Preliminary Clinical Evaluation of Glybenclamide in Treatment of Diabetes Mellitus

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SUMMARY

An initial clinical trial with a new hypoglycemic sulfonylurea is described. In most patients, significant reductions in blood sugar and elevations of plasma insulin levels were produced throughout the day, by doses ranging from 2.5 to 15 mg. twice daily. The greatly enhanced activity compared to other sulfonylureas may be due to increased hydrophobic bonding to the receptor vicinity. Two patients developed hypoglycemic reactions. Transient elevations in transaminase levels were the only other toxic effect noted. Our results to date do not suggest that this compound is clinically advantageous to other orally active hypoglycemic agents. DIABETES *19*:579-84, August, 1970.

Since their introduction in 1955, sulfonylureas have become well established in the treatment of diabetes mellitus of maturity onset. None of the several analogues introduced to date into the practice of medicine have appreciably increased the number of patients in whom they can replace insulin. Although toxicity is rarely severe, gastrointestinal disturbances, skin eruptions, bone marrow depression and hepatocellular damage have occurred. Limited application and side effects account in part for the continued interest in new preparations.

Glybenclamide (U26452, HB-419) is a recently developed sulfonylurea. Its chemical formula is N-4 [2-(5-chlor-2 methoxybenzamido)-ethyl]-phenylsulfonyl-N'-cyclohexyl urea. Compared on the basis of weight, it is approximately 1,000 times more potent in the dog.[1] It is similar to other sulfonylureas in that it has no independent action in totally pancreatectomized animals, but potentiates the effect of exogenous insulin. Enhanced insulin secretion has been demonstrated in the perfused rat pancreas, the pancreatico-duodenal vein of intact dogs[1] and in rats following subtotal pancreatectomy.[2] The present study was undertaken to investigate the usefulness and possible side effects of the new agent in the control of maturity-onset diabetes.

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METHODS

Selection of patients

Twelve maturity-onset diabetics were selected on the basis of stability of their disease over several months. Ages ranged from thirty-nine to seventy-one years, and weight from 122 to 269 pounds. There were four men and eight women; six were white and six nonwhite. Although all patients were receiving oral hypoglycemic agents, the degree of control ranged from good to poor. Two of the patients had previously received insulin for periods up to two years. One patient (E. F.) never had significant elevation of fasting blood sugar levels. The remaining eleven patients were overt diabetics, with mean fasting blood sugar ranging from 106 to 392 mg./100 ml. while not receiving treatment. No patient had a history or evidence of ketosis, liver disease, azotemia, senility or alcoholism. The only important coincident disease was essential hypertension in five of the patients. Treatment for this was continued throughout the study with a thiazide diuretic in three, and either methyldopa or guanethidine in the two others.

Design of the clinical study

The current hypoglycemic treatment was replaced by placebo for seven days before admission to a metabolic ward. A weighed diet of 2200 calories with 180 gm. of carbohydrate for men, and 1800 calories with 160 gm. of carbohydrate for women was administered during the two-week in-patient study. The carbohydrate content of each meal was the same each day. Moderate activity was permitted.

On Days 1, 2, and 3 a placebo capsule was administered twelve-hourly, and blood sugar and plasma insulin determined at 8 a.m., noon, 4 p.m. and 8 p.m. During this control period, base-line estimations of complete blood count, sedimentation rate, alkaline phosphatase, serum glutamic oxalo-acetic and pyruvic transaminases, serum triglycerides, cholesterol, direct and indirect bilirubins, serum electrolytes, blood urea nitrogen, creatinine, uric acid and prothrombin time were made. On each day, urine was collected from 8 a.m. to 2 p.m., 2 p.m. to 8 p.m., and 8 p.m. to 8 a.m. for determination of urinary sugar and insulin. On Day 4 each patient drank a liter of water at 7 a.m. and a further 200 cc. each hour for the next six hours. A placebo capsule was taken at 8 a.m. immediately before breakfast. Patients received no lunch. Blood and urine were collected each hour until 2 p.m. for determination of sugar, insulin and creatinine. On Day 5, the procedure was identical, but glybenclamide was administered instead of placebo. The dose varied between 2.5 and 15 mg., the higher doses being given to patients with higher fasting blood sugar levels during the control period. Between Days 6 and 12 the procedure was followed as on Days 1 to 3, with glybenclamide given instead of placebo. The dosage selected on Day 5 was given at 8 a.m. and 8 p.m. Blood and urine collections were performed on Days 6, 8, 10 and 12, with toxicity studies on Days 8 and 12.

