Biochemical Pharmacology, Vol. 23, pp. 1693–1700. Pergamon Press, 1974. Printed in Great Britain.

BLOCKADE OF INSULIN RELEASE BY CERTAIN PHENOTHIAZINES*

ANTHONY G. PROAKIS† and JOSEPH L. BOROWITZ

Department of Pharmacology and Toxicology, Purdue University, West Lafayette, Ind. 47907, U.S.A.

(*Received* 31 *May* 1973; *accepted* 9 *November* 1973)

Abstract—Chlorpromazine lowered the ratio of plasma insulin to blood glucose in adrenalectomized rats fed a glucose load. Desmethylchlorpromazine also lowered this ratio 30 min after glucose administration, but elevated it at 60 min. By contrast, fluphenazine had no significant effect on the ratio of plasma insulin to blood glucose. Chlorpromazine and desmethylchlorpromazine were more effective than fluphenazine in blocking insulin release from the isolated rat pancreas. Although adrenal epinephrine release and blockade of the effect of insulin may also be involved, the results of this study show that the hyperglycemic effect of certain phenothiazines is mainly determined by blockade of insulin release.

HYPERGLYCEMIA after the administration of chlorpromazine has been observed in several animal species including man.[1–9] Other phenothiazine derivatives have exhibited similar glycemic effects when administered to mice or rats.[10,11] It has been suggested that the primary mechanism of phenothiazine-induced hyperglycemia is through the release of epinephrine from the adrenal medulla, resulting in the mobilization of liver glycogen.[10,12–14] However, the relative effectiveness of phenothiazine analogs in raising blood sugar does not correlate with their ability to release adrenal catecholamines.[10]

Although chlorpromazine-induced hyperglycemia is abolished in adrenalectomized animals,[10,11,13] chlorpromazine-induced glucose intolerance is not prevented by prior adrenalectomy or adrenaldemedullation.[13,15] This decrease in glucose tolerance does not appear to result from a drug-linked disturbance of glucose transport,[16] but is considered to occur through a chlorpromazine-induced impairment of cellular utilization of the sugar[11,17,18] possibly by inhibition of insulin secretion.[19] Accordingly, this study was aimed at evaluating the glycemic effects of three phenothiazines and their influence on the insulin secretory activity of the pancreas.

MATERIALS AND METHODS

Male albino rats (Laboratory Supply Co., Indianapolis), weighing 300–350 g, were used throughout the study. Prior to the experiments, the rats were housed in groups of 10 with free access to food and water.

Two studies *in vivo* were conducted to assess the effects of phenothiazines on plasma glucose. The first involved the effect of phenothiazines on blood sugar in intact animals and the other evaluated the effect of phenothiazines on glucose tolerance. Each of the studies was carried out in a single day. Adrenalectomized and

* Supported in part by National Institutes of Health Grants GM 43,285 and GM 15005.

† Present address: A. H. Robins Research Laboratories, Richmond, Va.

sham-operated rats were employed in the glucose tolerance study. Bilateral adrenalectomy was performed via a midline dorsal incision under light ether anesthesia 5 days before any drug treatment, and the animals were maintained on a regular diet with 0·9% sodium chloride in the drinking water. Sham-operated rats were subjected to a similar surgical manipulation but the adrenals were left intact. Groups of rats were pretreated with the phenothiazines (10 mg/kg, i.p.) 30 min prior to the administration of a loading dose of glucose (2 g/kg, i.p.). Solutions of glucose were prepared with distilled water to a concentration of 250 mg/ml. At several predetermined intervals, the animals were sacrificed by decapitation, and free-flowing blood was collected in beakers wetted with oxalate. Blood plasma was obtained by centrifugation using a refrigerated centrifuge and frozen until analysis. Plasma glucose was determined enzymatically by the glucose oxidase method* using a Beckman Glucose Analyzer (model 2001). The plasma was also assayed for immunoreactive insulin (IRI) levels by the method† of Hales and Randle.[20] All plasma samples were diluted 1:5 with the recommended buffer for the IRI analysis against a human insulin standard. The plasma IRI and plasma glucose values from each animal were used to determine the insulinogenic index which is the ratio of peripheral insulin relative to blood glucose concentration.

