Estimation of the Secretion Rate of Insulin from the Urinary Excretion Rate of C-Peptide

Study in Obese and Diabetic Subjects

MARY T. MEISTAS, MARC RENDELL, SIMEON MARGOLIS, AND A. AVINOAM KOWARSKI

SUMMARY

Direct methods for measuring the secretion rate of insulin are too cumbersome for clinical application. Since C-peptide is secreted in an equimolar ratio with insulin and is excreted into the urine, measuring the urinary excretion rate of C-peptide (U-C) could serve as an indicator of its secretion rate (SR-C) if its urinary clearance (UCl-C) is constant and unaffected by plasma C-peptide concentration, body mass, or diabetes.

We measured clearance ratios of C-peptide/creatinine (CR) in the fasting state and integrated 0–1, 1–3, and 3–5 h after 100 g of glucose p.o. as well as over a full 24-h in eight obese, eight lean, and six maturity-onset diabetic subjects. CR did not differ significantly when values in the fasting state were compared with those in the postprandial periods and was therefore unaffected by plasma C-peptide concentration. Furthermore, CR was similar in the lean, obese, and diabetic subjects. SR-C, determined as the product of the metabolic clearance rate of C-peptide and its fasting or integrated plasma concentrations, correlated significantly with U-C in all the subjects ($r = 0.87$, $P < 0.0001$). The correlation of U-C with SR-C in the diabetic subjects alone was also significant ($r = 0.88$, $P < 0.0001$). In conclusion, our data support the use of U-C as an indirect measure of SR-C and therefore of SR-I. DIABETES *31*:449–453, May 1982.

The secretion rate of insulin can be determined from the product of its metabolic clearance rate (MCR) and integrated concentration (IC),[1] but this method is both cumbersome and time-consuming. Since C-peptide is secreted in an equimolar ratio with insulin,[2–4] it is possible to determine the secretion rate of insulin from the secretion of C-peptide (SR-C). While very small amounts of insulin are found in the urine,[5–7] the kidney excretes C-peptide and its concentration is considerably higher in urine than in plasma.[8–10] If it can be shown that the fraction of secreted C-peptide in the urine is fairly constant, the insulin secretion rate can be estimated from the urinary excretion rate of C-peptide. We have previously reported a statistically significant correlation ($r = 0.86$, $P < 0.0001$) between the 24-h urinary excretion of C-peptide and the rate of insulin secretion in 50 normal lean subjects[1] and 4.0 ± 1.5% (mean ± 1 SD) of the secreted C-peptide appeared in their urine. These findings enabled us to estimate the secretion rate of insulin in lean subjects from their C-peptide excretion rate. We have now tested the applicability of our estimation of the insulin secretion rate in obese and diabetic patients.

Urinary C-peptide excretion (U-C) equals the product of the integrated plasma concentration of C-peptide (IC-C) and its urinary clearance rate (UCl-C):

$$U\text{-}C = IC\text{-}C \times UCl\text{-}C$$

and the secretion rate of C-peptide (SR-C) equals the product of its MCR and IC-C:

$$SR\text{-}C = IC\text{-}C \times MCR\text{-}C$$

Therefore:

$$U\text{-}C/SR\text{-}C = UCl\text{-}C/MCR\text{-}C.$$

We can conclude that U-C is a fixed fraction of SR-C if the ratio UCl-C/MCR-C is similar in the three groups of subjects and unaffected by the plasma C-peptide concentration. MCR-C is similar in lean diabetic and nondiabetic subjects and is constant over a wide range of plasma C-peptide.[11] There is also evidence indicating that MCR-C is constant in lean and obese nondiabetic subjects as well.[12]

Urinary C-peptide clearance measurements incorporate the variability in glomerular filtration rates that exist in both normal (mean creatinine clearance 150 L/day; range 104–

This study was published in abstract form in Diabetes *30* (Suppl. 1): 121A, 1981.

