Studies on Diazoxide Hyperglycemia

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SUMMARY

Studies on diazoxide hyperglycemia in rats have shown the blood glucose response to orally administered diazoxide to be dose-related with a peak effect at five hours following dosage. Diazoxide hyperglycemia could be reversed by the administration of tolbutamide. Plasma insulin-like activity was not decreased by diazoxide nor was the insulin-mediated increase in L-arabinose distribution affected. Both hypophysectomy and adrenalectomy reduced the hyperglycemic response to diazoxide. In addition, plasma corticosteroid levels were found to be significantly elevated five hours following the drug. The hyperglycemic response to diazoxide could be reduced by prior treatment with the beta-adrenergic blocking drug, MJ 1999 [4-(2-isopropylamino-l-hydroxyethyl) methane-sulfonanilide HCl]. Further studies showed that potassium deficiency resulted in an exaggerated hyperglycemic response to diazoxide; this response, as well as that produced in normal animals, could be antagonized by administration of potassium chloride. In addition, glucose tolerance was somewhat decreased by subhyperglycemic doses of diazoxide, especially in potassium deficient animals. It is suggested that adrenergic influences are a significant component of diazoxide hyperglycemia. The potassium changes are thought merely to alter the sensitivity of the sympathetic elements or receptors.

Considerable clinical experience has shown the benzothiadiazine diuretics as a class to be relatively free from side effects other than those resulting from extensions of their renal actions. However, in a small percentage of patients, administration of these compounds has been reported to precipitate or worsen the diabetic state in an apparently reversible fashion. This diabetogenic action was again noted during clinical trials of diazoxide,* a nondiuretic benzothiadiazine compound, as a hypotensive agent. This was noted particularly when the compound was given in combination with other benzothiadiazines.[1] This action was subsequently confirmed when the compound was given in combination with other thiazides to dogs or rats[2-4] or when it was administered alone to rats.[4] Additional evidence has now accumulated showing that the compound is acutely hyperglycemic when administered alone by a variety of routes to several species.[5,6]

The hyperglycemic effect of diazoxide has been investigated in some detail in the present study and an attempt has been made to elucidate the possible mechanism of this phenomenon further.

METHODS

Female Wistar rats weighing between 150 to 250 gm. were fasted for eighteen hours prior to the start of all experiments. Potassium deficiency was established by placing the animals on a potassium deficient diet (Nutritional Biochemical Company) for ten days. Adrenalectomized rats were prepared by excision of the adrenals through a retroperitoneal incision under ether anesthesia. The animals were used two days following adrenalectomy and were maintained on 0.9 per cent sodium chloride solution until use. Hypophysectomized rats were purchased from Hormone Assay Laboratories, Inc.

Blood sampling was accomplished in most studies on unanesthetized, lightly restrained rats by means of an indwelling aortic catheter implanted according to the technic described by Weeks and Jones.[7] Departures from this sampling technic are noted in the description of the individual experiment.

Insulin-like activity was determined using rat epididymal fat pads as described by Leonards et al.[8] using glucose uptake as the measure of activity. Activity of the serum was determined by comparison of the uptake obtained to a dose-response curve obtained using glucagon-free insulin.

Determination of the insulin effect on L-arabinose distribution was carried out as previously described.[9]

Blood glucose was measured in samples diluted 1:10 with 4 per cent sodium fluoride - 0.9 per cent sodium chloride solutions by means of a ferricyanide technic

*3-Methyl-7-chloro-1,2,4-benzothiadiazine-1,1-dioxide, furnished through the courtesy of The Schering Corporation.

From the Mead Johnson Research Center, Evansville, Indiana.

in an AutoAnalyzer.[10] Plasma corticosteroid values were obtained fluorometrically as described by Guillemin et al.[11] Determination of plasma potassium levels was accomplished directly utilizing a flame attachment to an AutoAnalyzer.[12]

Statistical analysis was by means of Students t test[13] or by analysis of variance and detection of differences using Duncan's new multiple range test.[14]

RESULTS

When diazoxide was given orally to fasted rats at a dose of 100 mg./kg. the results shown in figure 1A were obtained. Blood glucose values were elevated at one hour following administration of the compound, and continued to increase over the entire five-hour period. Blood sugar values had returned to control levels when determined eighteen hours after drug administration. This response to diazoxide was dose-related as shown in figure 1B. The values depicted in this figure are increases obtained five hours after administration of the compound.

