Studies in Patients with Chlorpropamide-Induced Hypoglycemia

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SUMMARY

Six patients with hypoglycemia due to chlorpropamide are reported. The common characteristics were old age and/or poor nutritional state.

The serum concentration of chlorpropamide during hypoglycemia was not higher than that seen in a group of chlorpropamide-treated diabetics without hypoglycemia. In both groups, the serum level declined in a rectilinear fashion after the drug was stopped, and the rate of decline was similar in the two groups. The serum level of chlorpropamide in the control group covered a fourfold range at the same dose level, and had no correlation with the degree of diabetic control.

The serum insulin concentration was in two instances somewhat high compared to the low blood sugar, but most of the time was found to be at a low (fasting) level. In three patients no evidence of insulin secretion in peripheral venous blood was found even after stimulation with glucagon, glucose, or meals. In two of them, however, the absence of insulin was transient and present only during the day of hypoglycemia.

It is suggested that diabetics in negative calorie balance are predisposed to hypoglycemia if they continue to take chlorpropamide, and that they suffer this complication even with a serum concentration of the drug that is within therapeutic range. The pathogenesis probably does not involve increased peripheral utilization of glucose, but more probably an inhibiting effect on production of glucose by the liver. DIABETES *19*:930-37, December, 1970.

The clinical features of hypoglycemia caused by chlorpropamide in diabetic patients have been well described in case reports[1-9] and reviews.[10,11] None of these reports contributes much to the understanding of the pathogenesis of this complication, however, which probably is far more common than the low number of reported cases would indicate. We have seen six patients with hypoglycemia caused by chlorpropamide during the last eighteen months. In these, we have measured serum chlorpropamide and serum insulin levels with the intention to elucidate possible pathogenetic factors of the hypoglycemia.

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METHODS

Blood glucose was determined with the ortho-toluidine method.[12] Normal fasting blood sugar levels in our laboratory range between 65 and 95 mg. per 100 ml. (mg. per cent). Serum chlorpropamide was determined according to Toolan and Wagner[16] with minor modifications. It proved very difficult to get chlorpropamide into solution in distilled water, and therefore a stock solution was made up of chlorpropamide in chloroform, 2,000 μg./ml. From this the daily working standard was prepared. We found it unnecessary to include a blank in the analytical procedure, as the O.D. of the blank is negligible.

The insulin determinations were performed in the Hormone Laboratory, Aker Hospital. The radioimmunoassay employed resembles that of growth hormone reported from that laboratory[13-15] with minor technical modifications. The lower sensitivity level of the insulin method is 5μU/ml., and accuracy of determination as judged by duplicate samples of the ordinary routine is $\pm$ 7 per cent.

Intravenous (I.V.) glucose tolerance test was performed according to Kienholz.[17] Thirty-three hundredths grams of glucose was given per kilogram of body weight in a 40 per cent solution, injection being completed within four minutes. K-values lower than 0.7 for the age group sixty to seventy years are clearly diabetic.

Glucagon was given intravenously in a dose of 1 mg., and blood was drawn at intervals for sixty minutes.

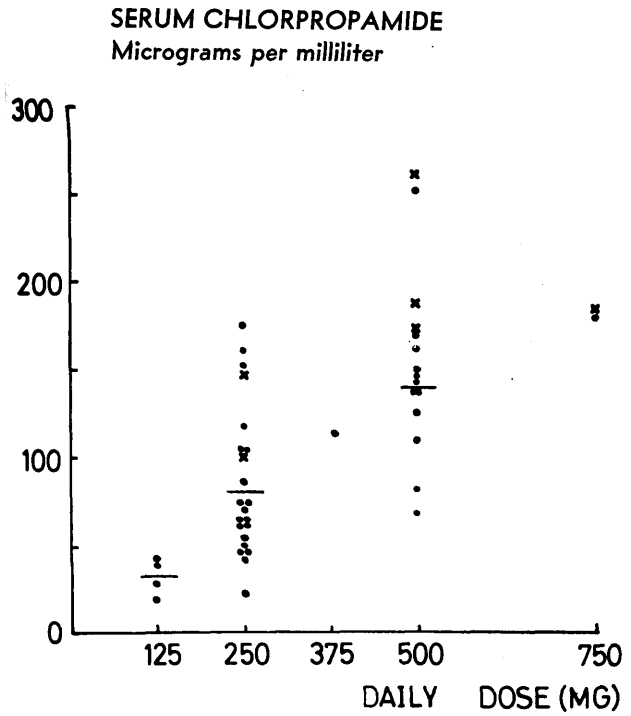

FIG. 1. Serum chlorpropamide concentration at varying dose levels in diabetic patients with (X) and without (●) hypoglycemia. Horizontal line at doses 125, 250, and 500 mg. indicates mean of patients without hypoglycemia.