After the in-patient study, the dose of glybenclamide was gradually increased to a maximum of 25 mg. twelve-hourly, or until diabetic control was judged adequate. Patients were seen weekly and blood collected two hours after breakfast for determination of sugar, insulin, and toxicity studies. Patients also brought urine collected during the preceding twenty-four hours for determination of sugar. In one patient (E. F.), hypoglycemic treatment was believed unnecessary and glybenclamide was discontinued after the in-patient period. Another patient (R. M.) suffered a cerebrovascular accident soon after leaving the hospital, and was withdrawn from further study. The other ten attended the clinic at weekly intervals for two months. Each patient then replaced glybenclamide by chlorpropamide 250 mg. each morning, and attended the clinic twice for the same investigations at two-weekly intervals. Chlorpropamide was replaced by placebo for the final four weeks, during which the patients attended the clinic twice.

Chemical methods

Sugar was determined by the Hoffman modification of the ferricyanide method on a Technicon Auto-Analyzer.[3] Plasma insulin was assayed as immunoreactive insulin by the method of Hales and Randle[4] with use of pork insulin standards. Urinary insulin was determined according to the method of Jorgensen.[5] However, because of the appearance of a precipitate after thawing of frozen urine samples, a second centrifugation was carried out.

Statistical evaluation

Statistical significance was assessed by means of the t test for paired observations, using the one-tailed method.

RESULTS

Single dose

Figure 1 illustrates the differences in blood sugar and plasma insulin following placebo or glybenclamide on Days 4 and 5. Significant decrease in blood sugar and elevation in plasma insulin occurred between two and six hours after glybenclamide ($p < 0.05$). As shown in figure 2, significant reductions in urinary sugar

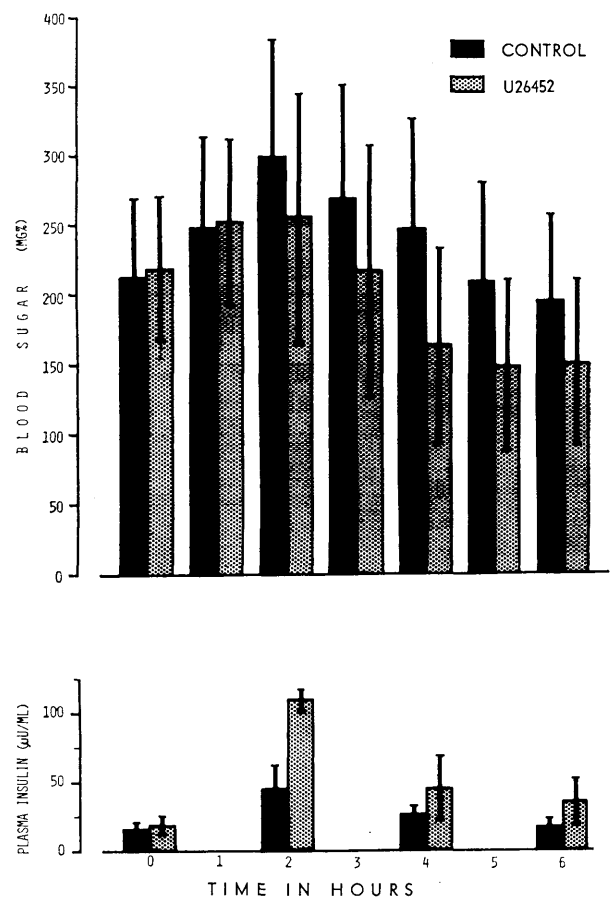


FIG. 1. Comparison of blood sugar and plasma insulin levels following a single dose of placebo or glybenclamide.