The rise in blood glucose induced by diazoxide (200 mg./kg., p.o.) was successfully antagonized by tolbutamide (100 mg./kg., s.c.) even when the latter agent was given after the hyperglycemia had developed to a marked degree (figure 2). These results are in agreement with those obtained by others in which

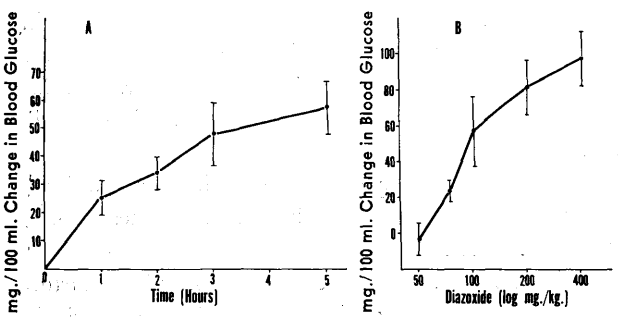

FIG. 1A. Time course of diazoxide hyperglycemia. Diazoxide (100 mg./kg.) was administered orally at zero time suspended in 0.25 per cent methocel. Per cent increases in blood glucose were obtained by comparison with zero time values obtained in the same animal. Each point represents the mean of values obtained from five animals. Vertical lines represent the standard error of the mean.

FIG. 1B. Diazoxide-blood glucose dose-response relationship. Diazoxide was administered at zero time in the indicated dosages suspended in 0.25 per cent methocel. Values for blood glucose increases are those obtained five hours after the drug by comparison with zero time values from the same animals. Each point represents the mean of values from five animals. Vertical lines represent the standard error of the mean.

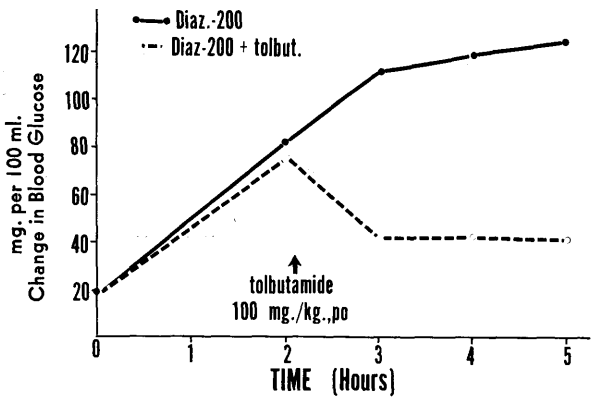

FIG. 2. Effect of tolbutamide on diazoxide hyperglycemia. Diazoxide (200 mg./kg.) was given orally at zero time as a suspension in 0.25 per cent methocel. Tolbutamide treated animals were given the compound dissolved in 0.25 per cent methocel; the others received an equivalent volume of 0.25 per cent methocel. The blood sugar increases in the tolbutamide treated animals are significantly different ($P = 0.05$) from those of the animals which received only diazoxide at the three-, four-, and five-hour intervals. Each curve represents the mean of values from five animals.

tolbutamide was capable of preventing the hyperglycemia produced by diazoxide[4,6] or by combinations of thiazide drugs.[4]

As seen in table 1 serum insulin-like activity in rats receiving diazoxide (400 mg./kg., p.o.) was not significantly lower than control rats even though a marked hyperglycemia is produced by this dose of diazoxide at this interval after administration (figure 1B). In addition, the results shown in table 2 provide evidence that diazoxide does not interfere with the peripheral effects of insulin. It is apparent that the effect of insulin on L-arabinose distribution is unaffected by the prior administration of diazoxide.

Results presented in table 3 demonstrate that diazoxide-induced hyperglycemia was modified to a con-

TABLE 1

Serum insulin-like activity following diazoxide treatment

Treatment	Number of rats	Serum insulin-like activity microunits/ml. Mean ± S.E.
Control	10	709.5±140.7
Diazoxide, 400 mg./kg., p.o.	10	566.0±102.6

Diazoxide was suspended in 0.25 per cent methocel and administered by stomach tube at zero time. Control animals received an equivalent amount (0.5 ml./kg.) of 0.25 per cent methocel at zero time. Blood was collected by decapitation and exsanguination five hours following treatment. The mean value for diazoxide treated animals is not significantly different ($P = >0.05$) than the mean for control animals.

