Plasma Insulin: Fluctuations in the "Big" Insulin Component in Man after Glucose and Other Stimuli

PHILLIP GORDEN and JESSE ROTH

From the Diabetes Section, Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT Plasma was filtered on G50 Sephadex, and two components of circulating insulin, "little" insulin and "big" insulin, were measured by immunoassay. The former component is indistinguishable from insulin, whereas the latter more closely resembles proinsulin and the other insulin-like substances isolated from the pancreas. In thin normal subjects, the fraction of plasma insulin that was big insulin (per cent "big"), 15–30 min after oral glucose, was less than 5%; per cent big rose 2- to 8-fold over the next hour and by 90–120 min represented 5–29% of the plasma insulin. In young thin subjects with idiopathic glucose intolerance associated with normal concentrations of plasma insulin, an identical pattern of big insulin was observed. In thin subjects in whom elevations of per cent big at 90–120 min during the standard test were only modest, starvation for 48 hr before the glucose administration resulted in a more pronounced rise in the per cent big insulin. Early after glucose administration to obese and acromegalic subjects, the per cent big was higher than in thin subjects. The magnitude of the elevation was roughly correlated with elevations in the fasting plasma insulin concentration. By 90–120 min, the per cent big in obese and acromegalic patients was the same range as in the thin subjects. Intravenously administered arginine and tolbutamide in a small number of subjects yielded a response that was similar to oral glucose; the per cent big was low early after stimulation and increased with time. In two patients with islet cell carcinomas, the per cent big was in the same range as in normal subjects.

INTRODUCTION

Steiner and Oyer (1) and Steiner, Cunningham, Spigelman, and Aten (2) have established that insulin is synthesized in the islets of Langerhans as a larger single chain polypeptide, proinsulin, that is converted intra-

cellularly into insulin. Proinsulin and at least two other insulin-like substances (peak "b" material) have been isolated from commercial bovine insulin (3). Chance, Ellis, and Bromer have demonstrated similar components in commercial porcine insulin (4). Bovine proinsulin and other peak b components (3), as well as porcine proinsulin (5), have substantially greater immunologic than biologic activity.

We have reported that circulating insulin has at least two components, both of which are secreted by the pancreas. The major component, "little" insulin, is indistinguishable from pancreatic insulin on gel filtration and in its reactivity with insulin antibody. "Big" insulin is less retarded on gel filtration and has a distinctive reactivity with insulin antibody (6). Rubenstein, Cho, and Steiner, using guinea pig anti-bovine proinsulin serum, have demonstrated substances in acid ethanol extracts of human urine and plasma that have immunoreactivity and gel filtration properties comparable to proinsulin or other components of peak b (7).

As yet we have insufficient data on the chemical and biological properties of big insulin to be certain of its identity. Temporarily we have postulated that big insulin represents the circulating form of proinsulin or of other peak b substances and, therefore, probably has greater immunological reactivity than biological activity (8).

At present, big insulin measurements cannot be quantitated precisely because of the distinctive reactivity of big insulin and the absence of a suitable standard (8). Therefore, we have measured big insulin in terms of a porcine insulin standard. We studied the fluctuations in big insulin after oral glucose administration to normal subjects, as well as the altered responses associated with starvation, diabetes, obesity, acromegaly, and islet cell tumors. Also, preliminary observations are included on big insulin responses after the administration of tolbutamide and arginine.

Received for publication 28 March 1969.

METHODS

Subjects. Healthy volunteers and selected patients were studied as inpatients on a metabolic unit (Table I). All were ambulatory and well nourished. "Thin" subjects were less than 110% and "obese" subjects were greater than 110% of ideal body weight. None had ever been treated with insulin.

Tests. Drugs were discontinued at least 48 hr before the studies. All tests were performed in the morning after an overnight fast, except where noted. Standard 100 g oral glucose tolerance tests (GTT) were performed after a normal diet that contained 300 g of carbohydrate had been given daily for at least 3 days. Starvation 100 g oral glucose tolerance tests were performed in the morning after 48 hr of food deprivation. Sodium tolbutamide, 1 g in normal saline, was infused over a 2 min period. Arginine HCl, 12.5% in hypotonic Ringer's solution, at a dose of 0.5 g/kg body weight, was infused over a 30 min period. Venous blood was drawn into heparin at intervals through an indwelling No. 19 gauge needle connected to a polyethylene cannula.