The procedure used for the assessment *in vitro* of phenothiazine-induced alteration of insulin secretion employed the isolated perfused rat pancreas preparation. After anesthesia with sodium pentobarbital (40 mg/kg, i.p.), surgical isolation of the pancreas was done using the method of Grodsky *et al.*[21] which involves removal in one block of the stomach, spleen, pancreas and duodenum. The pancreas was perfused via an arterial cannula in the aorta at the level of the celiac axis, and effluent was collected from a cannula attached to the portal vein. Prior to aortic cannulation and subsequent removal of the preparation from the abdominal cavity, 300 units of heparin was administered to the rat via the ileolumbar vein. A 5-min interval was allowed to assure the presence of heparin within the pancreatic area. Perfusion of the tissue was initiated immediately after aortic cannulation. The preparation was then placed in a tissue chamber bath maintained at a constant temperature of 37°. Krebs–Ringer bicarbonate buffer[22] (KRB) containing 1% fraction V bovine albumin was used as the bathing medium as well as the perfusion medium. The perfusion medium was delivered to the pancreas by means of a multichannel piston-type metering pump (Harvard Apparatus Co., no. 1504). In the glucose (3 g/l)-containing buffer (G-KRB), the appropriate quantity of sodium chloride was deleted to maintain isotonicity. Prior to use, the buffers were recycled for 20 min from their respective reservoirs through a glass lung which was gassed with a humidified mixture of 95% O_2 and 5% CO_2. The buffer reservoirs were also maintained at 37° and after the equilibration period the pH ranged from 7·2 to 7·5. In the drug-containing perfusion media, the phenothiazines were added from stock solutions to the buffer media immediately prior to the perfusion of the tissue. The effluent flow rate ranged from 4·0–4·5 ml/min at a pulse rate of 20/min. A 15-min perfusion period was allowed for tissue equilibration and for establishment of a constant flow rate. Samples of the effluent were collected in graduated tubes at minute intervals. Analysis of the effluent fraction from the isolated pancreas for IRI content was carried out in the same manner as

* Glucostat, Worthington Biochemical Corp., Freehold, N.J.

† Insulin Radioimmunoassay Kit, Amersham/Searle Corp.

previously described for plasma IRI. The presence of phenothiazines in the effluent did not interfere with the immunoassay of insulin. Prior to determination of the drug-induced changes in insulin output, the insulin values were corrected for "resting secretion" by subtraction of the mean value derived from the 2-min pre-stimulus effluent fractions. The perfusion studies were carried out over a period of days; therefore, the control glucose experiments and the glucose-free perfusions were spaced throughout the experimental phase such that at least one control tissue was perfused on days when phenothiazines were evaluated.

Chlorpromazine hydrochloride and mono-*N*-desmethyl-chlorpromazine hydrochloride (desmethylchlorpromazine) were supplied by Smith, Kline & French Laboratories, Philadelphia, Pa. Fluphenazine dihydrochloride was donated by E. R. Squibb & Co., Princeton, N.J. Bovine albumin fraction V was obtained from Sigma Chemical Co. All other chemicals used in the preparation of buffers were of analytical grade.

RESULTS

The effect of graded intraperitoneal doses of the phenothiazines on plasma glucose levels 1 hr after treatment is shown in Fig. 1. Chlorpromazine at 5 and 10 mg/kg, i.p., and desmethylchlorpromazine at 10 mg/kg, i.p., significantly elevated glucose levels above those associated with saline treatment. Fluphenazine was not effective at any of the dosage levels tested. At the 10 mg/kg dose, the elevation of blood sugar caused by chlorpromazine was significantly greater than that produced by desmethylchlorpromazine and fluphenazine. This same ordered relationship exists among these phenothiazine derivatives when evaluated for hyperglycemic effect in the mouse.[10]