From the Departments of Pediatrics and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Address reprint requests to A. Avinoam Kowarski, M.D., University of Maryland School of Medicine, Howard Hall Tower 10-047, Baltimore, Maryland 21201.

Received for publication 1 September 1981 and in revised form 14 December 1981.

203 L/day;[13] inulin clearance range 104–253 L/day)[14] and obese subjects (mean creatinine clearance 140 L/day; range 60–260 L/day).[15] Despite this variability, the mean glomerular filtration rate (GFR) is similar in lean and obese subjects without evidence of renal insufficiency. In addition to intersubject variability, rates of GFR have also been reported to display diurnal[16,17] and exercise-induced changes,[18] resulting in intrasubject variability as well. Thus, to observe the effect of plasma C-peptide on its urinary clearance, it is necessary to minimize the variable of GFR. In this study, this was achieved by reporting urinary C-peptide clearance measurements as the urinary C-peptide clearance/creatinine clearance ratio (CR). We first compared the CR in obese and diabetic subjects at varying levels of plasma C-peptide with the ratios found in lean subjects. We then calculated the quantity of C-peptide secreted over each time interval and correlated it with the C-peptide excreted over the same time interval. The methodology that we employed was the continuous blood withdrawal system, which is uniquely suited for the determination of urinary clearance,[19] as it provides accurate integrated plasma concentrations coincident with the period of urine collection.

Patient population. A body mass index (BMI = wt/ht^2) was calculated for eight lean and eight obese subjects, recruited on a volunteer basis. Subjects with a BMI less than 25 were classified as lean; those whose BMI exceeded 25 were classified as obese. The mean BMI for the lean group was 20.7 ± 2.2 and 36.1 ± 10.7 for the obese group. All subjects were in good medical health and had been on no medications for at least 1 mo prior to the study. Fasting plasma glucose levels, measured on the day of the study, were normal in all nondiabetic subjects. Six adult-onset diabetic patients were also studied. The mean BMI of the diabetic subjects was 31.3 ± 7.6. None of the diabetic subjects had been on insulin therapy for at least 6 mo prior to study. Informed consent was obtained from each subject and the study was performed in accordance with the principles in the Declaration of Helsinki.

Each subject was admitted to the Clinical Research Unit of the Johns Hopkins Hospital for a 24-h period of continuous blood withdrawal in synchrony with a 24-h measured urine collection. Nondiabetic subjects received a standard hospital diet, while diabetic subjects received a 1,000-calorie ADA diet. All subjects were fasted for the last 6 h of the 24-h period in preparation for a glucose tolerance test. Urine specimens were collected in a plastic container and refrigerated. A small aliquot of the final urine specimen obtained at the end of the 24-h period served as a baseline for the glucose tolerance test; the remainder was used to complete the 24-h urine collection. After a discrete fasting blood sample was obtained, each subject ingested 100 g of glucola within a period of several minutes. Continuous blood withdrawal was then resumed for an additional 3 h. Urine collections were obtained 1 and 3 h after glucose ingestion.

Continuous venous blood withdrawal system (Cormed, Inc., Middleport, New York). Continuous blood withdrawal was accomplished with a nonthrombogenic catheter and a peristaltic minipump.[20] Blood was withdrawn at the constant rate of 10 ml/h and collected into EDTA-containing test tubes immersed in ice. The tubes were changed at 30-min intervals for the duration of the study; the plasma was separated and frozen at 3-h intervals. Upon completion of the study, equal volumes of plasma were removed from each tube and pooled. The 24-h pool was formed by combining aliquots from each of the 48 30-min collections; the 0–1, 1–3, and 3–5 h pools were formed from the corresponding plasma collections after glucose ingestion.

Plasma C-peptide concentration was determined using a double antibody radioimmunoassay.[21] The intraassay coefficients of variation (CV) for C-peptide were 1.8% at 5 ng/ml, 2.4% at 10 ng/ml, and 8.1% at 1 ng/ml. The interassay CV was 10% at a concentration of 3 ng/ml. The lowest level of sensitivity of the method was 0.1 ng/ml.