Patients

The control group consisted of thirty-eight diabetic patients maintained in reasonably good diabetic control with chlorpropamide. They had never had hypoglycemia. In six of these patients, with serum chlorpropamide levels in excess of 100 μg./ml., the drug was stopped and the serum concentration examined daily in order to study the disappearance rate from the blood. A glucagon test was performed in five patients in the control group.

The hypoglycemic group

K. K., male aged sixty years, had had diabetes mellitus for six months. Upon detection of his diabetes, an oral glucose tolerance test showed a maximal blood sugar of 235 mg./100 ml. after one hour, with a corresponding serum insulin level of 41 μU/ml., which in our experience is a low response compared to that of the blood sugar. One gram tolbutamide I.V. given the next day gave a serum insulin level of 82 μU/ml. after five minutes, which is a rapid and vigorous response. He had been treated with 250 mg. chlorpropamide daily for four months when he was admitted in coma, and had not used other medication except occasionally opiates for abdominal pain. Such pains had been present for several days before admission, he had had poor appetite, but had kept on taking his hypoglycemic drug.

Upon admission in coma he was in poor general condition, slightly jaundiced and dehydrated. Data concerning serum chlorpropamide, serum insulin, and blood sugar are given in table 1. He responded quickly to intravenous glucose, but had repeated episodes of hypoglycemia during the next four days. Thus his blood sugar on two later occasions was measured at 30 and 44 mg. per 100 ml., with serum insulin of 13 and 7 μU/ml. respectively. He was given 1 mg. glucagon intravenously on two occasions: the first time his blood sugar rose from 34 to 48 mg. per 100 ml. within thirty minutes; and the next time it rose from 85 to 89 mg. per 100 ml. within the same time period.

Six days after admission his blood sugar was 60 mg. per 100 ml., and he died on Day 8. Postmortem examination showed hepatic cancer, probably adenocarcinoma of the bile ducts. The liver weighed 5 kg. and was densely packed with tumorous tissue. The pancreas and kidneys were normal.

A. P., female aged 75, had had diabetes mellitus for four

TABLE 1

Serum chlorpropamide, serum insulin and blood glucose in six diabetic patients on their day of admission in hypoglycemia

	K.K.	Aa.P.	H.K.	T.K.	A.L.P.	S.S.
Dose of chlorpropamide, mg./day	250	500	500	500	250	750
Duration of therapy with chlorpropamide	4 months	4 months	3 months	3 weeks	2 years	6 months
Last dose, hours before admission	20	8	11	9	29	24<
Serum chlorpropamide concentration, microg./ml.	149	190	262	176	104	185
Mean daily reduction in serum chlorpropamide concentration, microg./ml.	19.0	46.0	24.0	25.3	21.5	32.3
Blood glucose concentration, mg./100 ml.	17	40	34	45	53	62*
Serum insulin concentration, microunits/ml.	5	Not done	5<	38	5<	10*

*Values obtained ninety minutes after the intravenous injection of 20 ml. 40 per cent glucose.

months, maintained good control with 500 mg. chlorpropamide daily, and taken no additional medication. She had lately been having epigastric distress, and the last two to three days before admission, diarrhea and anorexia with scant food intake, but she had kept on taking chlorpropamide regularly. Upon admission she was deeply soporous, in fair general condition, and somewhat overweight. (See table 1 for blood sugar and serum chlorpropamide.) She responded promptly to intravenous glucose, but thirty-two hours later hypoglycemia recurred with a blood sugar of 28 mg. per 100 ml. Insulin analyses were unfortunately not done for this patient.

Thereafter the course was uneventful. She was dismissed with 1 gm. metformin daily, which kept her fasting blood sugar in the range of 93 to 125 mg. per 100 ml. No evidence was found of liver or renal disease.

H. K., female aged eighty-six, had had diabetes for sixteen years. She had been treated with insulin until three months prior to admission, when she was switched to 500 mg. chlorpropamide daily and 50 mg. phenformin daily. She was taking no other drug.

The last few days before admission she had been anorectic and nauseated, possibly related to the use of phenformin. She took her tablets regularly. She was admitted in coma, with levels of blood sugar, serum insulin, and chloropropamide as seen in table 1. She immediately regained consciousness after intravenous glucose, and there was no recurrence of the hypoglycemia. A nonclassified anemia with hemoglobin of 9.5 gm./100 ml. was present. No liver or kidney disease was found.

One day after admission her blood sugar was 210 mg. per 100 ml. with 6 μU./ml. serum insulin, and three days later blood sugar was 172 mg. per 100 ml. and serum insulin less than 5 μU./ml. Insulin therapy was started again.

T. K., female aged seventy-three, had had diabetes for a few months, the last twenty-one days on 500 mg. chlorpropamide daily. She used 0.25 mg.-t.i.d. Talucin (Proscillaridin) for cardiac insufficiency, and received injections of vitamin B_{12} for pernicious anemia.

