C-peptide Immunoreactivity (CPR) in Urine

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SUMMARY

Human urinary C-peptide immunoreactivity (CPR) was measured by a double-antibody radioimmunoassay using synthetic human C-peptide. Urine CPR was stable as long as one year, if stored frozen. Urine CPR was eluted by gel filtration as a single peak in the region that corresponded to ^{125}I-labeled C-peptide. Even in a patient with insulin antibody whose serum contained a CPR fraction with higher molecular weight, urine CPR was eluted in its expected region.

Normal subjects excreted 81 ± 36 μg. CPR per day. Urine CPR excretion was very low in juvenile-onset diabetics, variable in adult-onset diabetics and liver disease patients, and increased in patients on corticosteroid treatment. Diurnal changes and a 50-gm. glucose tolerance test revealed that urine CPR excretion rate increased in parallel with plasma CPR levels. The responses of diabetic patients were smaller and more sluggish. Urine CPR excretion tended to be decreased in patients with renal diseases and extremely low in those on regular hemodialysis. CPR was positively correlated with serum creatinine. Urine CPR was not increased in patients with marked β_2-microglobulin excretion.

Highly significant correlations were observed between 24-hour urine CPR values and fasting plasma CPR or summed CPR after glucose load. Thus, urine CPR seems to provide a good means to assess B-cell function, and it is particularly suitable to monitor B-cell function continuously or when multiple blood samplings are not practical. Continuous measurement of daily urine CPR in diabetic patients on glibenclamide treatment revealed that improvement of blood sugar control could occur without preceding increases in total daily B-cell secretion. DIABETES *27* (Suppl. 1):210-15, 1978.

It is now well established that the proinsulin connecting peptide (C-peptide) is released into blood from the beta cells together with insulin.[393,451] Assay of serum C-peptide, as a substitute for the insulin assay, provides an additional means to evaluate the endocrine function of the pancreatic beta cells even in the presence of circulating insulin antibody.[172,301] Until recently, however, the C-peptide assay was not readily available for clinical use because of the limited availability of human C-peptide. This problem was solved by the development of a radioimmunoassay system using synthetic human C-peptide.[209] A kit of this assay system has recently become available commercially in Japan.

Katz and Rubenstein[214] reported that both the liver and the kidney played important roles in the clearance of insulin in rats, while the kidney represented the major site for the clearance of proinsulin and C-peptide. Although C-peptide was excreted at a higher rate than insulin in the urine of rats, most of its renal clearance was attributed to degradation rather than excretion.[214] In man, Kaneko et al.[210] found that urine C-peptide immunoreactivity (CPR) was very high as compared with that in the other body fluids and that it increased following an oral glucose load paralleling the changes in serum. We found that the assay of CPR in diluted human urine samples could be performed adequately by the commercial kit, and we confirmed their findings. We also observed that 24-hour urine CPR was low in juvenile diabetics and high in patients on corticosteroid treatment.[243]

More recently, Horwitz et al.[190] reported on the validity of urine CPR assay and presented some clinical data on 24-hour urine CPR in diabetics and in patients with renal diseases. They found that urine CPR of two adult-onset diabetics increased during acute infection and that CPR clearance was independent of creatinine clearance over a wide range.

Our present report deals with the results of 24-hour

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urine CPR excretion, diurnal changes in CPR excretion rate, and week-to-week changes of urine CPR during sulfonylurea treatment.

MATERIALS AND METHODS

Assay Methods

C-peptide immunoreactivity (CPR) was measured by use of a radioimmunoassay kit (Daiichi Radioisotope Laboratories, Tokyo) as described in detail previously.[243] It is based on a double-antibody technique that uses rabbit antiserum against human synthetic C-peptide as the first antibody and goat anti-rabbit gamma globulin as the second antibody. According to the supplier, cross-reaction with human proinsulin is 7 per cent on a molar basis. Total volume of the first incubation is 500 μl. including 100μl. standard C-peptide or unknown sample. The optimal range of this assay system lies between 0.5 and 8 ng. per milliliter.

Unextracted fresh urine was diluted with 0.1 M phosphate buffer containing 0.5 per cent bovine albumin. When urine was diluted at 1:10 or higher, the same final CPR values were obtained. Recovery tests were performed by assaying the diluted urine with or without additions of known amounts of standard C-peptide. In dilutions higher than 1:16, the assay gave the expected values.[243] Urine samples were usually assayed at 1:20 dilutions, since normal samples at 1:20 to 1:40 dilutions gave CPR values within the optimal range of the standard curve.