TABLE 2

Effect of diazoxide on insulin enhancement of L-(+) arabinose distribution in the rat

Treatment	Number of rats	Volume distribution of L-(+) arabinose (ml./100 gm.)
Control	6	40.7
Diazoxide, 100 mg./kg.	6	44.7
Insulin, 2 units/kg.	6	62.2
Insulin, 2 units/kg. + diazoxide, 100 mg./kg.	6	58.0
		C.V. = 6 per cent

Insulin was administered subcutaneously in saline. Diazoxide was given orally one hour prior suspended in 0.25 per cent methocel. Blood samples were taken two hours following insulin and/or L-(+) Arabinose administration from retro-orbital vessels by capillary pipette. Any two means joined by the same vertical line are not significantly different (P = 0.05). Variation in this experiment is expressed by the term coefficient of variability (C.V.).

TABLE 3

Effect of adrenalectomy and hypophysectomy on diazoxide hyperglycemia

Treatment	Number of rats	Blood glucose per 100 ml. Zero time	Five hrs. post-drug
Intact + diazoxide 200 mg./kg., p.o.	5	92.4±3.4	172.0±15.0
Adrenalectomized + diazoxide, 200 mg./kg., p.o.	5	57.6±1.3	77.0±3.9
Hypophysectomized + diazoxide, 200 mg./kg., p.o.	5	55.2±4.0	104.4±5.8

Diazoxide administered orally in 0.25 per cent methocel at zero time. Blood samples were removed from retro-orbital vessels by means of a capillary pipette.

siderable degree in adrenalectomized or hypophysectomized animals. In addition, studies in intact rats (table 4) indicate that plasma corticosteroid levels were significantly elevated following diazoxide administration.

Experiments in which rats were administered the beta-adrenergic blocking agent MJ 1999 [4-(2-isopropylamino-1-hydroxyethyl) methane-sulfonanilide HCl] indicated that this compound is capable of modifying the hyperglycemia produced by diazoxide (table 5). All blood glucose values obtained in the presence of MJ 1999 were significantly different (P = 0.05) from the values obtained at the corresponding time interval in animals receiving diazoxide alone. Previous studies[15] had shown MJ 1999 to be capable of antagonizing the hyperglycemia resulting from epinephrine administration in the rat.

Additional studies were suggested by the observation that diazoxide hyperglycemia could be prevented by the administration of potassium chloride.[4] In this regard diazoxide-evoked hyperglycemia was compared in normal and potassium deficient animals. In these experiments (figure 3) potassium deficiency strikingly exaggerated the hyperglycemic actions of diazoxide. Glucose tolerance curves from potassium deficient animals (figure 4) showed no greater peak response to the glucose load (0.5 gm./kg., i.a.) than did normal animals, but diazoxide in subhyperglycemic doses exaggerated the peak response obtained in glucose tolerance studies in both normal and potassium deficient animals. In addition the time required for return to normal blood sugar values was prolonged in those potassium deficient animals which had received diazoxide despite the fact that these doses produce no hyperglycemia by themselves.

Figure 5 illustrates the effect of prior administration of potassium chloride (750 mg./kg., p.o.) on diazoxide hyperglycemia. Modification of the hyperglycemic response is clearly evident in the potassium deficient animals, but is also present in the case of normal animals.

TABLE 4

Effect of diazoxide treatment on levels of plasma corticosteroids in the rat

Treatment	Number of rats	Plasma corticosteroids micrograms/ml. Mean ± S.E.
Controls	10	0.29±0.06
Diazoxide, 200 mg./kg., p.o.	10	0.62±0.05

Control animals received 0.25 per cent methocel orally at zero time, treated animals received diazoxide suspended in 0.25 per cent methocel at zero time. Blood samples were taken by decapitation and exsanguination into heparinized tubes five hours following treatment. Corticosteroid values following diazoxide are highly significantly different from control (P = 0.01).