The stability of plasma C-peptide without trasylol was assessed in the following manner: a pool of blood was separated into two pools, and 10% trasylol (10,000 U/ml) was added to one pool. After both pools were maintained on ice for 3 h, the plasma was separated and aliquots of each plasma pool were measured in the same assay. No significant difference was found in the levels of C-peptide in the two pools.

C-peptide immunoreactivity was also stable in plasma frozen without trasylol for several weeks, the maximum period prior to immunoassay in our study.

Urinary C-peptide concentration was determined using a modification of the immunoassay for plasma C-peptide.[1] After the urine was centrifuged at 3,000 rpm at 4°C for 30 min using an IEC International Centrifuge, the pH was adjusted to 7.6 with 10 N NaOH and diluted 1:10 with 0.01 M phosphate buffered saline, pH 7.6, containing 1% bovine serum albumin. The diluted urine was then assayed in the same assay system as plasma C-peptide.

Plasma and urinary creatinine were determined by the picric acid colorimetric method.

Urinary C-peptide clearance/creatinine clearance ratio (CR) was determined using the following expression:

$$\frac{{}^{P}\text{creat} \times {}^{U}\text{Cpep}}{{}^{U}\text{creat} \times {}^{P}\text{Cpep}}$$

where ^{P}creat and ^{P}Cpep are the plasma concentrations and ^{U}creat and ^{U}Cpep are the urinary concentrations of creatinine and C-peptide, respectively. The fasting ratio was determined using discrete specimens of fasting plasma and urine. The postprandial and 24-h ratios were calculated using plasma that was integrated 0–1, 1–3, and in some cases, 3–5 h after the ingestion of 100 g of glucose as well as over a full 24 h. Urine measurements were made from a sample of all urine collected over the appropriate time interval. Calculations were made using a computer program.

Urinary C-peptide excretion (U-C) was determined as the product of the urinary volume and the concentration of C-peptide in the 1:10 dilution of urine, after correcting for the dilution factor.

Secretory rate of C-peptide (SR-C). The secretion rate of C-peptide is the product of its metabolic clearance rate (MCR-C) and its average, integrated plasma concentration (IC-C). MCR-C, 4.4 ml/min/kg (175 ml/min/m^2),[11] was demonstrated to be a function of body mass and similar in lean, obese, and adult-onset diabetic subjects.[11,12] Therefore,

$$\text{SR-C} = \text{IC-C} \times 175 \times \text{m}^2 \text{ (surface area)}.$$

Statistical analysis. The Mann-Whitney nonparametric test was used to test the difference between CR of the various groups of subjects as well as the CR of the fasting, post-

prandial, and 24-h time intervals of each group of subjects. The Student's *t* test was used to test the differences between U-C and SR-C among the three groups of subjects. Correlations between SR-C and U-C were analyzed by linear regression methods.

RESULTS

The ratios of C-peptide clearance/creatinine clearance (CR) of the lean, obese, and diabetic subjects are presented in Table 1. In each group of subjects, fasting CR was not significantly different from postprandial or 24-h CR. The mean CR in the lean subjects was not significantly different from that of obese or diabetic subjects.

Calculated rates of C-peptide secretion and excretion, with mean and SD for each group, are presented in Table 2. The percent of C-peptide excretion was not significantly different in each time interval or between each of the three groups of subjects. The mean percent excretion is lower, but not significantly different from our previous estimate of 4.0 ± 1.5% in 50 normal, lean subjects.[1]

Urinary C-peptide excretion correlated with SR-C calculated for each of the postprandial and 24-h time intervals in the lean (0.938, $P < 0.0001$), obese ($r = 0.874$, $P < 0.0001$), and diabetic ($r = 0.879$, $P < 0.0001$) groups of subjects, as well as in all subjects when combined in one group ($r = 0.870$, $P < 0.0001$). The 24-h SR-C of one obese subject was more than 2 SDs above the others (SR-C = 4.21 mg). Without this subject, the correlation between 24-h rates of C-peptide secretion and excretion in all the remaining subjects was also highly significant ($r = 0.74$, $P < 0.0001$) (Figure 1).