The effects of phenothiazine pretreatment (10 mg/kg, i.p.) on plasma glucose levels in adrenalectomized and sham-operated rats after the administration of a glucose load (2 g/kg, i.p.) are shown in Table 1. Chlorpromazine pretreatment caused a pronounced delay in the removal of glucose from the blood in adrenalectomized and

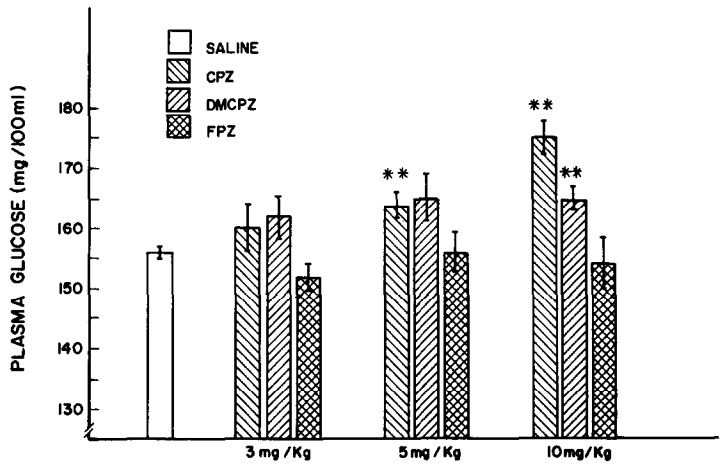

FIG. 1. Dose-related effect of phenothiazine administration on plasma glucose in rats 1 hr after treatment. Each bar represents the mean plasma glucose levels ± S. E. of five rats treated with chlorpromazine (CPZ), desmethylchlorpromazine (DMCPZ) or fluphenazine (FPZ). The double asterisk (**) indicates significantly different from control ($P < 0.01$).

TABLE 1. EFFECT OF PHENOTHIAZINE PRETREATMENT ON PLASMA GLUCOSE IN ADRENALECTOMIZED AND SHAM-OPERATED RATS AFTER A GLUCOSE LOAD*

Treatment	Time, after glucose load (min) 15	30	60	120
Adrenalectomized				
Saline	328 ± 36	257 ± 15	152 ± 15	148 ± 4
CPZ†	384 ± 54	421 ± 31	343 ± 48	181 ± 9
DMCPZ†	544 ± 39	378 ± 34	184 ± 3	178 ± 7
FPZ†	385 ± 34	369 ± 34	279 ± 23	177 ± 14
Sham-operated				
CPZ	456 ± 18	510 ± 49	325 ± 21	263 ± 55
DMCPZ	438 ± 75	494 ± 65	224 ± 17	166 ± 6
FPZ‡	498 ± 42	412 ± 59	371 ± 69	271 ± 49

* Groups of adrenalectomized or sham-operated rats were pretreated with chlorpromazine (CPZ), desmethylchlorpromazine (DMCPZ) or fluphenazine (FPZ), 10 mg/kg, i.p., 30 min prior to the administration of a glucose load (2 g/kg, i.p.). Control adrenalectomized groups received saline followed by glucose. Each value represents the mean plasma glucose level ± S. E. of four animals.

† Glucose curve of phenothiazine treatment is significantly different from saline treatment $P < 0.05$.

‡ Glucose curve of sham-operated phenothiazine-treated groups is significantly different from adrenalectomized phenothiazine treatment $P < 0.05$.

sham-operated groups. However, with chlorpromazine treatment, prior adrenalectomy caused no significant deviation from the level of glucose intolerance demonstrated by the sham-operated animals. Similar findings resulted from desmethylchlorpromazine treatment. No significant differences were detected between the glucose curves generated from adrenalectomized and sham-operated animals, although both were significantly greater than the corresponding saline-treated groups. Fluphenazine was less effective than the other analogs in altering the disposition of glucose in adrenalectomized animals, since the level of intolerance was significantly less than that observed in the sham-operated group.