Insulin was assayed by a double-antibody method.[208] Blood glucose was determined by a glucose-oxidase method or by a Technicon Auto-Analyzer using neocuproine copper reagent.[470] Creatinine in plasma or urine was measured by the alkali-picrate method.[41]

Sampling

Urine samples were collected in plastic bottles, which were kept in a refrigerator (4° C.) during the collection period when possible. In some normal subjects and ambulatory patients, urine samples were collected in plastic bottles containing 0.5 gm. NaN_3. An aliquot was taken and frozen within 24 hours after collection. We have previously confirmed that these procedures are adequate to preserve urine CPR.[243] Blood was withdrawn from an antecubital vein. Plasma or serum was kept frozen until assayed.

Gel Filtration

Gel filtration of unextracted urine (1 ml.) was performed on a Sephadex G 100 column (1.5×84 cm.) equilibrated in 3 M acetic acid.[33] An acid-ethanol extract of 2 ml. serum of an insulin-treated diabetic patient, dissolved in 1 ml. 3 M acetic acid, was gel-filtered similarly. Fractions of 2.5 ml. were collected, dried in vacuo, redissolved in phosphate-albumin buffer (pH 7.4), and assayed for CPR.

Subjects

Control subjects were nonobese healthy students and hospital personnel. Diabetic patients were selected on the basis of documented fasting blood glucose values above 140 mg./dl., who had no obvious signs of renal dysfunction. Most of the 24-hour urine samples were collected from hospitalized patients without discontinuation of their drug treatment. All medications were withheld on the morning the glucose tolerance test was performed. Patients with anorexia nervosa, liver diseases, renal diseases (except for diabetic nephropathy), and insulinoma and patients who were taking 20 to 30 mg. prednisolone for various disorders were included for the study of 24-hour urine CPR. The sex, age, and some other features of these patients are shown in table 1.

RESULTS

Plasma and Urine CPR Changes During 50 gm. Glucose Tolerance Test in Normal Subjects and Diabetic Patients (Figure 1)

After the administration of 50 gm. glucose to normal subjects, plasma CPR increased promptly, almost paralleling the changes in plasma insulin. Urine CPR excretion per milligram creatinine in hourly samples also increased after the glucose load, reaching a maximum of 407 per cent of the fasting level at the second hour and decreasing thereafter. In adult-type diabetic patients, the absolute amount of urine CPR per milligram creatinine was not subnormal, but the relative increase after the glucose load from baseline was smaller (maximum = 256 per cent of the basal) and more sluggish than normal. Urine CPR was low and the response after glucose was very small (maximum = 160 per cent of the basal) in juvenile-onset diabetics. These patterns roughly reflected the changes in plasma CPR and IRI.

Twenty-four-hour Urine CPR Values in Various Disease States

As shown in table 1, normal subjects excreted 81 ± 36 μg. CPR or 56 ± 27 μg. per gram creatinine per day. Urine CPR excretion was markedly decreased in juvenile-onset diabetics and to some extent in nonobese adult-onset patients but was not significantly decreased in obese diabetics. Both fasting plasma CPR and urine CPR were increased in patients

TABLE 1

Fasting plasma CPR concentration and 24-hour urine CPR in various diseases (Mean ± S.D.)