DISCUSSION

The urinary excretion of C-peptide may be used to estimate the secretory rate of insulin if the fraction of C-peptide excreted in the urine is constant. In the present study, this was confirmed both directly from U-C and SR-C, and indirectly by the demonstration of a constant CR. CR was measured during periods of feeding, when levels of plasma C-peptide are high, as well as during the fasting state when the level of plasma C-peptide is low. The CR was not affected by these maximal changes in the plasma concentration of C-peptide. The mean CR of 0.07 in our subjects indicates that the urinary clearance of C-peptide is only 7% of the creatinine clearance. These results could be interpreted to indicate that most of the filtered C-peptide is reabsorbed by the renal tubule. This rate of tubular resorption is similar to the tubular resorption rate for other small polypeptides such as gastrin,[22] parathyroid hormone,[23–25] angiotensin,[26] beta-2 microglobulin,[27] lysozyme,[28,29] growth hormone,[28,30] insulin,[28,31,32] and glucagon.[33]

In rats, the clearance of urinary C-peptide was 0.6% of the C-peptide infused into the renal artery.[32] Since renal blood flow in rats is greater than 40% of the total blood flow,[32] we estimate that the fraction of C-peptide excreted in the urine by the rat is only 0.2%, a value considerably lower than the 2–3% found in our human subjects.

Our results are in agreement with the urinary C-peptide/creatinine clearance ratio reported by Kajinuma et al.,[34] who found no significant difference in the ratio in 11 normal subjects, 16 patients with mild renal failure, and 8 patients with moderate renal failure. Our finding of similar CR ratios in lean, obese, and diabetic subjects suggests that the urinary excretion rate of C-peptide can be used to estimate the secretory rate of insulin. The report by Kajinuma indicates that the method may even be valid in mild or moderate renal failure. Since more severe renal failure or disorders of tubular function may affect the excretion of C-peptide, caution must be exercised in the use of urinary C-peptide to predict

TABLE 1
Urinary C-peptide/creatinine clearance ratios in the fasting and postprandial states of lean, obese, and adult-onset diabetic subjects

		Fasting	0–1 h	1–3 h	24 h
Lean	1	0.054	0.064	0.064	0.050
	2	0.068	0.064	0.050	0.075
	3	0.069	0.076	0.045	0.070
	4	0.056	0.066	0.064	0.064
	5	0.112	0.088	0.101	0.074
	6	0.062	0.068	0.066	0.058
	7	0.052	0.076	0.045	0.068
	8	0.068	0.067	0.077	0.085
Mean ± SD		0.068 ± 0.019	0.071 ± 0.008	0.064 ± 0.019	0.068 ± 0.011
Obese	1	0.024	0.015	0.018	0.029
	2	0.047	0.044	0.042	0.054
	3	0.065	0.043	0.066	0.070
	4	0.060	0.081	0.076	0.078
	5	0.030	0.032	0.031	0.031
	6	0.072	0.100	0.107	0.121
	7	0.042	0.045	0.057	0.054
	8	0.095	0.078	0.083	0.119
Mean ± SD		0.054 ± 0.023	0.055 ± 0.029	0.060 ± 0.029	0.070 ± 0.035
Diabetic	1	0.122	0.144	0.119	0.117
	2	0.031	0.034	0.032	0.033
	3	0.084	0.122	0.140	0.139
	4	0.074	0.063	0.058	0.075
	5	0.042	0.039	0.052	0.053*
	6	0.020	0.020	0.031	0.022*
Mean ± SD		0.062 ± 0.038	0.070 ± 0.051	0.072 ± 0.046	0.073 ± 0.047

* 3–5 h rather than 24 h.

