Williamson, J. R., Browning, E. T., Scholz, R., Kreisberg, R. A., and Fritz, I. B.: Inhibition of fatty acid stimulation of gluconeogenesis by (+)—decanoylcarnitine in perfused rat liver. Diabetes *17*:194, 1968.