TABLE 5

Effect of β-adrenergic blockade on the hyperglycemic response to diazoxide

Treatment	Number of rats	Increase in blood glucose (mg. per 100 ml.) Mean ± S.E. 2 hrs.	3 hrs.	5 hrs.
Diazoxide, 100 mg./kg.	5	20.4±5.1	36.6±6.2	47.2±6.1
Diazoxide, 100 mg./kg. + MJ 1999 20 mg./kg.	5	8.8±2.1	18.6±3.4	34.4±4.8
P =		0.05	0.05	0.05

MJ 1999, 20 mg./kg., s.c., was administered thirty minutes prior to the diazoxide which was given orally as a suspension in 0.25 per cent methocel. Probability values refer to the probability of the response to diazoxide + MJ 1999 being significantly less than the response to diazoxide alone.

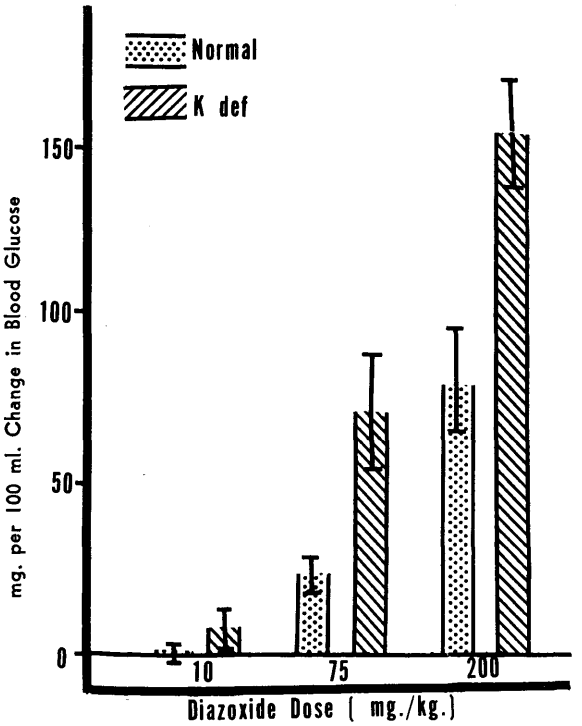

FIG. 3. Effect of potassium deficiency on diazoxide hyperglycemia. Diazoxide was administered at zero time suspended in 0.25 per cent methocel. Increases in blood glucose are those obtained at five hours by comparison to zero time values obtained in the same animal. Each bar represents the mean of values obtained from five animals; the vertical lines represent the standard error of the mean.

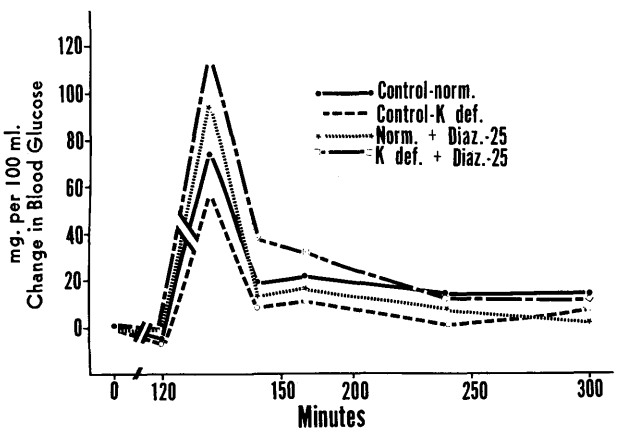

FIG. 4. Effect of potassium deficiency and/or diazoxide on glucose tolerance. Diazoxide (25 mg./kg.) was given orally at zero time as a suspension in 0.25 per cent methocel. Two hours later a glucose load (0.5 gm./kg.) was rapidly injected intra-arterially. Each curve is the mean obtained from five animals.