For three days prior to admission she had been having diarrhea and vomiting, did not eat or drink, but kept on taking her tablets. Upon admission she was comatose, but rapidly regained consciousness after intravenous glucose. (See table 1 for blood sugar, serum insulin, and serum chlorpropamide.) Although chlorpropamide was stopped and she was able to eat, hypoglycemia recurred twenty-six hours later (blood sugar 31 mg. per 100 ml.), again with immediate response to intravenous glucose. The course was then uneventful, with fasting blood sugar in the range of 98 to 135 mg. per 100 ml. on low carbohydrate diet alone. Except for moderate cardiac failure she was in good general condition, her anemia was well compensated, and there was no evidence of liver or kidney damage.

Six days after admission, on no hypoglycemic drugs, her fasting blood sugar was 122 mg. per 100 ml. with a serum insulin level of 6 μU./ml. At 12 noon, blood sugar was 135 mg. per 100 ml. with serum insulin 34 μU./ml., and at 3:30 p.m. blood sugar was 135 mg. per 100 ml. and serum insulin 17 μU./ml. Thus her insulin response to hyperglycemia was subnormal.

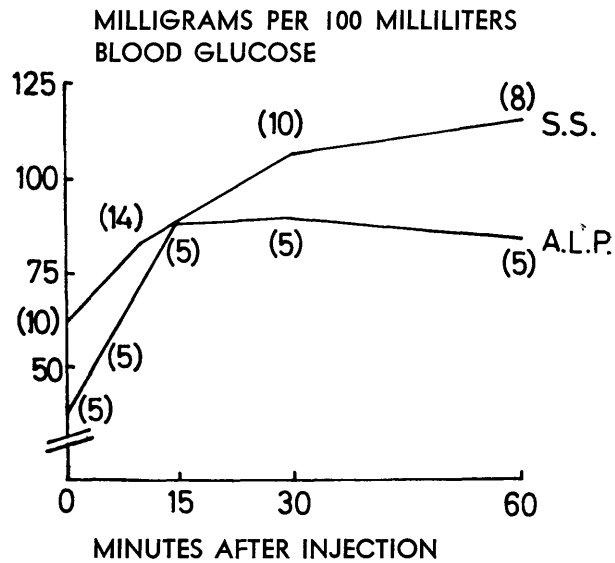

FIG. 2. Blood glucose in two hypoglycemic patients (S.S., and A.L.P.) before and after injection of 1 mg. glucagon intravenously. Serum insulin concentration (microunits/ml.) in brackets.

A. L. P., male aged sixty-six, had diabetes for two years, always treated with 250 mg. chlorpropamide daily, and no other medication.

He had been ill with pneumonia for two weeks prior to admission, febrile and anorectic. The last three days he had scarcely been eating at all, but had taken his tablets. He had felt increasingly weak and dizzy. When admitted to hospital he was soporous, perspiring, and dehydrated, with a temperature of 38.2° Centigrade. (Table 1 shows blood sugar, serum insulin and serum chlorpropamide.) Shortly after admission he was given intravenous glucagon with a rise in blood sugar but not in serum insulin (figure 2), and his level of consciousness improved. Two hours later, an intravenous glucose tolerance test was performed as seen in figure 3. Still no insulin could be demonstrated in peripheral venous blood, and peripheral utilization of glucose, as represented by the K-value, was very low.

The rest of the stay was uneventful. No evidence of liver or kidney disease was found. Two weeks later, on low carbohydrate diet and no hypoglycemic drugs, the glucagon and intravenous glucose tolerance tests were repeated. This time a modest insulinemia was present, with a maximum of 16 μU./ml. five minutes after glucagon, and a maximum of 22 μU./ml. after glucose. Oral glucose tolerance test gave a maximal insulinemia of 64 μU./ml. His fasting blood sugar remained under 122 mg. per 100 ml. and he was dismissed on a low calorie diet.

S. S., female aged sixty-one, had had diabetes for two years, the last six months on chlorpropamide in a daily dose of 750 mg. She received no other medication.

For one week prior to admission she had suffered from pneumonia, and the last two days had been stuporous. She was dyspnoeic, dehydrated—with a rectal temperature of 38.0° Centigrade, and slightly overweight. There was no