TABLE 2. EFFECT OF PHENOTHIAZINE PRETREATMENT ON THE INSULINOGENIC INDICES OF ADRENALECTOMIZED RATS AFTER THE ADMINISTRATION OF A GLUCOSE LOAD*,†

Treatment	Time after glucose load (min) 15	30	60	120
Saline	0·35 ± 0·05	0·90 ± 0·27	0·38 ± 0·06	0·24 ± 0·06
CPZ	0·14 ± 0·01	0·36 ± 0·15	0·15 ± 0·03	0·23 ± 0·02
DMCPZ	0·20 ± 0·06	0·31 ± 0·08	1·05 ± 0·23	0·37 ± 0·04
FPZ	0·69 ± 0·16	0·93 ± 0·18	0·43 ± 0·20	0·31 ± 0·02

* Insulinogenic index = [plasma immunoreactive insulin (μU/ml)]/[plasma glucose (mg/100 ml)].

† Groups of adrenalectomized rats were pretreated with chlorpromazine (CPZ), desmethylchlorpromazine (DMCPZ) or fluphenazine (FPZ), 10 mg/kg, i.p., 30 min prior to the administration of a glucose load (2 g/kg, i.p.). Control adrenalectomized groups received saline followed by glucose. Each value represents the mean ratio ± S. E. of plasma immunoreactive insulin to the corresponding plasma glucose levels from the same groups of rats shown in Table 1. CPZ produced a decrease ($P < 0.05$) in the indices compared to saline controls. DMCPZ produced a lower index ($P < 0.05$) at 30 min and a higher index ($P < 0.05$) at 60 min when compared to saline controls.

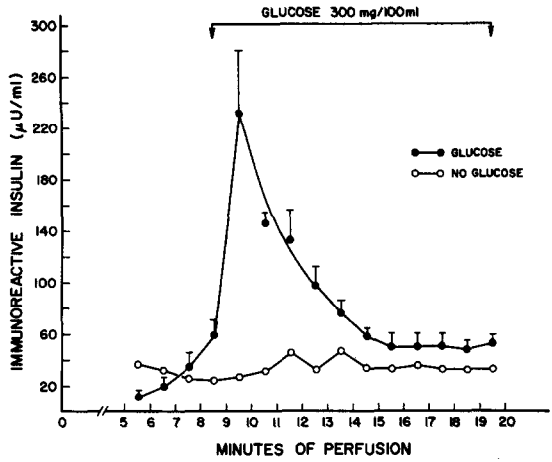

FIG. 2. Effect of glucose (300 mg/100 ml) on insulin secretion from the isolated perfused rat pancreas. Each point for the glucose-stimulated insulin release is the mean ± S. E. obtained from six glands. Control glands (N = 2) were perfused with glucose-free Krebs–Ringer bicarbonate buffer.

The insulinogenic indices determined for the various phenothiazine treatments are shown in Table 2. A low index indicates a reduced capacity of the pancreas to respond to a glucose stimulus.[23] Chlorpromazine produced a significant reduction in the ratios compared to saline controls when tested over the whole time period involved. However, no differences were detected between fluphenazine, 10 mg/kg, and saline-treated groups. Desmethylchlorpromazine treatment caused no significant change in the total insulin secreted compared to control. However, the lack of parallelism of the curves is reflected in a significant interaction term (F = 7·35, df = 3·24,