	n	Age (yr.) mean (range)	Sex M:F	Weight index	Fasting plasma CPR (ng./ml.)	Total 24-hr. urine CPR (μg.)	24-hr. urine CPR per creatinine (μg./gm.)
Normal	26	33 (20-56)	21:5	1.01 ± 0.08	1.93 ± 0.80	81 ± 36	56 ± 27
Diabetes							
Juvenile-type[a]	12	34 (17-56)	3:9	0.88 ± 0.12	0.46 ± 0.38†	8 ± 6†	10 ± 10†
Adult-type							
nonobese[b]	19	45 (29-72)	11:8	0.95 ± 0.11	1.63 ± 0.55	45 ± 21†	53 ± 34
obese[c]	16	52 (40-67)	13:3	1.20 ± 0.06	1.72 ± 0.42	64 ± 35	61 ± 35
Steroid treatment[d]	7	35 (20-52)	5:2	1.04 ± 0.21	4.37 ± 1.18†	195 ± 102†	178 ± 67†
Anorexia nervosa	6	24 (16-34)	1:5	0.64 ± 0.08	1.48 ± 0.97	52 ± 17	120 ± 85†
Liver diseases[e]	12	47 (24-67)	5:7	0.99 ± 0.08	1.89 ± 0.91	85 ± 38	101 ± 49*
Kidney diseases							
Serum creatinine < 5 mg./dl.[f]	18	42 (16-69)	10:8		2.47 ± 1.47	41 ± 29†	57 ± 41
Serum creatinine ≥ 5 mg./dl.[g]							
Hemodialysis (−)	8	51 (28-72)	5:3		5.11 ± 2.17†	31 ± 22†	46 ± 32
Hemodialysis (+)	21	46 (26-76)	9:12		6.70 ± 3.46†	8 ± 10†	46 ± 50
Insulinoma	3	46 (26-60)	2:1	1.11 ± 0.12	4.43 ± 0.29†	71 ± 16	63 ± 22

Weight index = weight/ideal body weight.

* and † represent significant differences from the normal group ($p < 0.05$ and $p < 0.01$, respectively).

[a]All patients were ketotic and were treated with insulin.

[b]14 treated with insulin and five with sulfonylureas.

[c]Four treated with insulin, eight with sulfonylureas, and four by diet.

[d]Two with acute leukemia, two with aplastic anemia, two with systemic lupus erythematosus, and one with sympathic ophthalmia.

Four with acute hepatitis, four with chronic hepatitis (two chronic aggressive, the other two not specified), two with liver cirrhosis, one with cholelithiasis, and one with obstructive jaundice.

[f]Eight chronic glomerulonephritis, two with chronic pyelonephritis, and four with nephrotic syndrome are included.

[g]The cause of chronic renal failure could not be specified in many cases.

on corticosteroid treatment. Urine CPR per milligram creatinine was increased in patients with anorexia nervosa and liver diseases, while the absolute amount of urine CPR did not differ significantly from normal in these patients. Patients with renal disease whose serum creatinine was above 5 mg./dl. had markedly higher fasting plasma CPR concentrations than did controls. There was a significant correlation between serum creatinine and plasma CPR levels in these patients ($p < 0.01$). Total 24-hour urine CPR was decreased in these patients, most strikingly in patients on hemodialysis, whether or not they had marked oliguria (< 500 ml. per day). Urine CPR/creatinine ratios were highly variable, and the mean values did not differ from controls. No correlation was observed between serum creatinine and twenty-four-hour urine CPR excretion in patients who were not undergoing hemodialysis, but the correlation became significant when all the kidney-disease patients were combined ($p < 0.01$).

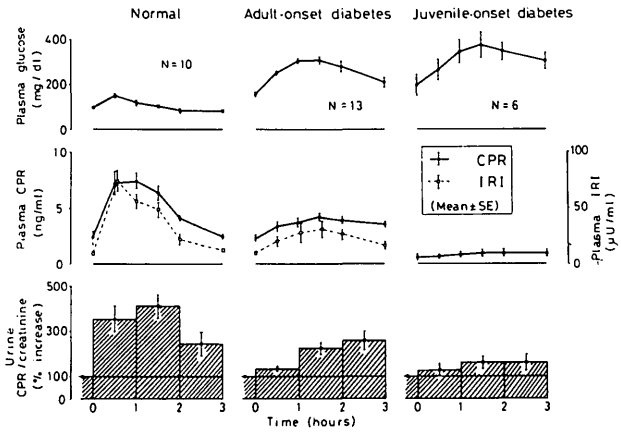

FIG. 1. Changes in plasma glucose, CPR, insulin (IRI), and urine CPR/creatinine ratio during 50-gm. glucose tolerance tests in normal and diabetic subjects.

There were highly significant correlations between 24-hour urine CPR and fasting plasma CPR or summed CPR values during the 50-gm. glucose tolerance test, but patients with renal insufficiency tended to have lower urine CPR excretion in relation to their elevated plasma CPR (figure 2). Correlations between plasma and urine CPR were improved further when the patients with renal disease were excluded. Four of six patients who were suspected of chronic cadmium poisoning excreted large amounts of β_2 microglobulin in urine (690-71,700 μg. per day; normal range 30-150 μg. per day), but their urine CPR excretion was not increased, being in the range of 32-174 μg per day.