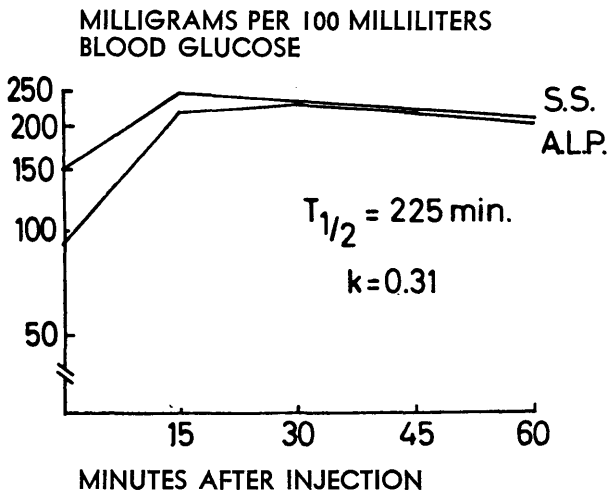

FIG. 3. Blood glucose in the same two patients as in figure 2 on their day of admission in hypoglycemia and after intravenous injection of 0.33 gm. glucose per kg. of body weight. Semilogarithmic scale.

evidence of liver or kidney disease. Her blood sugar was 35 mg. per 100 ml., and she was given 20 ml. of 40 per cent glucose intravenously. One and a half hours later, a new blood sample gave the value seen in table 1. Shortly thereafter she was given 1 mg. glucagon intravenously, and the result is seen in figure 2. One hour later, an intravenous glucose tolerance test was performed as seen in figure 3. During this procedure, serum insulin reached a maximum of 14 μU./ml. fifteen minutes after injection. Her level of consciousness returned to normal, and hypoglycemia did not recur.

One week after stopping chlorpropamide, her fasting blood sugar was 204 mg. per 100 ml. and serum insulin was 14 μU./ml. One-hour postprandial blood sugar was 270 and 223 mg. per 100 ml. on two occasions, with serum insulin levels of 16 and 11 μU./ml. respectively. A repeated glucagon test, after chlorpropamide was stopped for one week, gave a rise in blood sugar from 240 to 300 mg. per 100 ml. and a maximal serum insulin of 73 μU./ml. after five minutes. Later, when therapy with chlorpropamide had been reinstituted, glucagon again gave a blood sugar rise from 150 to 244 mg. per 100 ml. with a maximal rise in serum insulin after five minutes to 63 μU./ml.

None of the patients had ingested alcohol prior to onset of hypoglycemia.

SPECIAL MEASUREMENTS

1. *Serum chlorpropamide levels.* Six of the patients with hypoglycemia are summarized in table 1. In figure 1 are given serum levels for these patients and the thirty-eight control patients on different dose levels. In the control patients taking 250 mg. daily, the mean serum chlorpropamide concentration was 80.6 μg./ml. (S.D. ± 41.6). Thus, both hypoglycemic patients at this dose level had a serum chlorpropamide concentration which was less than 2 S.D. above the control mean. For control patients on 500 mg. daily, mean serum concentration was 139.4 μg./ml. (S.D. 36.2). One out of three hypoglycemic patients at this dose level had a serum chlorpropamide concentration which exceeded control mean plus two S.D.

2. *Disappearance of chlorpropamide from the blood.* In both the hypoglycemic and control groups, the serum chlorpropamide concentrations diminished in a rectilinear fashion after the drug was discontinued (figure 4). For the hypoglycemic group (table 1), mean daily reduction in serum level was 28.0 μg./ml., while it was 27.0 for the six control patients. This difference is not significantly different ($p > 0.5$).

3. *Glucagon tests* were performed in five patients with good diabetic control on chlorpropamide. Their maximal rise in blood sugar after thirty minutes amounted to 39, 43, 46, 48, and 82 mg. per 100 ml., with a mean of 52 mg. per 100 ml. Insulin analyses were not performed.

DISCUSSION

1. *Serum chlorpropamide.*

The values found for serum chlorpropamide in control patients at varying dose levels, and also the wide spread of these values in patients using the same dose, agree well with the results of other investigators.[18-20] The fact that good diabetic control was nevertheless

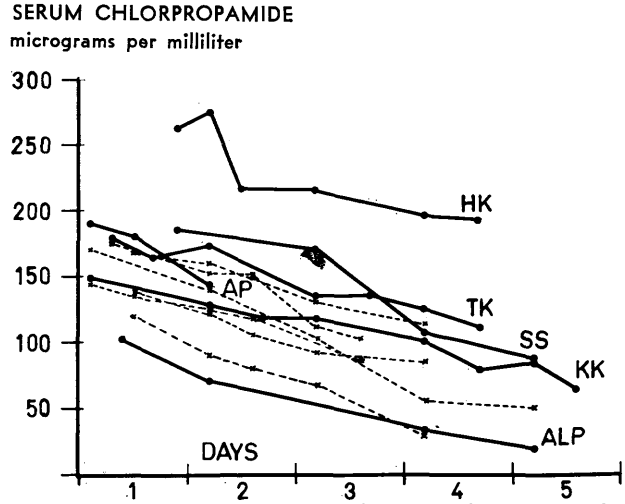

FIG. 4. Serum concentration of chlorpropamide on successive days after stopping the drug in patients with hypoglycemia (with initials, solid lines), and in six control patients (stippled lines). Day 1 is the day of admission in hypoglycemia and, for the control patients, the day after chlorpropamide was stopped. The spacing of crosses and filled circles (blood sampling) indicates the approximate hour.

achieved supports the impression of Knauff et al.[21] and Sackner and Balian[22] of poor correlation between serum levels and the degree of diabetic control. The determination of serum chlorpropamide, therefore, has minor importance in the practical management of the diabetic patient.