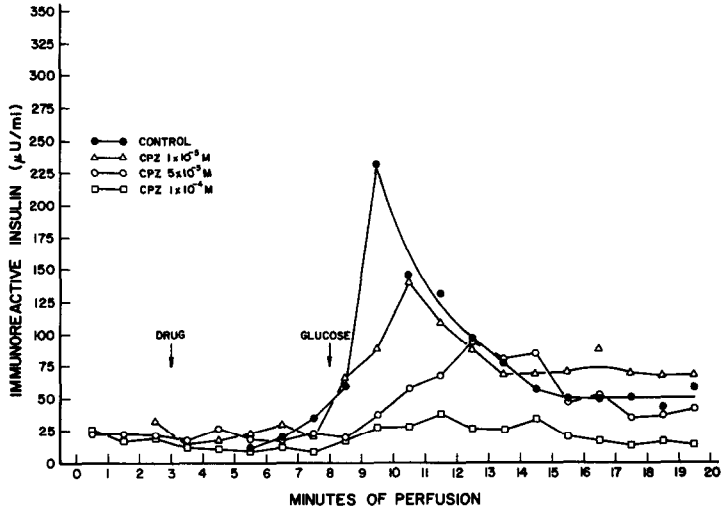

FIG. 3. Effect of chlorpromazine (CPZ) on the glucose-stimulated insulin secretion from the isolated perfused rat pancreas. CPZ was added to the perfusion buffer to attain the appropriate concentration and was present 5 min before and 12 min during the glucose stimulus. Control points represent the mean from six glands; n = 5 for 10^{-5} M CPZ; n = 3 for all others.

$P < 0.025$). Differences in means occur at the 30- and 60-min time intervals where a significant decrease in the insulinogenic index resulted at 30 min but increased significantly above controls at the 60-min period.

Figure 2 shows the pattern of insulin secretion by the isolated pancreas when perfused with a medium containing 300 mg/100 ml of glucose. Insulin output by the pancreas was increased sharply during the first several min, and the secretory rate declined during the subsequent 1-min intervals. No significant increase in the secretory level was observed during perfusion with the glucose-free buffer.

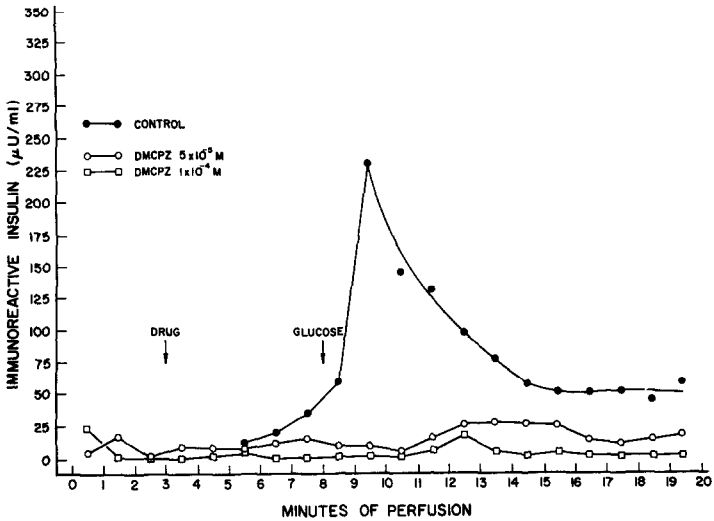

FIG. 4. Effect of desmethylchlorpromazine (DMCPZ) on the glucose-stimulated insulin secretion from the isolated perfused rat pancreas. DMCPZ was added to the perfusion buffer to attain the appropriate concentration and was present 5 min before and 12 min during the glucose stimulus. Control points represent the mean from six glands; n = 3 for each concentration of DMCPZ.

Chlorpromazine (10^{-5} to 10^{-4} M) produced a concentration-dependent inhibition of glucose-evoked insulin output (Fig. 3). The presence of 10^{-4} M chlorpromazine in the perfusion medium was effective in reducing by slightly over 80 per cent the efflux of insulin induced by glucose stimulation. Desmethylchlorpromazine demonstrated greater activity in this respect (Fig. 4). Whereas chlorpromazine at 5×10^{-5} M reduced the efflux by approximately 50 per cent, desmethylchlorpromazine produced a decrement from control values on the order of 90 per cent. In contrast, fluphenazine was less effective in altering pancreatic secretory activity (Fig. 5). The presence of 1×10^{-4} M fluphenazine caused about the same degree of inhibition as did 5×10^{-5} M chlorpromazine (47 vs 49 per cent). Furthermore, a 90 per cent reduction from control insulin values occurred at 5×10^{-4} M fluphenazine, whereas comparable inhibitory activity was observed with desmethylchlorpromazine at one-tenth that concentration.