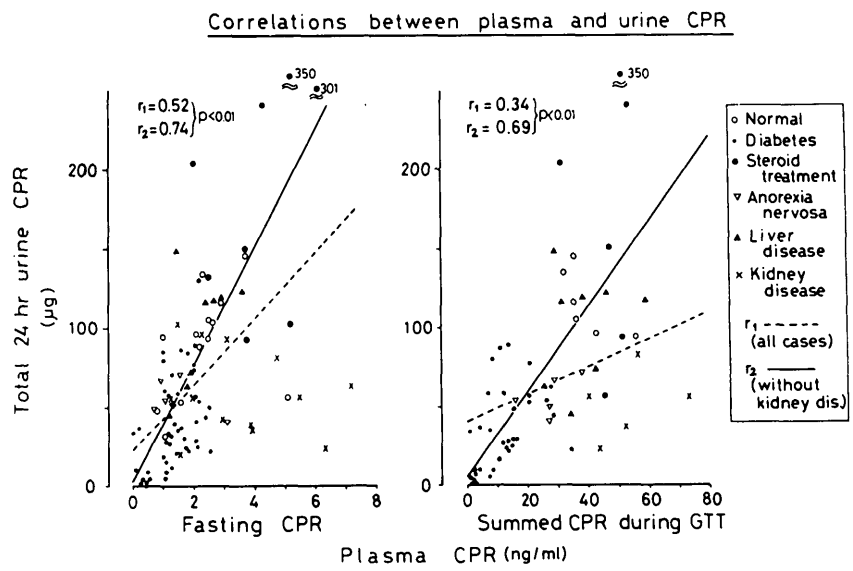

FIGURE 2

Correlations between plasma CPR (fasting and summed plasma CPR values during 50-gm. glucose tolerance test) and 24-hour urine CPR. Correlations are highly significant, but correlation coefficients excluding patients with kidney disease (r_2) are greater than those including all cases (r_1).

Three insulinoma patients excreted normal amounts of CPR per day despite their raised fasting plasma CPR levels.

Gel Filtration Profile of CPR in Urine

As reported previously by other authors,[190,210] urine CPR eluted as a single peak from Sephadex G 100 columns in the region corresponding to the ^{125}I-C-peptide marker (figure 3). In one insulin-treated diabetic patient with elevated fasting plasma CPR, gel filtration of an acid-ethanol extract of serum revealed that a major part of CPR was of higher molecular weight than that of ^{125}I-C-peptide. Most of the urine CPR of this patient, however, appeared as a single peak coinciding with the elution position of CPR in normal urine.

Diurnal Changes in Urine CPR Excretion Rate

In eight healthy subjects and 20 diabetic patients, divided urine samples between meals and bedtime were collected throughout one day. Plasma glucose and CPR levels were also measured at timed intervals. In normal subjects, as shown in figure 4, plasma glucose and CPR concentrations were lowest before breakfast, increased following each meal, and decreased before the next meal. Urine CPR excretion rate was lowest between midnight and breakfast, increased after each meal, and decreased during sleep. In adult-onset diabetics, the general pattern of diurnal changes in plasma and urine CPR was similar to that in controls. The ratio of the average CPR excretion rate from breakfast to bedtime to that from bedtime to breakfast was 3.28 ± 1.89 in normal subjects, 3.35 ± 1.34 in adult-onset diabetics, and 1.47 ± 0.74 in