The serum levels of chlorpropamide in control and hypoglycemic patients cannot be fairly compared due to the uncertainty in determining the exact hour of the last dose in the latter group. According to table 1, the interval between last dose and the collection of blood usually was well below twenty-four hours, which was the exact interval in the control group. Serum levels in the hypoglycemic group are therefore, if anything, slightly overestimated in comparison with the controls.

Serum levels of chlorpropamide during hypoglycemia are rarely reported in the literature. In three patients on ordinary therapeutic doses the levels were 100 μg./ml. seventy-two hours after the last dose,[28] 154 μg./ml. seventy-seven hours after the hypoglycemia in a newborn baby of a diabetic mother on chlorpropamide,[29] and 184 μg./ml.[25] Two cases have been reported with very large doses: 350 μg./ml. after 2 gm.[22] and 500 μg./ml. after 5 gm. in suicidal intent.[30]

The present results tend to indicate that serum level of chlorpropamide is not the important denominator for the development of hypoglycemia. Only one of the hypoglycemic patients had a serum concentration that was more than 2 S.D. higher than the control mean at similar dose levels. Furthermore, disregarding the dose level, serum concentration in individual control patients often was as high as or even higher than in patients with hypoglycemia. We are of the opinion that hypoglycemia may be induced by chlorpropamide in certain susceptible individuals even if their serum level of chlorpropamide is within ordinary therapeutic range. The nature of this individual predisposition will be discussed later.

This assumes that none of our patients had been taking drugs that would interfere with the effect of chlorpropamide by increasing the "free," non-protein-bound fraction of the hypoglycemic drug in serum. Potentiation of chlorpropamide effect has been reported with the concomitant use of sulphaphenazole,[23] salicylates,[24] dicoumarol,[25] and phenylbutazon,[26] all of which are able to cause displacement of chlorpropamide from its carrier protein. The standpoint is subject, furthermore, to the following theoretical objection: Brotherton et al. recently reported[27] that, contrary to what has been believed, significant amounts of chlorpropamide are converted to metabolites that might possibly have reduced hypoglycemic activity. The method of Toolan and Wagner measures both chlorpropamide and these metabolites. If the differential extraction method of Brotherton et al. had been available, it is conceivable that there would be proportionately more active chlorpropamide contributing to the total serum measurement in the hypoglycemic patients than in the measurement of the control group.

On the basis of the reported serum chlorpropamide levels, it was not unexpected to find that the disappearance rate from serum was similar in the control and the hypoglycemic group. The rectilinear fall in serum concentration was, however, not expected, although the findings of Carlozzi et al.[20] were similar. It has, however, become customary to quote the findings of Johnson et al.[31] and of Knauff et al.[21] that the major part of chlorpropamide is being cleared from the serum in an exponential fashion with a half-time of thirty-two to thirty-six hours. These conclusions were based, however, on experiments with acute loading of previously untreated patients with either radioactively labeled[31] or unlabeled[21] drug. The possibility that prolonged use of the drug might alter the pattern of excretion, as was also suggested by the quoted authors, has received little attention. The rectilinear fall in serum concentration may indicate an overloaded excretion system.

2. *Serum insulin.*

To our knowledge, serum insulin levels during hypoglycemia caused by therapeutic dosage of chlorpropamide have not been reported. Soeldner and Steinke[35] found serum insulin levels of 17 and 6 μU./ml. when the blood sugar was 8 and 24 mg. per 100 ml., respectively, in two patients who had taken tolbutamide. Grunberg et al. observed a level of 90 μU./ml. in a child who ingested an unknown amount of chlorpropamide.[54]

If hypoglycemia were caused by excessive insulin secretion, as in insuloma of the pancreas or after intravenous injection of tolbutamide, one might expect to find high levels of insulin in peripheral venous blood. Botterman et al.[32] found a mean serum insulin of 211 μU./ml. in eleven patients with insuloma during hypoglycemia. A transient rise to almost similar levels may occur during intravenous tolbutamide tests in normal and obese people in our own experience. Such high levels were not approached in our present hypoglycemic patients.

On two occasions (K.K., blood sugar 30 mg. per 100 ml. with serum insulin 13 μU./ml., and T.K., blood

sugar 45 mg. per 100 ml. and serum insulin 38 μU./ml.), serum insulin was higher than would be expected during hypoglycemia. In our laboratory, normal fasting serum insulin is rarely found to exceed 5(-10) μU./ml. These two values therefore point in the direction of a disrupted relationship between blood sugar and serum insulin, and may be explained by continuing beta-cell stimulation by chlorpropamide in the presence of low blood sugar.