DISCUSSION

There is general agreement that phenothiazine-induced blood glucose elevation involves adrenomedullary epinephrine release, since removal of the adrenal medullae

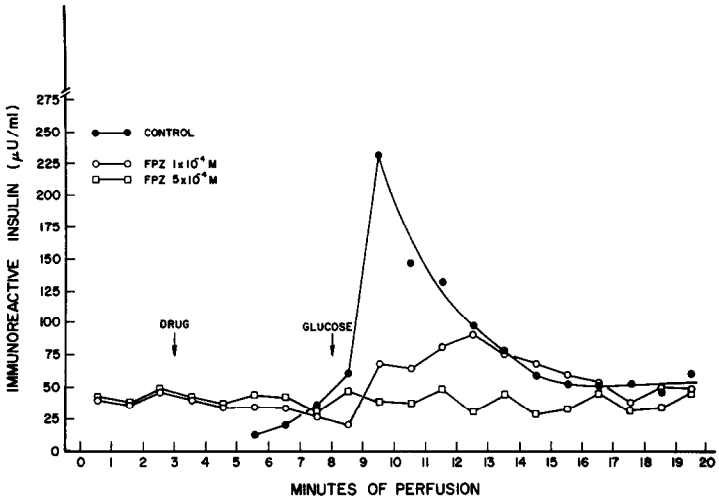

FIG. 5. Effect of fluphenazine (FPZ) on the glucose-stimulated insulin secretion from the isolated perfused rat pancreas. FPZ was added to the perfusion buffer to attain the appropriate concentration and was present 5 min before and 12 min during the glucose stimulus. Control points represent the mean from six glands; n = 3 for each concentration of FPZ.

abolishes or attenuates the glycemic effects.[10,11,13] However, despite the obvious role of the adrenals in initiating the hyperglycemia, extra-adrenal mechanisms appear to be more important in the over-all effect on blood sugar. Thus, the magnitude of hyperglycemia produced by the phenothiazine analogs cannot be explained on the sole basis of their catecholamine-releasing activities.[10] This was most apparent in the case of fluphenazine, which demonstrated better activity in releasing adrenal catecholamines[10] but was less effective than chlorpromazine in elevating blood sugar.[10]

Data from the present study show that inhibition of insulin release is a major factor in determining the extent of the hyperglycemic effect of certain phenothiazine analogs. Thus, chlorpromazine and its desmethyl derivatives were shown to inhibit insulin secretion in response to the high levels of glucose as evidenced by the resulting low insulinogenic indices. Consistent with the observations *in vivo*, chlorpromazine and desmethylchlorpromazine decreased the glucose-evoked increase in insulin output from the isolated perfused rat pancreas, the latter agent being more active in this respect at equivalent concentrations. In contrast to the other two agents, fluphenazine exerted no apparent influence on the pancreatic secretory activity in adrenalectomized animals.

The inhibition of secretion by desmethylchlorpromazine may be significant since this analog is recognized as a metabolite of the parent drug chlorpromazine.[24] Accordingly, the observed inhibitory effect by chlorpromazine on pancreatic activity *in vivo* may reflect the contribution of some of its metabolites as well.

Although the exact nature and site of inhibition of insulin secretion is unknown, phenothiazines have been shown to exert inhibitory effects on a number of enzyme and energy-generating systems.[25–27] Interestingly, the monodesmethyl metabolites of tricyclic psychoactive drugs have recently been shown to exert a stronger inhibition of the Na–K–ATPase activity as well as a higher affinity for binding protein than the parent compounds.[28]

The glucose intolerance observed in fluphenazine-treated adrenalectomized rats cannot be attributed to a drug effect on the pancreas since no alteration to insulin activity was observed. Thus, this slight intolerance must be extra-pancreatic as well as extra-adrenal in origin. This finding also suggests that phenothiazines may act to block the effect of insulin activity. Antagonism of insulin activity by certain phenothiazines is also implied from studies in which chlorpromazine was shown to afford protection against the hypoglycemic convulsions and death in mice after insulin injection.[2]

Alterations in glucose metabolism by chlorpromazine, and other phenothiazines, have been encountered clinically.[6,9] Fluphenazine may be more useful in these situations, since it appears to have little effect on insulin release from the pancreas.