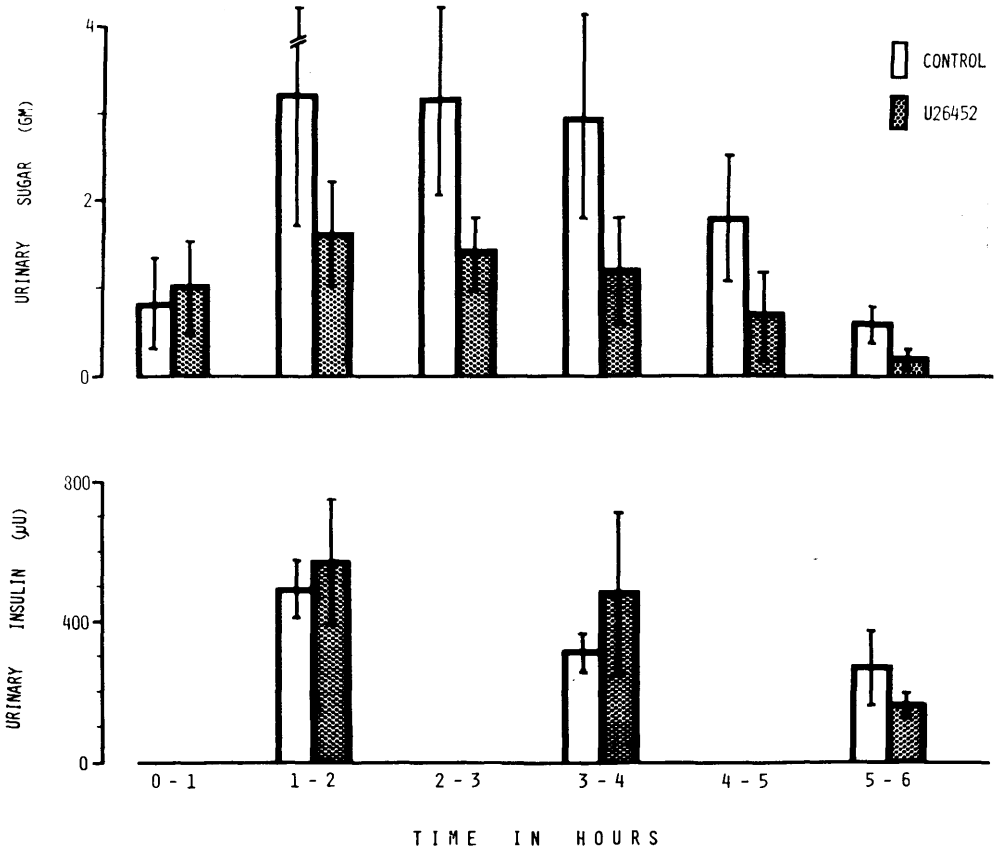


FIG. 2.

Urinary sugar and insulin excretion following a single dose of placebo and glybenclamide.

excretion ($p < 0.05$) occurred between three and six hours, without significant change in urinary excretion of insulin.

Twelve-hourly administration

Determinations obtained at each collection time were averaged for each patient in both control (Days 1-3) and test periods (Days 6-12). As shown in figure 3, significant ($p < 0.005$) decrease in blood sugar occurred throughout the day. By averaging all values obtained for each patient, figures representative of the total control and test periods were obtained. A significant reduction from a mean value of 234 ± 33 to 192 ± 31 mg./100 ml. was demonstrated ($p < 0.001$). By the same methods of calculation, significant elevations in plasma insulin were demonstrated at each collection time ($p < 0.05$), and a significant increase from mean values of 30 ± 4 to 41 ± 5 μU. per ml. was demonstrated ($p < 0.001$). As shown in figure 4, similar changes were demonstrated less clearly in the urine. Average twenty-four-hour excretion figures were calculated for each patient. A decrease in mean values of sugar from 35 ± 12 to 23 ± 12 gm. was just below the level of significance ($0.05 < p < 0.10$). An increase in urinary insulin from 5,065 ± 1,620 to 7,160 ± 1,910 μU. was significant ($p < 0.025$).

When the figures representative of control and test periods are compared, there was a decrease in blood sugar ranging from 7 to 43 per cent, and a 12 to 36 per cent increase in plasma insulin. As demonstrated in figure 5, however, there was no correlation between the percentage changes in blood sugar and plasma insulin ($r = 0.11$ $p > 0.05$).

There was a highly significant correlation between blood sugar and urinary sugar excretion in both control ($r = 0.93$, $p < 0.001$) and test ($r = 0.86$, $p < 0.001$) periods. There was no significant correlation, however, between plasma insulin and urinary insulin excretion ($r = 0.02$, $p > 0.05$) in control or test periods.