The most striking finding in our patients, however, was the very low levels of serum insulin during hypoglycemia. Even after stimulation by glucagon and intravenous glucose in two patients, significant amounts of insulin could not be demonstrated in the blood on the day of admission. The finding of Marri et al.[32] of exaggerated serum insulin response to glucagon during chlorpropamide-induced hypoglycemia could thus not be confirmed. Also in a third patient, H. K., significant amounts of insulin could not be demonstrated in serum, and insulin therapy had to be started. In these three patients functional hypo- or an insulinemia was present, at least on their day of admission in hypoglycemia.

It is our opinion that the hypoglycemia of diabetic patients on long-term treatment with chlorpropamide is usually not due to systemic hyperinsulinemia caused by excessive stimulation of the beta cells. The very low K-value in two of our patients supports this contention. This explanation is in line with studies which show that chlorpropamide has an extrapancreatic action.[35,37-40] Danowski et al.[41] in addition failed to find evidence of increased glucose utilization during these circumstances. As discussed below, the present results do not rule out the possibility of hyperinsulinemia in the portal vein, however.

In two patients the lack of serum insulin seemed to be temporary. In S.S., although serum insulin remained low in the presence of hyperglycemia, insulin secretion was promoted by glucagon when this test was repeated several days later. This dissociation between the beta-cytotropic effect of glucose and of glucagon in non-obese, noninsulin-dependent diabetics has also been noted by Simpson et al.[34] In patient A.L.P., oral glucose administered two weeks after the hypoglycemic episode resulted in a definite insulinemia in peripheral venous blood. To explain the apparently transient refractoriness of the beta cell to stimulation during chlorpropamide-induced hypoglycemia, one may consider the possibility that long-term use of the drug per se results in blunting of the insulin response, as pointed out by Reaven and Dray.[35] The insulin secretion, as described by these authors, was however not nearly diminished to the extent that it alone could account for our findings. It is more plausible that the prolonged period of negative calorie balance and low blood sugar kept the need for and the production of insulin at a low level. Refeeding would revert this pattern.

3. *The role of the liver.*

The liver probably plays a key role, not only in the mechanism of action of chlorpropamide during uncomplicated long-term treatment, but also in the development of hypoglycemia. It is well known that chlorpropamide reduces glucose production by the liver. This may be mediated through the release into the portal vein of small doses of insulin,[42-45] may be a direct effect of the sulfonylurea on the liver[46] or may be the result of suppression of endogenous glucagon.[47] This last theory has been doubted by Buchanan et al.[48]

We suggest that the hypoglycemia during treatment with chlorpropamide is also due to insufficient glucose production by the liver. The fact that most patients reported with this complication are old and/or in poor nutritional state,[1,4,5,49] as all our patients were, favors contention. This common characteristic probably represents the predisposition to hypoglycemia reactions alluded to earlier, when it was shown that the serum level of chlorpropamide in the hypoglycemic individuals was not higher than that of many controls. These patients probably have a low hepatic glycogen content. Admittedly, two of our patients reacted to exogenous glucagon with a normal rise in blood sugar, as did the hypoglycemic patients reported by others.[49-51] This finding is, however, probably insufficient evidence for reduced hepatic glycogen stores.[52] In our patient K. K., however, the response was seriously blunted, corresponding to his severely damaged liver. In this particular patient, his hepatic carcinoma may have directly contributed to the development of hypoglycemia.[53]

The present observations do not indicate in what manner chlorpropamide might affect the liver. Slight hyperinsulinemia in the portal vein might have been present without high insulin levels in peripheral venous blood. A positive response to a rather large dose of glucagon does not exclude the possibility that suppression of endogenous glucagon might have taken place. The manner in which starvation sensitizes the liver to the antiglycogenolytic action of chlorpropamide is not known.

In practical consequences of our concept, we would not start therapy with chlorpropamide when states of negative calorie balance were present or to be expected;

and, in addition, when a state of negative calorie balance supervenes as during an intercurrent illness, the dose of chlorpropamide ought to be reduced or temporarily stopped.

REFERENCES

[1] Coates, J. R., and Robbins, J. J.: Severe hypoglycemic shock due to chlorpropamide. JAMA *170*:941-43, 1959.

[2] Lindeman, R. D.: Severe hypoglycemia caused by chlorpropamide. Diabetes *9*:110-13, 1960.

[3] Levin, E. B.: Severe hypoglycemic reaction with chlorpropamide in therapeutic dosage. California Med. *98*:279-80, 1963.

[4] Vogl, A.: Chlorpropamide-induced hypoglycemic coma. Postgrad. Med. *36*:400-03, 1964.