REFERENCES

1. S. COURVOISIER, J. FOURNEL, R. DUCROT, M. KOLSKY and P. KOETSCHOT, *Archs int. Pharmacodyn. Thér.* **92,** 305 (1953).
2. D. NORMAN and W. A. HIESTAND, *Proc. Soc. exp. Biol. Med.* **90,** 89 (1955).
3. J. A. LEBLANC, *Proc. Soc. exp. Biol. Med.* **103,** 621 (1960).
4. M. S. SIMOES and W. OSSWALD, *Metabolism* **4,** 333 (1955).
5. S. K. GUPTA, M. A. PATEL and A. D. JOSEPH, *Archs int. Pharmacodyn. Thér.* **128,** 82 (1960).
6. B. W. HILES, *J. Am. med. Ass.* **162,** 1651 (1956).
7. C. KORENYI and B. LOWENSTEIN, *Dis. nerv. Syst.* **29,** 827 (1968).
8. J. MOYER, V. KINROSS-WRIGHT and R. M. FINNEY, *Arch. Intern. Med.* **95,** 202 (1955).
9. A. AMDISEN, *Acta psychiat. scand.* **40** (180), 411 (1964).
10. A. G. PROAKIS, J. H. MENNEAR, T. S. MIYA and J. L. BOROWITZ, *Proc. Soc. exp. Biol. Med.* **137,** 1385 (1971).
11. A. BONACCORSI, A. GARATTINI and A. JORI, *Br. J. Pharmac. Chemother.* **23,** 93 (1964).
12. M. MRAZ and L. TRINER, *Archs int. Pharmacodyn. Thér.* **141,** 434 (1963).
13. T. GHAFGHAZI, T. S. MIYA, J. H. MENNEAR and R. K. CHALMERS, *J. pharm. Sci.* **57,** 1690 (1968).
14. J. H. MENNEAR and T. S. MIYA, *Proc. Soc. exp. Biol. Med.* **133,** 770 (1970).
15. A. JORI, D. BERNARDI and S. GARATTINI, *Int. J. Neuropharmac.* **3,** 553 (1964).
16. A. S. SUSTEN, J. H. MENNEAR and T. S. MIYA, *Biochem. Pharmac.* **20,** 3145 (1971).
17. J. BERNSOHN, I. NAMAJUSKA and L. S. COCHRANE, *Archs Biochem. Biophys.* **62,** 274 (1956).
18. R. V. CHAGOVETS and T. M. SHTUTMAN, *Fedn Proc.* **22**(2), 935 (1963).
19. A. S. SUSTEN and J. H. MENNEAR, *Toxic. appl. Pharmac.* **24,** 364 (1973).
20. C. D. HALES and P. J. RANDLE, *Biochem. J.* **88,** 137 (1963).
21. G. M. GRODSKY, A. A. BATTS, L. L. BENNETT, C. VCELLA, N. B. MCWILLIAMS and D. F. SMITH, *Am. J. Physiol.* **205,** 638 (1963).
22. W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER, in *Manometric Techniques*, p. 149. Burgess, Minneapolis (1957).
23. H. S. SELTZER and V. L. HARRIS, *Diabetes* **13,** 6 (1964).
24. J. L. EMMERSON and T. S. MIYA, *J. pharm. Sci.* **52,** 411 (1963).
25. L. G. ABOOD, *Proc. Soc. exp. Biol. Med.* **88,** 688 (1955).
26. M. BERGER, H. J. STRECKER and H. WAELSCH, *Nature, Lond.* **177,** 1234 (1956).
27. A. SKINNER and R. G. SPECTOR, *Br. J. Pharmac. Chemother.* **33,** 129 (1968).
28. H. HACKENBERG and J. KRIEGLSTEIN, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **274,** 63 (1972).