TABLE 2
Secretion and excretion rates of C-peptide in lean, obese, and adult-onset diabetic subjects

		C-peptide secretion (IC-C × MCR-C) mcg			C-peptide excretion (mcg)			$\frac{\text{C-peptide excreted}}{\text{C-peptide secreted (\%)}} \times 100$		
		0–1 h	1–3 h	24 h	0–1 h	1–3 h	24 h	0–1 h	1–3 h	24 h
Lean	1	69.4	138.9	1708.5	1.9	4.7	30.9	2.7	3.4	1.8
	2	60.7	121.4	1263.3	1.4	1.4	40.9	2.3	1.2	3.2
	3	79.1	158.1	1674.0	1.3	1.5	29.5	1.6	1.0	1.8
	4	51.0	102.0	727.3	0.7	4.4	16.5	1.4	4.3	2.3
	5	83.1	166.1	1314.1	2.8	9.7	40.0	3.4	5.8	3.0
	6	44.4	88.9	978.0	1.8	3.1	32.0	4.0	3.5	3.3
	7	86.7	173.5	1585.8	3.2	4.0	53.7	3.7	2.3	3.4
	8	65.8	131.5	1228.9	2.0	2.8	40.8	3.0	2.1	3.3
Mean ± SD		67.5 ± 15.1	135.1 ± 30.2	1310.0 ± 343.4	1.9 ± 0.8	4.0 ± 2.6	35.6 ± 11.0	2.8 ± 0.9	3.0 ± 1.6	2.8 ± 0.7
Obese	1	266.7	533.3	4209.0	1.2	7.9	57.5	4.5	1.5	1.4
	2	112.5	225.0	1600.4	0.9	1.5	24.6	0.8	0.7	1.5
	3	86.8	173.6	2035.4	1.0	8.0	54.1	1.2	4.6	2.7
	4	79.4	158.8	1058.4	4.0	4.0	37.7	5.0	2.5	3.6
	5	70.9	141.7	1246.6	1.0	1.7	15.1	1.4	1.2	1.2
	6	142.4	284.8	2566.7	4.0	9.7	77.5	2.8	3.4	3.0
	7	39.9	79.8	1190.7	0.8	1.3	20.8	2.0	1.6	1.8
	8	130.6	261.1	1974.1	5.5	6.0	73.3	4.2	2.3	3.7
Mean ± SD		116.1 ± 69.3	232.2 ± 138.6	1985.1 ± 1033.0	2.3 ± 1.9	5.0 ± 3.4	45.1 ± 24.0	2.7 ± 1.6	2.2 ± 1.3	2.4 ± 1.0
Diabetic	1	98.5	197.1	2162.0	2.6	4.4	88.2	2.6	2.2	4.1
	2	99.0	198.0	1794.2	1.4	2.3	23.0	1.4	1.2	1.3
	3	87.0	174.0	2229.0	6.2	7.0	94.7	7.1	4.0	4.2
	4	142.6	285.2	2248.5	3.6	10.1	51.4	2.5	3.5	2.3
	5	78.6	157.3	252.5*	1.5	5.6	3.3*	1.9	3.6	1.3
	6	50.5	101.1	169.2*	0.2	0.6	0.6*	0.4	0.6	0.4
Mean ± SD		92.7 ± 30.2	185.4 ± 60.5	2108.5 ± 212.8	2.6 ± 2.1	5.0 ± 3.4	64.3 ± 29.0	2.6 ± 2.3	2.5 ± 1.4	2.3 ± 1.4

* Represent 3–5-h collections rather than 24-h and were not included in the calculation of the 24-h means of C-peptide secretion and excretion.

insulin secretion in these situations. In fact, Horwitz et al.[9] found no correlation between C-peptide and creatinine clearance when subjects with advanced renal failure were included.