[5] Bauer, H. G.: Severe and prolonged hypoglycemic shock during sulfonylurea treatment. Metabolism *14*:220-28, 1965.

[6] Karon, E. H., and Kromer, J. L.: Severe, recurrent hypoglycemia due to chlorpropamide therapy. Minn. Med. *48*:1017-19, 1965.

[7] Rothfeld, E. L., Crews, A. H., Jr., Ribot, S., and Bernstein, A.: Severe hypoglycemia. Result of renal retention of chlorpropamide. Arch. Intern. Med. *115*:468-69, 1965.

[8] Boman, H.: Hypoglykemisk coma under klorpropamidbehandling. Norske Laegeforen *86*:472-73, 1966.

[9] Dall, J. L., Conway, H., and McAlpine, S. G.: Hypoglycemia due to chlorpropamide. Scot. Med. J. *12*:403-04, 1967.

[10] Bergman, H.: Hypoglycemic coma during sulfonylurea therapy. Acta Med. Scand. *177*:287-98, 1965.

[11] Ostman, J., and Grønberg, A.: Biverkningar vid oral diabetes-behandling med sulfonylurea. Nord. Med. *72*:1388-96, 1964.

[12] Hyvärinen, A., and Nikkilä, E. A.: Specific determination of blood glucose with o-toluidine. Clin. Chim. Acta. *7*:140-43, 1962.

[13] Yalow, R. S., and Berson, S. A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. *39*:1157-75, 1960.

[14] Hunter, W. M., and Greenwood, F. C.: Preparation of iodine-131-labeled human growth hormone of high specific activity. Nature *194*:495-96, 1962.

[15] Norman, N., and Turter, Aa. R.: Radioimmunoassay studies with human growth hormone and a pituitary lipid mobilizing factor. Acta Endocr. *58*:318-38, 1968.

[16] Toolan, T. J., and Wagner, R. L.: The physical properties of chlorpropamide and its determination in human serum. Ann. N.Y. Acad. Sci. 73 art. *3*:449-58, 1959.

[17] Kienholz, M.: Der intravenöse glukosetoleranztest. Med. Welt. *18*:2760-63, 1967.

[18] Beaser, S. B.: The correlation between oral dosage, blood levels, and clinical and metabolic activity of chlorpropamide in the treatment of diabetes mellitus. Ann. N.Y. Acad. Sci. 74 art. *3*:701-07, 1959.

[19] Beaser, S. B.: Further studies on the action of chlorpropamide in diabetes mellitus. Clin. Pharmacol. Therap. *2*:157-62, 1961.

[20] Carlozzi, M., Iezzani, D. C., and Silver, L.: Blood levels of chlorpropamide in normal men following chronic administration. Ann. N.Y. Acad. Sci. 74 art. *3*:788-93, 1959.

[21] Knauff, R. E., Fajans, S. S., Ramirez, E., and Conn, J. W.: Metabolic studies of chlorpropamide in normal men and in diabetic subjects. Ann. N.Y. Acad. Sci. 74 art. *3*:603-17, 1959.

[22] Sackner, M. A., and Balian, L. J.: Severe hypoglycemia after the ingestion of a sulfonylurea compound. Amer. J. Med. 28 *1*:135-42, 1960.

[23] Korsgaard Christensen, L., Mølholm Hansen, J., and Kristensen, M.: Sulphaphenazole-induced hypoglycemic attacks in tolbutamide-treated diabetics. Lancet *2*:1298-1301, 1963.

[24] Høglund, N. J.: Hypoglykemi vid tablettbehandling av diabetes mellitus. Nord. Med. *1*:93, 1963.

[25] Kristensen, M., and Mølholm Hansen, J.: Accumulation of chlorpropamide caused by dicoumarol. Acta. Med. Scand. *183*:83-86, 1968.

[26] Dalgas, M., Christiansen, I., and Kjerulf, K.: Fenylbutazoninduceret hypoglykaemitilfeldev hos klorpropamidbehandlet diabetiker. Ugeskr. Laeg. *127*:834-36, 1965.

[27] Brotherton, P. M., Grievson, P., and McMartin, C.: A study of the metabolic fate of chlorpropamide in man. Clin. Pharmacol. Therap. *10*:505-14, 1969.

[28] Laroche, C., Nenna, A., Cremer, G., and Caquet, R.: Hypoglycémies graves chez des diabétiques traités par les sulfamides hypoglycemiants. Bull. Mém. Soc. Méd. Hop. Paris *116*:461-66, 1965.

[29] Zucker, P., and Simon, G.: Prolonged symptomatic neonatal hypoglycemia associated with maternal chlorpropamide therapy. Pediatrics *42*:824-25, 1968.

[30] Davies, D. M., MacIntyre, A., Miller, E. J., Bell, S. M. and Mehra, S. K.: Need for glucagon in severe hypoglycemia induced by sulfonylurea drugs. Lancet *1*:363-64, 1967.