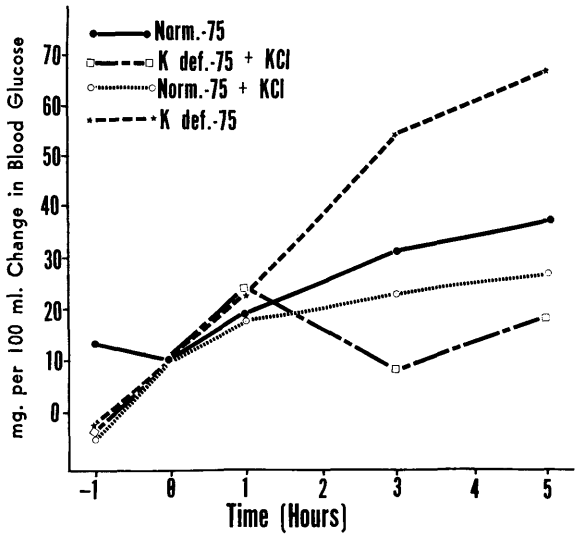

FIG. 5. Effect of potassium chloride on diazoxide-induced hyperglycemia in normal and potassium deficient rats. Rats were given potassium chloride (750 mg./kg.) or an equivalent volume of water orally one hour prior to receiving diazoxide. Diazoxide (75 mg./kg.) was administered orally in 0.25 per cent methocel at zero time. Each curve is the mean of values from five animals. Values from animals receiving potassium chloride are significantly different from those receiving diazoxide alone at five hours in the case of normals ($P = 0.05$) and at three and five hours in the case of potassium deficient animals ($P = 0.01$).

DISCUSSION

Studies to date appear to exclude the pancreas or insulin from participation in diazoxide hyperglycemia. The drug has been found to be effective in pancreatectomized animals,[6] adding its hyperglycemic effect to the already elevated blood sugar, and is also effective in alloxan diabetic animals.[5,6] These observations suggest that there is no interference by diazoxide with insulin release. Further, based upon the experiments on L-arabinose distribution in the present study there is no antagonism of insulin effect at the periphery. These results agree well with those of others who have shown that animals receiving diazoxide remain sensitive to insulin.[6] One clinical study has reported a reduction in serum insulin-like activity in patients receiving thiazide diuretics.[16] In the present investigation, insulin-like activity did not increase and may have actually decreased somewhat (not statistically significant) in the face of the hyperglycemia induced by diazoxide. This might be interpreted as evidence that diazoxide inhibited insulin release. However, the effectiveness of tolbutamide in preventing diazoxide-induced hyperglycemia casts some doubt upon this in-

terpretation. Furthermore, other investigators have been unable to demonstrate a decrease in circulating levels of serum insulin-like activity and insulin antibodies are apparently not formed during treatment with this class of drugs.[17]

The remaining evidence therefore suggests either adrenergic or pituitary involvement as a mechanism of action. Studies from another laboratory have revealed that the decreased hyperglycemic response to diazoxide observed in hypophysectomized animals may be due to a state of inanition which results in decreased glycogen stores since forcefeeding of the animals will restore the response.[18] If these findings are adequate to explain the loss of response in these animals, then involvement of the sympathetic nervous system may be postulated as the primary mechanism. The increased levels of plasma corticosteroids observed during the present investigations might be explained as the result of adrenergic stimulation.[19] However, unpublished observations from this laboratory (Kvam, 1964) indicated that the elevation of plasma corticosteroids induced by the administration of epinephrine to rats could not be antagonized by MJ 1999. It seems unlikely, therefore, that the release of corticosteroids is brought about through direct stimulation of adrenergic *beta* receptors. It is possible that the elevated levels of corticosteroids contribute to the hyperglycemic response themselves or that the adrenal cortex and hypophysis are permissive for the hyperglycemic effect of diazoxide.

Studies from other laboratories showing a reduction in liver glycogen following diazoxide administration[5,6] as well as modification of the hyperglycemic response by prior administration of a ganglionic blocking agent[18] are certainly compatible with adrenergic involvement. Additional evidence is afforded by the finding of elevated levels of plasma-free fatty acids following diazoxide administration.[18] This latter response is known to be produced by adrenergic stimulation but may be produced by a variety of other factors as well.[20] However, the data in the present report which demonstrate modification of diazoxide hyperglycemia by MJ 1999, a beta-adrenergic blocking agent, also suggest an adrenergic mechanism.