We further evaluated the usefulness of urinary C-peptide for estimating the secretion of C-peptide (SR-C) by correlating the SR-C in the 0–1, 1–3, and 3–5-h postprandial periods and over a 24-h period with the quantity of C-peptide excreted over the corresponding time interval (U-C). The correlation of SR-C with U-C was highly significant in all three of our subject groups. The SR-C for the 0–1- and 1–3-h intervals after glucose ingestion was only marginally significantly higher in the obese than in the lean subjects ($P < 0.05$). After standardizing for body mass, however, the differences in SR-C were no longer significant. When integrated over 24 h, the SR-C/m² of the three groups of subjects was almost equal. The similarity between the SR-C of the obese and lean subjects agrees with our previous contention that the hyperinsulinemia of obesity is mainly due to a diminished hepatic clearance of insulin in obese subjects.[12] The 24-h integrated plasma concentrations of insulin were 69% higher in 23 obese, when compared with 45 lean sub-

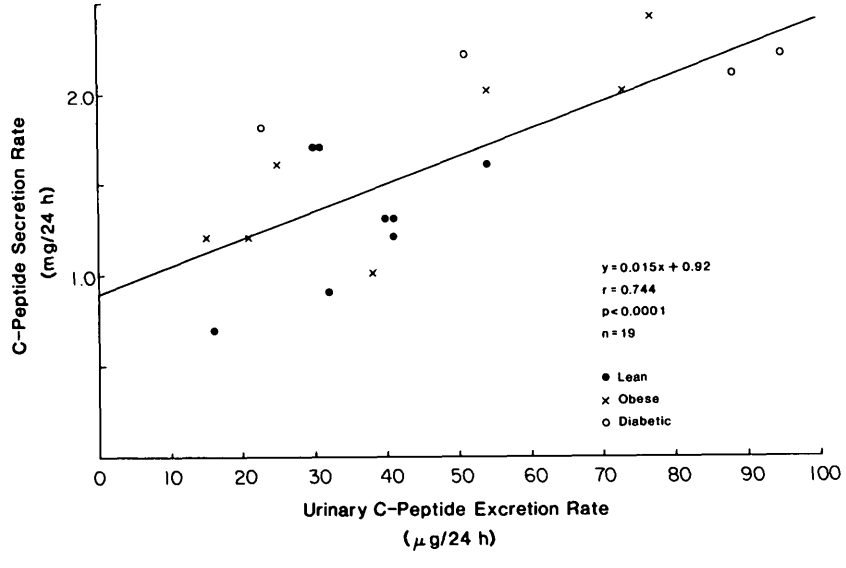

FIGURE 1. Linear regression of 24-h secretion and excretion rates of C-peptide in lean, obese, and diabetic subjects.

jects ($P < 0.0001$), while 24-h integrated concentrations of C-peptide were only 13% higher. The latter difference was only marginally significant. Since the peripheral clearance rate of insulin and the metabolic clearance rate of C-peptide are similar in lean and obese subjects, we concluded that a diminished rate of hepatic insulin extraction was responsible for the higher 24-h integrated insulin concentrations observed in the obese subjects. This conclusion is in agreement with a recent report of similar plasma C-peptide levels after 100-g glucose ingestion in lean, obese, and maturity-onset diabetic subjects.[35]

The 24-h and postprandial rates of urinary C-peptide excretion were each similar in our lean, obese, and diabetic subjects. C-peptide excretion constituted 2.6 ± 1.4% of the secreted C-peptide. Our results are in agreement with Kuzuya et al., who also reported similar 24-h excretion rates of C-peptide in normal and in obese, diabetic subjects.[10]

The mean fraction of secreted C-peptide that was excreted in our present study is lower but not significantly different from our previous report of 4.0 ± 1.5% in 50 normal, lean subjects. The results of our present study indicate that urinary C-peptide is a useful indirect measure of insulin secretion in obese and adult-onset diabetic subjects over a wide physiologic range of plasma C-peptide concentrations.