Toxicity studies

Two subjects (E. F. and R. M.) experienced one episode each of symptomatic hypoglycemia. Whereas R. M. was taking 10 mg. twelve-hourly, E. F. was taking only 2.5 mg. twelve-hourly when hypoglycemia occurred. Neither had further trouble following dose

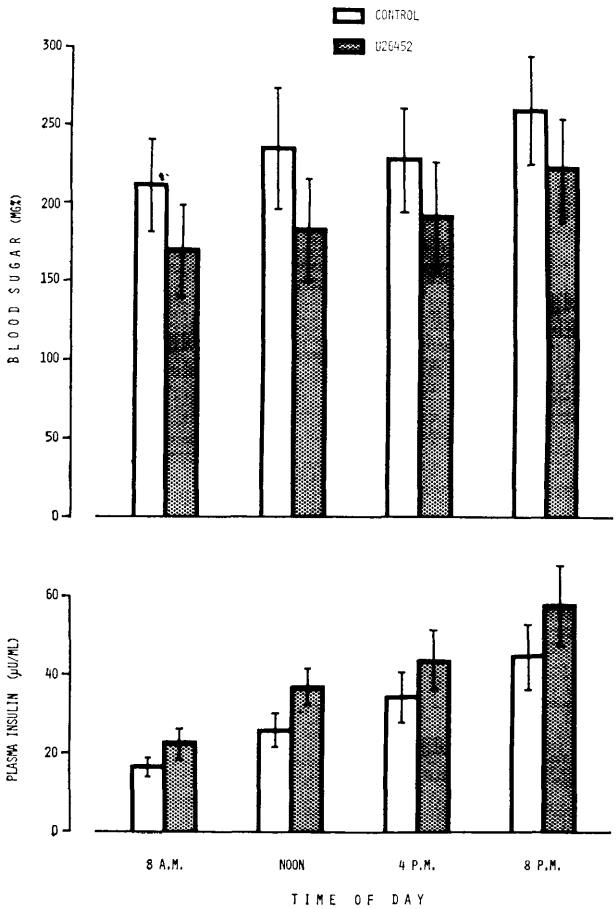


FIG. 3. Mean blood sugar and plasma insulin levels during several days of twelve-hourly administration of placebo or glybenclamide.

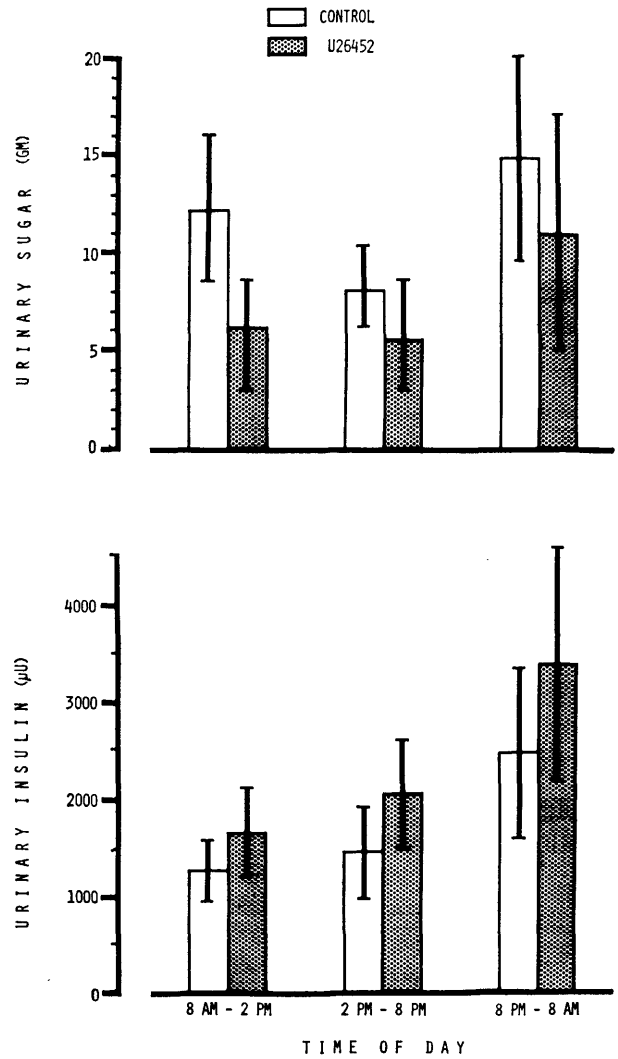


FIG. 4. Mean urinary sugar and insulin excretion during several days of twelve-hourly administration of placebo or glybenclamide.

reduction. The subsequent cerebrovascular accident in R.M. was not related to hypoglycemia. No gastrointestinal, or neurological symptoms were reported, and patients taking antihypertensive drugs showed no change in blood pressure on glybenclamide. No change in creatinine clearance was observed after glybenclamide administration.