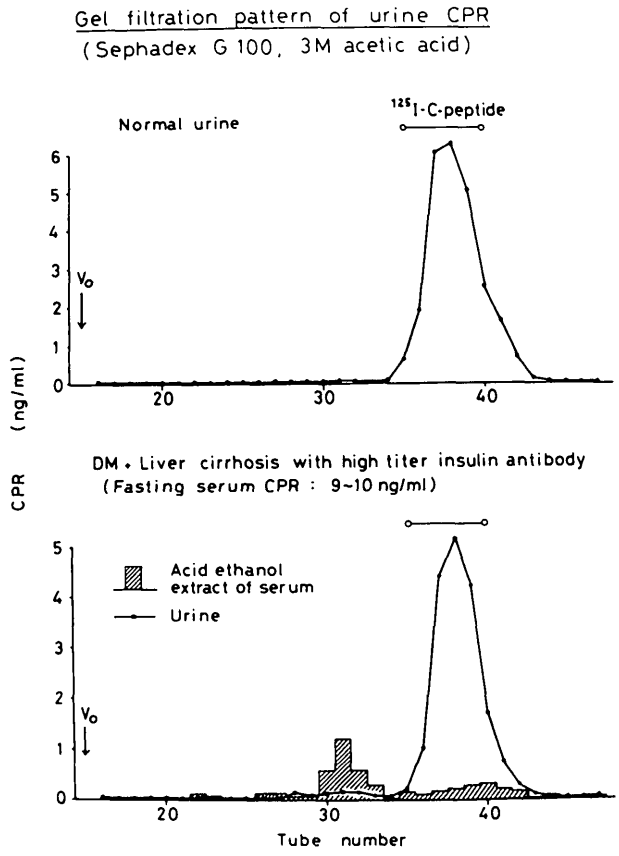

FIG. 3. Gel filtration (Sephadex G 100, 3 M acetic acid) of urine CPR in a normal and in an insulin-treated diabetic patient who had a high fasting serum CPR value. Urine CPR profiles are similar in both cases, but the acid-ethanol extract of serum of the latter patient contains a CPR fraction with a higher molecular weight.

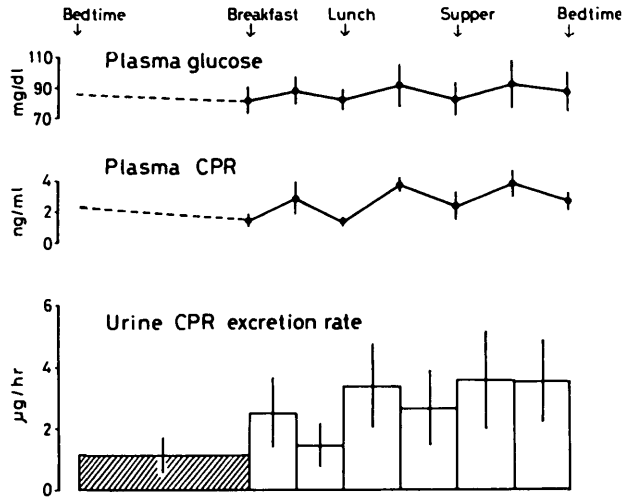

FIG. 4. Diurnal changes of plasma glucose, CPR, and excretion rate of urine CPR in eight healthy subjects. Mean ± S.D.

juvenile-onset diabetics.

Urine CPR Changes During Glibenclamide Treatment of Adult-onset Diabetics

Adult-onset diabetics who were to be treated with glibenclamide were hospitalized, and their daily excretion of CPR was followed. After a period of diet treatment for one to two weeks, 2.5 to 7.5 mg. glibenclamide was administered. Figure 5 shows the weekly changes in urine CPR excretion before and after the initiation of drug treatment. In all cases there was a decrease in fasting blood glucose, and in four cases daily CPR excretion was significantly increased after glibenclamide administration. In three cases, however, urine CPR remained unchanged despite the decrease in fasting blood glucose. Even in patients whose CPR excretion increased after treatment, increase in urine CPR did not necessarily precede the fall in fasting blood glucose. The coefficient of variation of urine CPR in a week ranged from 5 to 40 per cent (mean = 19 per cent).

DISCUSSION

The present results confirm that the assay of urine CPR can be adequately performed by the usual radioimmunoassay method. Urine CPR excretion rates roughly paralleled plasma CPR levels, and there were good correlations between the 24-hour urine CPR and fasting plasma CPR values or summed plasma CPR levels after a glucose load. Exceptions are insulinoma and kidney disease patients.[243] Urine CPR excretion in insulinoma may vary in individual cases, since Shima et al.[429] reported such a patient who excreted a large amount of CPR in his urine.[429] The 24-hour urine CPR data in patients with diabetes and other diseases are mostly consistent with previous information on insulin secretion.[364] In diabetic patients, the relative increase in the CPR excretion rate following a glucose load was more sluggish and smaller than in healthy subjects. Some insulin-treated adult-onset diabetics occasionally have erroneously high plasma CPR values because of the binding of endogenous proinsulin to insulin antibodies.[33] As only a little proinsulin is excreted in the urine,[74] assay of urine CPR may give more reliable information about the true C-peptide value in such cases. The gel-filtration profile of urine CPR revealed that most of the peptide eluted in the 3,000-Mol.-wt. zone even in the patient whose serum contained substantial amounts of endogenous proinsulin (figure 3).