ACKNOWLEDGMENTS

This study was aided by research grants HD-16077 and training grant AM-07109 of the National Institutes of Health, United States Public Health Service. The patients were studied at the Clinical Research Center of Pediatrics, supported by grant RR-0052 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.

REFERENCES

[1] Meistas, M., Zadik, Z., Margolis, S., and Kowarski, A. A.: Correlation of urinary excretion of C-peptide with the integrated concentration and secretion rate of insulin. Diabetes *30*:639–43, 1981.

[2] Rubenstein, A. H., Clark, J. L., Melani, F., and Steiner, D.: Secretion of proinsulin and C-peptide by pancreatic beta cells and its circulation in blood. Nature *224*:697–99, 1969.

[3] Melani, F., Rubenstein, A. H., Oyer, P. E., and Steiner, D. F.: Identification of proinsulin and C-peptide in human serum by a specific immunoassay. Proc. Natl. Acad. Sci. USA *67*:148–55, 1970.

[4] Heding, L. G., Larsen, U. D., Markusson, J., Jorgensen, K. H., and Hallund, O.: Radioimmunoassay for human, pork and ox C-peptides and related substances. Horm. Metab. Res. (Suppl.) *5*:40–44, 1974.

[5] Chamberlain, M. J., and Stimmler, L.: The renal handling of insulin. J. Clin. Invest. *46*:911–19, 1967.

[6] Rabkin, R., Simon, N. M., Steiner, S., and Colwell, J. A.: Effect of renal disease on renal uptake and excretion of insulin in man. N. Engl. J. Med. *282*:182–87, 1970.

[7] Rubenstein, A. H., Lowy, C., Welborn, T. A., and Fraser, T. R.: Urine insulin in normal subjects. Metabolism *16*:234–44, 1967.

[8] Kaneko, T., Munemura, M., Oka, H., Oda, T., Suzuki, S., Yasuda, H., Yanaihara, N., Nakagawa, S., and Makabe, K.: Demonstration of C-peptide immunoreactivity in various body fluids and clinical evaluation of the determination of urinary C-peptide immunoreactivity. Endocrinol. Jpn. *22*:207–12, 1975.

[9] Horwitz, D. L., Rubenstein, A. H., and Katz, A. I.: Quantitation of human pancreatic beta-cell function by immunoassay of C-peptide in urine. Diabetes *26*:30–35, 1977.

[10] Kuzuya, T., Matsuda, A., Sakamoto, Y., Tanabashi, S., and Kajinuma, H.: C-peptide immunoreactivity (CPR) in urine. Diabetes *21* (Suppl. 1):210–15, 1978.

[11] Faber, O., Hagen, K. C., Binder, C., Markusson, J., Naithani, V. K., Blix, P. M., Kuzuya, H., Horwitz, D. L., Rubenstein, A. H., and Rossing, N.: Kinetics of human connecting peptide in normal and diabetic subjects. J. Clin. Invest. *62*:197–203, 1979.

[12] Meistas, M., Margolis, S., and Kowarski, A. A.: Hyperinsulinemia of obesity is due to decreased clearance of insulin. Submitted for publication.

[13] Koushanpour, E.: Renal Physiology: Principles and Functions. Philadelphia, W. B. Saunders, 1976, p. 103.

[14] Smith, H. W.: The Kidney: Structure and Function in Health and Disease. New York, Oxford University Press, 1951.

[15] Schteingart, D. E., and Conn, J. W.: Characteristics of the increased adrenocortical function observed in many obese patients. Ann. NY Acad. Sci. *131*:398–400, 1965.

[16] Wesson, L. G.: Electrolyte excretion in relation to diurnal cycles of renal function. Medicine *43*:547–92, 1964.

[17] Doolan, P. D., Alpen, E. L., and Theil, G. B.: A clinical appraisal of the plasma concentration and endongenous clearance of creatinine. Am. J. Med. *32*:65–79, 1962.