Moderate elevations in serum glutamic oxalo-acetic transaminase occurred in four patients at varying times within the period of glybenclamide administration. All four had original values at the upper limits of normal, ranging from 35 to 45 U./ml. The maximal value determined was 83 U./ml. No important changes were demonstrated in other tests of liver function. One patient (W. R.) developed a transient elevation of blood urea nitrogen with a temporary increase in hydrochlorothiazide dosage. This elevation disappeared without changing the dosage of glybenclamide. No significant changes were seen in the blood counts or other determinations.

Out-patient study

Six patients treated on an out-patient basis maintained adequate control as judged by values of blood sugar persistently below 160 mg./100 ml. two hours after a meal. These patients required less than 20 mg. of glybenclamide per day. Inadequate control of the other six patients was not improved by increasing the dosage to 50 mg. per day.

The mean blood sugar levels determined two hours after a meal following administration of glybenclamide, chlorpropamide, or placebo are recorded in table 1. Whereas three patients had lower values while taking

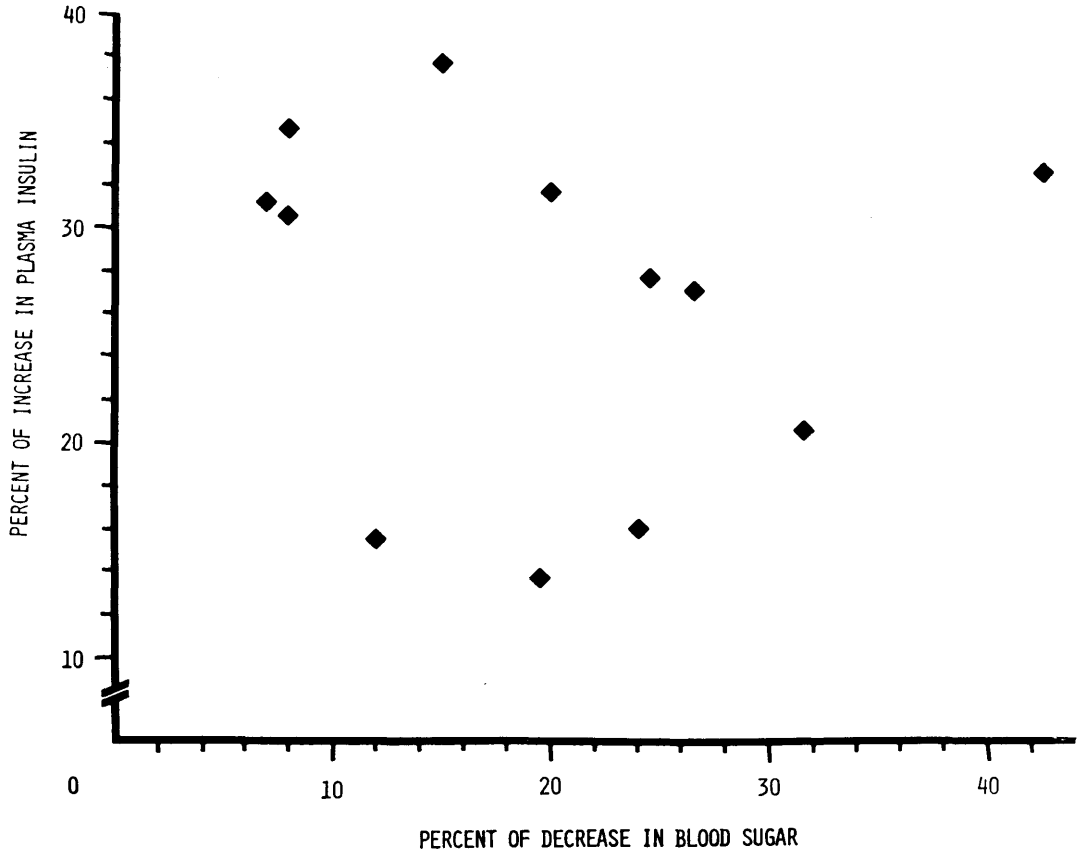


FIG. 5. Scattergram indicating the lack of correlation between percentage changes in blood sugar and plasma insulin levels during several days of glybenclamide administration.