It is tempting to speculate that the involvement of the sympathetic nervous system in diazoxide hyperglycemia is the result of reflexes triggered by the hypotensive response to the drug. In this regard in a study in which several hypotensive drugs were administered to human subjects[21] it was found that only diazoxide was capable of eliciting a hyperglycemic response even though hypotension occurred with all agents tested.

The results demonstrating increased sensitivity to diazoxide in potassium deficient animals and the prevention of the hyperglycemic response in both normal and potassium deficient animals by the administration of potassium salts do not appear entirely compatible with the other observations. Wolff et al.[4] have theorized that the hyperglycemic effects of the thiazides might be correlated with their ability to deplete potassium and a similar correlation has been proposed by investigators studying chlorthalidone[22] and chlorothiazide[23] in humans. In the latter study abnormalities in glucose tolerance observed during chlorothiazide therapy were significantly reversed by potassium supplementation. It is difficult, however, to apply the same reasoning to diazoxide-induced hyperglycemia since this drug tends to conserve sodium and does not deplete potassium[24] as occurs with other drugs in this class. It is entirely possible that the modification of the hyperglycemic effects of diazoxide by alterations in potassium content of body fluids merely reflects changes in sensitivity of the sympathetic nerve elements or receptor substances involved in eliciting the hyperglycemic response and has little bearing on the basic mechanism.

It should be noted that in the animal studies designed to elucidate the mechanism of action of diazoxide-induced hyperglycemia, the doses of drug needed to elicit the response were quite large compared with those used clinically. Hence, although certain conclusions may be drawn concerning the mechanism by which diazoxide hyperglycemia is induced in animals these findings may have little relevance to the situation in man where clinical doses are employed.

ADDENDUM

Since the preparation of this manuscript, the following report has appeared: Wolff, F. W., and Parmley, W. W.: Further observations concerning the hyperglycemic activity of benzothiadiazine. Diabetes *13*:115, 1964. These authors observed that diazoxide-induced hyperglycemia occurred in adrenal demedullated rats indicating that this organ does not play a major role in the effect. Potassium chloride (750 mg./kg.) interfered with diazoxide hyperglycemia which is in agreement with the results obtained in the present study.

SUMMARIO IN INTERLINGUA

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monstrate que le responsa de glucosa sanguinee a oralmente administrate diazoxida es un function del dose e attinge su maximo cinque horas post le administration. Le hyperglycemia inducite per diazoxida poteva esser recertite per le administration de tolbutamida. Le activitate insulinoide in le plasma non esseva reducite per diazoxida, e etiam le augmento, mediate per insulina, in le distribution de L-arabinosa non esseva afficite. Tanto hypophysectomia e adrenalectomia reduceva le responsa hyperglycemic a diazoxida. In plus, il esseva trovate que le nivellos de corticosteroide in le plasma esseva significativemente elevate cinque horas post le administration del pharmaco. Le responsa hyperglycemic a diazoxida poteva esser reducite per le previe tractamento con le pharmaco de blocage beta-adrenergic, MJ 1999, i.e., 4-(2-isopropylamino-1-hydroxyethyl)-methano-sulfonanilida a HCl. Altere studios ha monstrate que un carentia de kalium resultava in un exaggerate responsa hyperglycemic a diazoxida. Iste responsa, e etiam le responsa producite in animales normal, poteva esser inhibite per le administration de chloruro de kalium. A parte isto, le tolerantia pro glucosa esseva levemente reducite per doses subhyperglycemic de diazoxida, particularmente in animales in carentia de kalium. Es postulate que influentias adrenergic es un componente significative in le disveloppamento de hyperglycemia per diazoxida. Es opinate que le alterationes in kalium affice mermente le sensibilitate del elementos sympathic o del receptores.

Depost le preparation del presente communication, le sequente reporto ha essite publicate: Wolff, F. W., e Parmley, W. W.: "Observationes Additional Concernente le Activitate Hyperglycemic de Benzothiadiazinas", *Diabetes* 13:115, 1964. Iste autores observava que hyperglycemia inducite per diazoxida occurre rattos con dismedullate adrenales, lo que indica que le adrenales non ha un rolo major in le effecto in question. Chloruro de kalium (750 mg per kg de peso corporee) interfereva in le causation de hyperglycemia per diazoxida. Isto es congrue con le resultatos obtenite in le presente studio.