[18] Van Pilsum, J. F., and Seljeskog, E. L.: Long-term endogenous creatinine clearance in men. Proc. Soc. Exp. Biol. Med. NY *97*:270–72, 1958.

[19] Vogelstein, B., Kowarski, A. A., and Litman, P. S.: The pharmokinetics of amikacin in children. J. Pediatr. *91*:333–39, 1977.

[20] Kowarski, A., Thompson, R. G., Migeon, C. J., and Blizzard, R. M.: Determination of the integrated plasma concentrations and true secretion rates of growth hormone. J. Clin. Endocrinol. Metab. *32*:356–60, 1971.

[21] Kaneko, T., Oka, H., Munemura, M., Oda, T., Yamashita, K., Suzuki, S., Yanaihara, N., Hashimoto, T., and Yanaihara, C.: Radioimmunoassay of human proinsulin and C-peptide using synthetic human connecting peptide. Endocrinol. Jpn. *21*:141–45, 1974.

[22] Thompson, J. C.: Renal inactivation of gastrin. Br. J. Surg. *57*:864, 1970.

[23] Melick, R. A., and Martin, T. J.: Parathyroid hormone metabolism in man: effect of nephrectomy. Clin. Sci. *37*:667–74, 1969.

[24] Kau, S. T., and Maack, T.: Transport and catabolism of parathyroid hormones by the normal kidney. Am. J. Physiol. *233*:F445–51, 1977.

[25] Martin, K. J., Hruska, K. A., Lewis, J., Anderson, C., and Slatopolsky, E.: The renal handling of parathyroid hormone. Role of peritubular uptake and glomerular filtration. J. Clin. Invest. *60*:808–14, 1977.

[26] Bailie, M. D., Rector, Jr., F. C., and Seldin, D. W.: Angiotensin II in arterial and renal venous plasma and renal lymph in the dog. J. Clin. Invest. *50*:119–26, 1971.

[27] Bernier, G. M., and Conrad, M. E.: Catabolism of human beta²-miroglobulin by the rat kidney. Am. J. Physiol. *217*:1359–62, 1969.

[28] Maack, T.: Renal handling of low molecular weight proteins. Am. J. Med. *58*:57–64, 1975.

[29] Maack, T., and Sigulem, D.: Renal handling of lysozyme. *In* Lysozyme (Proc. Int. Conf. Lysozyme). Osserman, E. F., Ed. New York, Academic Press, 1974, pp. 321–33.

[30] Johnson, V., and Maack, T.: Renal extraction, filtration, absorption and catabolism of growth hormone. Am. J. Physiol. *233*:F185–F96, 1977.

[31] Katz, A. J., and Emmanouel, D. S.: Metabolism of polypeptide hormones by the normal kidney and in uremia. Nephron *22*:69–80, 1978.

[32] Katz, A., and Rubenstein, A. H.: Metabolism of proinsulin, insulin and C-peptide in the rat. J. Clin. Invest. *52*:1113–21, 1973.

[33] Emmanouel, D. S., Jaspan, J. B., Rubenstein, A. H., Huen, A. H.-J., Fink, E., and Katz, A. I.: Glucagon metabolism in the rat. Contribution of the kidney to the metabolic clearance rate of the hormone. J. Clin. Invest. *62*:6–13, 1978.

[34] Kajinuma, H., Tanabashi, S., Ishiwata, K., and Kuzuya, N.: Urinary excretion of C-peptide in relation to renal function. *In* Proinsulin, Insulin, C-peptide. Babas, S., Kaneko, T., and Yanaihara, T., Eds. Amsterdam, Excerpta Medica, 1978, pp. 183–89.

[35] Rendell, M., Zarriello, J., Drew, H. M., and Ross, D. A.: Endogenous insulin secretion measured by C-peptide in maturity onset diabetics controlled by diet alone. Arch. Intern. Med. *141*:1617–22, 1981.