chlorpropamide, the majority appeared to have lower levels when receiving glybenclamide. There was considerable variation in the levels of plasma insulin and urinary sugar excretion, and no significant difference from control could be demonstrated for either chlorpropamide or glybenclamide.

TABLE 1

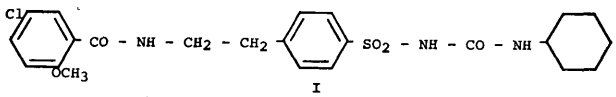
A comparison of blood sugar in mg./100 ml. two hours after food following glybenclamide, chlorpropamide, or placebo

Patient	Glybenclamide	Chlorpropamide	Placebo
B.I.	101	83	101
E.L.	86	91	169
M.F.	177	307	238
F.G.	116	136	158
L.Hi.	289	412	400
S.W.	168	223	303
W.R.	324	234	334
M.B.	365	331	552
A.W.	177	241	323
L.Ho.	225	308	380
Means	203	237	296

DISCUSSION

The sulfonylureas have a limited but assured place in the treatment of the maturity-onset diabetic. Their limitations are inherent in their mode of action, as they require viable beta cells to be effective. A new oral hypoglycemic agent of promise would have a different mechanism of action, and would extend the number of patients beyond those presently responsive to the well-tried sulfonylureas such as tolbutamide and chlorpropamide. Glybenclamide has been demonstrated to lower blood sugar effectively in maturity-onset diabetics. The increases in plasma and urinary insulin suggest that as with other sulfonylureas, the important mechanism is enhanced secretion of insulin. However, the lack of correlation between percentage changes in blood sugar and plasma insulin is compatible with a more complex mechanism. The suggestion that sulfonylureas also reduce pancreatic glucagon secretion[6] has not been supported by more recent studies.[7] Other possibilities include potentiation of the action of secreted insulin on peripheral cells.

Glybenclamide is a chemical extension of the previous work with sulfonylureas, and one of its interesting aspects lies in the remarkable increase in potency. As all studies to date suggest the same mechanism of action of tolbutamide and glybenclamide, the potency difference could be due to a quantitative difference in the interaction between the drugs and the same receptor. Other possibilities include different rates of transport, but the chemical structure of glybenclamide (I) does not suggest that either protein binding or lipid solubility would radically differ from tolbutamide (II).



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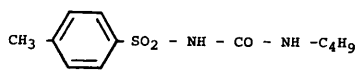

II

The principal mode of interaction between the sulfonylurea formation and receptor is probably hydrogen bonding, with the sulfonyl and carbonyl oxygens acting as donors and the urea hydrogens acting as acceptors. The chemical change should have very little influence on the strength of hydrogen bonding. Therefore, if increase of hypoglycemic activity is due to enhanced binding strength, increased hydrophobic bonding may be solely responsible for it. In glybenclamide the additional groups participating in hydrophobic bonding may be the benzene ring and chloride and methoxy groups of the anisamido formation, and the ethyl methylene group.

Although glybenclamide has much greater potency than other sulfonylureas, no other advantage has been noted. The potency may actually be disadvantageous in that hypoglycemic reactions would be expected to be frequent in mild diabetics. Whereas mild diabetics responded well to glybenclamide, more severe diabetics who had responded inadequately to previous oral therapy were also controlled inadequately by glybenclamide. It is possible that some diabetics inadequately controlled by presently available sulfonylureas may respond better to glybenclamide. In this preliminary study, control of two-hour postprandial blood sugar was better on high doses of glybenclamide than on 250 mg. of chlorpropamide, and a more detailed comparison would seem indicated. In animals, glybenclamide has been remarkably nontoxic. Although no evidence of serious toxicity was found in this study, more investigation will be required before the incidence relative to other sulfonylureas can be established.

ACKNOWLEDGMENT

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