The quantity of C-peptide in urine is greater than that of insulin, of which only 0.1 to 0.3 per cent of the total daily amount secreted is excreted.[59,74] Previously we estimated that 13 to 20 per cent of the secreted C-peptide appears in urine,[243] while Horwitz et al.[190] calculated the amount to be 5 per cent. This discrepancy arises mainly from difference in the normal values for 24-hour urine CPR; our normal values are higher than those (36 ± 4 μg. per day) of Horwitz et al.[190] This variability might be attributed, at least in part, to intrinsic differences in the assay systems including the preparation of standard C-peptide or antiserum. This conclusion seems plausible because

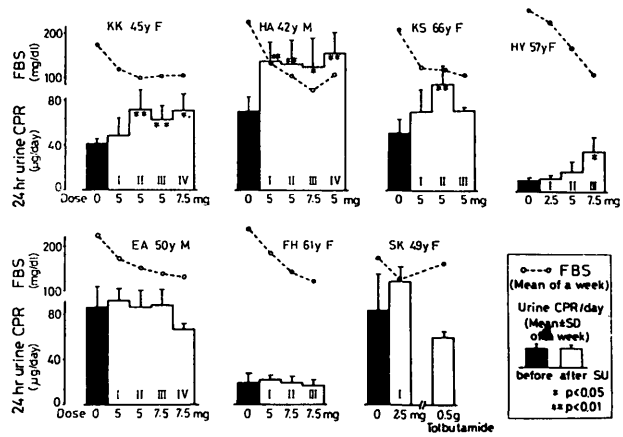

FIG. 5. Changes in 24-hour urine CPR before and after the initiation of glibenclamide treatment in seven diabetic patients. Each column represents the Mean ± S.D. daily urine CPR for each week. * and ** denote that the urine CPR excretion is significantly different from that before the administration of glibenclamide (* $p < 0.05$, ** $p < 0.01$).

our normal fasting plasma CPR levels also tend to give higher values than those reported by others.[243]

In patients with renal disease, fasting plasma CPR levels were increased when serum creatinine became elevated. Increases in basal and stimulated plasma insulin levels have been reported in uremia,[15] but the elevation of CPR that we found was even more striking than that of insulin.[243] This may be explained by the fact that the kidney represents the major site of C-peptide clearance, while both the kidney and liver are operative in insulin metabolism.[214] Urine CPR excretion tended to be lower in patients with kidney disease and was markedly decreased in dialyzed patients. Low-molecular-weight proteins are readily filtered at the glomerulus and are largely taken up and catabolized by tubular cells.[44,462] In rats, catabolism seems to be more important than urinary excretion for the organ clearance of insulin, proinsulin, and C-peptide, like many other low-molecular-weight proteins.[214] However, the renal handling of C-peptide appears to differ from that of β_2-microglobulin. CPR excretion was not increased in patients suspected of chronic cadmium poisoning who excreted strikingly increased amount of β_2-microglobulin.[355] The urine CPR/creatinine ratios were extremely variable in patients with renal diseases. Subdivision of kidney diseases into specified functional categories would seem to be necessary for further studies.

Except for patients with renal failure, urine CPR excretion seems to reflect the daily β-cell secretory activities fairly well. Thus, the urine CPR assay provides a means to assess β-cell function, and it is particularly suitable to monitor it over a certain length of time or when multiple blood samplings are difficult. Follow-up of 24-hour urine CPR in adult-onset diabetics on glibenclamide is an example of the application of the urine CPR assay. The mechanism by which sulfonylureas lower blood glucose remains controversial.[107,213] The present data demonstrate that improvement of blood glucose levels is associated with increased daily β-cell secretion in some but not all cases, suggesting that an increased total daily secretion of insulin may not be a prerequisite for the clinical efficacy of glibenclamide. On the other hand, it was also noted that the daily urine CPR excretion did not decrease despite the decline in blood glucose levels. This may be interpreted as indicating that glibenclamide exerted a betacytotropic action during the early period of blood glucose improvement.

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