[31] Johnson, P. C., Hennes, A. R., Driscoll, T., and West K. M.: Metabolic fate of chlorpropamide in man. Ann. N.Y Acad. Sci. 74 art. *3*:459-70, 1959.

[32] Botterman, N. P., Schwartz, K., Kollmannsberger, A. and Kopetz, K.: Zur klinischen diagnostik von inselzelladenomen. Med. Klin. *64*:479-88, 1969.

[33] Marri, G., Cozzolino, G., and Palumbo, R.: Glucagon in sulfonylurea hypoglycemia? Lancet *1*:303-04, 1968.

[34] Simpson, R. G., Benedetti, A., Grodsky, G. M., Karam J. H., and Forsham, P. H.: Early phase of insulin release Diabetes *17*:684-92, 1968.

[35] Reaven, G., and Dray, J.: Effect of chlorpropamide on serum glucose and immunoreactive insulin concentrations in patients with maturity-onset diabetes mellitus. Diabetes *16*: 487-92, 1967.

[36] Soeldner, J. S., and Steinke, J.: Hypoglycemia in tolbutamide-treated diabetes. JAMA *193*:398-99, 1965.

[37] Ping-Chi Chu, Conway, M. J., Krause, H. A., and Goodner, C. J.: The pattern of response of plasma insulin and glucose to meals and fasting during chlorpropamide therapy. Ann. Intern. Med. *68*:757-69, 1968.

[38] Davidoff, F. F.: Oral hypoglycemic agents and the mechanism of diabetes mellitus. New Eng. J. Med. *278*:148-55, 1968.

[39] Feldman, J. M., and Lebovitz, H. E.: An appraisal of the extrapancreatic action of sulfonylureas. Arch. Intern. Med. *123*:314-22, 1969.

[40] Feldman, J. M., and Lebovitz, H. E.: Biological activities

of tolbutamide and its metabolites. A dissociation of insulin releasing and hypoglycemic activity. Diabetes *18*:529-37, 1969.

[41] Danowski, T. S., Leinberger, M., and Mateer, F. M.: Chlorpropamide in single dosage and the responses to insulin or glucose in diabetics: the absence of evidence of increased glucose utilization. Ann. N.Y. Acad. Sci. *74* art. 3:979-96, 1959.

[42] Madison, L. L., Combes, B., Unger, R. H., and Kaplan, N.: The relationship between the mechanism of action of the sulfonylureas and the secretion of insulin into the portal circulation. Ann. N.Y. Acad. Sci. *74* art. 3:548-56, 1959.

[43] Craig, J. W., Drucker, W. R., Miller, M., Woodward, H., Jr., and Molzahn, V.: A comparison of the influence of tolbutamide and small doses of insulin on the splanchnic output and peripheral uptake of glucose in man. Ann. N.Y. Acad. Sci. *74* art. 3:537-47, 1959.

[44] Yalow, R. S., Black, H., Villazon, M., and Berson, S. A.: Comparison of plasma insulin levels following administration of tolbutamide and glucose. Diabetes 9:356-62, 1960.

[45] Ashmore, J., Cahill, G. F., Earle, A. S., and Zottu, S.: Studies on the disposition of blood glucose. A comparison of insulin and Orinase. Diabetes 7:1-8, 1958.

[46] Kaldor, A., and Pogatsa, G.: The effect of sulfonylureas on hepatic glucose release. Diabetes *11* Suppl. 1:16-18, 1962.

[47] Samols, E., Tyler, J. M., and Mialhe, P.: Suppression of pancreatic glucagon release by the hypoglycemic sulphonylureas. Lancet *1*:174-76, 1969.

[48] Buchanan, K. D., Williams, R. H., and Vance, J. E.: Pancreatic glucagon release and sulfonylureas. Lancet *1*:420-21, 1969.

[49] Dall, J. L., and Melrose, A. G.: Hypoglycemia from sulphonylurea. Brit. Med. J. *1*:1379, 1964.

[50] Alexander, R. W.: Prolonged hypoglycemia following acetohexamide administration. Diabetes *15*:362-64, 1966.

[51] Loehry, C., Chisholm, I., and Summerhayes, M.: Hypoglycemic coma with chlorpropamide. Postgrad. Med. J. 43: 462-64, 1967.

[52] Van Itallie, T. B., and Bentley, W. B. A.: Glucagon-induced hyperglycemia as an index of liver function. J. Clin. *Invest. 34*:1730-37, *1955*.

[53] Marks, V., and Rose, F. C.: Hypoglycemia. Blackwell Scientific Publications, Oxford, 1965.

[54] Greenberg, B., Weihl, C., and Hug, G.: Chlorpropamide poisoning. Pediatrics *41*:145, 1968.