REFERENCES

[1] Dollery, C. T., Pentecost, B. L., and Samaan, N. A.: Drug-induced diabetes. Lancet *2*:735, 1962.

[2] Langdon, R. G., and Wolff, F. W.: Action of diazoxide. Brit. Med. J. *2*:926, 1962.

[3] Wolff, F. W., Langdon, R. G., Ruebner, B. H., Hollander, C., and Skoglund, R. D.: A new form of experimental diabetes. Diabetes *12*:335, 1963.

[4] Wolff, F. W., and Parmley, W. W.: Etiological factors in benzothiadiazine hyperglycemia. Lancet *2*:69, 1963.

[5] Gulbenkian, A., Petillo, J. J., Schobert, L. J., Seidman, F., Yannell, A., and Tabachnick, I. I. A.: Hyperglycemic effect of diazoxide. Fed. Proc. *22*:543, 1963.

[6] Tabachnick, I. I. A., Gulbenkian, A., Zeman, W., and Black, J.: The effect of a benzothiadiazine on carbohydrate metabolism. Diabetes *12*:354, 1963.

[7] Weeks, J. R., and Jones, J. A.: Routine direct measurement of arterial pressure in unanesthetized rats. Proc. Soc. Exp. Biol. Med. *104*:646, 1960.

[8] Leonards, J. R., Landau, B. R., and Bartsch, G.: Assay of insulin and insulin-like activity with rat epididymal fat pad. J. Lab. Clin. Med. *60*:552, 1962.

[9] Kvam, D. C.: Lack of effect of tolbutamide upon some insulin-responsive sugars. Proc. Soc. Exp. Biol. Med. *115*: 904, 1964.

[10] Weller, C., Liner, M., Macaulay, A., Ferrari, A., and Kessler, G.: Continuous in vivo determination of blood glucose in human subjects. Ann. N. Y. Acad. Sci. *87*:658, 1960.

[11] Guillemin, R., Clayton, G. W., Lipscomb, H. S., and Smith, J. D.: Fluorometric measurement of rat plasma and adrenal corticosterone concentration. J. Lab. Clin. Med. *53*: 830, 1959.

[12] Isreeli, J., Pelavin, M., and Kessler, G.: Continuous automatic integrated flame photometry. Ann. N. Y. Acad. Sci. *87*:636, 1960.

[13] Snedecor, G. W.: Statistical Methods in Experiments, 5th ed. Ames, Iowa, Iowa State College Press, 1956, p. 45.

[14] Duncan, D. B.: Multiple range and multiple F tests. Biometrics *11*:1, 1955.

[15] Riggilo, D. A., and Kvam, D. C.: Inhibition of catecholamine-induced increases in blood glucose and free fatty acids by beta-adrenergic blocking agents. Fed. Proc. *23*:124, 1964.

[16] Samaan, N., Dollery, C. T., and Fraser, R.: Diabetogenic action of benzothiadiazines. Lancet *2*:1244, 1963.

[17] Greenberg, S. R., Dresner, M., and Gorczyca, R.: The effect of thiazide diuretics on insulin antibodies. Amer. J. Med. Sci. *246*:329, 1963.

[18] Gulbenkian, A., Seidman, F., and Tabachnick, I. I. A.: Diazoxide hyperglycemia and free fatty acid mobilization. Fed. Proc. *23*:542, 1964.

[19] Kraicer, J., and Logothetopoulos, J.: Adrenal cortical response to insulin-induced hypoglycemia in the rat. II. Mediating role of adrenaline and/or noradrenaline. Acta Endocr. *44*:259, 1963.

[20] Rudman, D.: The adipokinetic action of polypeptide and amine hormones upon the adipose tissue of various animal species. J. Lipid. Res. *4*:119, 1963.

[21] Okun, R., Wilson, W. R., and Gelfand, M. D.: The hyperglycemic effect of hypotensive drugs. J. Chron. Dis. *17*:31, 1964.

[22] Reutter, F., and Labhart, A.: Saluretic and glukosetoleran. Helv. Med. Acta *28*:487, 1961.

[23] Rapoport, M. I., and Hurd, H. F.: Thiazide-induced glucose intolerance treated with potassium. Arch. Int. Med. *113*:405, 1964.

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