TABLE I
Clinical Profile of Patients

Subject	Sex	Age	Height	Weight	Ideal	Comment
		yr	in.	lb.	%*	
Thin						
MCC	M	19	71	136	91	Normal volunteer
RUS	M	20	66.8	140	100	Normal volunteer
BRO	F	20	68.4	119	92	Normal volunteer
NOY	F	20	67.4	144	104	Normal volunteer
SHE	M	20	74.3	174	100	Normal volunteer
DOR	F	21	68	125	98	Idiopathic glucose intolerance
VOL	M	29	66.6	135	100	Idiopathic glucose intolerance
RAY	M	48	70.8	196	109	Glucose intolerance, nontoxic nodular goiter, history of alcoholism
Obese						
CAI	M	62	66.8	166	113	Glucose intolerance, capillary microaneurysms, rheumatoid arthritis, duodenal ulcer
WHI	F	45	63.4	142	114	Glucose intolerance, essential hypertension
MIG	F	14	69.2	185	117	Glucose intolerance, Papago Indian, resistant to insulin (0.3 U/kg), normal plasma growth hormone concentration
SMI	F	29	64.8	164	126	Normal GTT
FIS	M	35	74	245	126	Normal GTT, ? Bechet's syndrome
BRS	F	55	60	150	126	Glucose intolerance, idiopathic diabetes insipidus
SCH	M	24	73.2	248	131	Glucose intolerance, idiopathic diabetes insipidus
MIT	F	52	61	167	144	Glucose intolerance, capillary microaneurysms, proteinuria, hypertension, mild hypercapnia
ACA	M	26	70	370	212	Glucose intolerance, congenital alkalosis (9), gout, proteinuria
ABR	M	37	66.2	456	292	Glucose intolerance, hypoventilation
Acromegaly						
CAS	M	27	68.8	176	103	Glucose intolerance, growth hormone >24 mμg/ml
HOU	F	35	69.4	175	111	Normal GTT, growth hormone = 28 mμg/ml
MUL	M	59	71.4	207	114	Glucose intolerance, growth hormone = 26 mμg/ml
Islet cell carcinoma						
DRE	F	47	63.7	121	100	Severe fasting hypoglycemia
HAN	M	24	68.8	134	89	Severe fasting hypoglycemia

* Metropolitan Life Insurance Tables.

Blood glucose was determined by an autoanalyzer modification of the alkaline ferricyanide technique (10). In these studies, normal glucose tolerance was defined as fasting 120 mg/100 ml or less, 1 hr 170 mg/100 ml or less, and 2 hr 120 mg/100 ml or less.

Insulin was measured by radioimmunoassay (11) using guinea pig anti-porcine insulin, porcine insulin-¹²⁵I, and porcine insulin as the unlabeled hormone (Lilly, lot No. PJ5589). Bound and free hormone were separated by precipitation with sheep anti-guinea pig gamma globulin (6). The plasma insulin concentration, assayed on dilute (1:10 or more) unfractionated plasma, is referred to as "total plasma insulin concentration" and expressed in mμg/ml of plasma.¹

Big insulin measurements. To separate the two components of circulating insulin, plasma, 1-2 ml, was filtered on a column of G50 Sephadex (6). Each of the approximately 25 fractions was assayed for insulin. Little insulin was recovered as a peak in a position similar to purified crystalline insulin, i.e., approximately 0.45 of the distance between the albumin and iodide markers. Big insulin was recovered about halfway between the albumin and little insulin (Fig. 1). For best resolution, small volumes of plasma were filtered on tall narrow (1 × 50 cm) columns of fine grade gel at 4° over 12-18 hr. With occasional plasma specimens, the big insulin appeared as a shoulder on the little insulin peak and could not be resolved completely by three or more filtrations. In these instances, the point of separation between big and little was selected by superimposition of a comparable gel filtration pattern of a plasma that was assayed at the same time and that had clear resolution of its two peaks.

The per cent big insulin is defined as millimicrograms of insulin recovered in the big insulin peak divided by the millimicrograms of insulin recovered in both peaks × 100, i.e.,

$$\text{per cent big} = \frac{\text{big}}{\text{big} + \text{little}} \times 100. \text{ The big insulin concentration is the product of the per cent } \frac{\text{big}}{100} \text{ and the total plasma insulin concentration, i.e., "big" insulin concentration} = \frac{\text{per cent big}}{100} \times \text{total plasma insulin concentration, the latter having been determined by assay of dilute unfractionated plasma.}$$

Recovery of insulin

$$= \frac{(\text{big} + \text{little})/\text{milliliters of plasma filtered}}{\text{total plasma insulin concentration}} \times 100.$$

90% of the columns gave recoveries of from 50 to 100%. Columns with unexplained recoveries of less than 50% were retained if a repeat run, that gave a similar low recovery, yielded a similar distribution of big and little insulin. The few specimens with recoveries reproducibly below 50% were randomly distributed among the patients and the times in the tolerance tests. When multiple runs of the same specimen yielded different recoveries, the distribution of insulin between big and little was not altered significantly. The per cent big insulin ranged from 34 to 50% on a single plasma specimen (patient MIT) filtered and assayed on six separate occasions, and the recovery of total insulin ranged from 72 to 123%.

RESULTS

Thin subjects, standard oral glucose tolerance test.
After the administration of oral glucose to thin normal

¹ The porcine insulin standard has biological activity of approximately 25 U/mg.

subjects, the total plasma insulin concentration rose promptly with a peak at 30–60 min; by 2 hr total insulin concentration had fallen toward normal. At each time the concentrations observed varied widely among individual subjects (11) (Fig. 2).

Early (15–30 min) after oral glucose, per cent big was low (0–4%). Subsequently, the per cent big rose so that late after oral glucose (90–120 min), per cent big

was in the range of 5–29%. The individual responses in five subjects were quite variable with rises ranging from 2- to 8-fold (Fig. 2).

To test the effect of glucose intolerance on big insulin, three young thin patients were selected who had idiopathic glucose intolerance (Table I). All had manifest the glucose intolerance on all of at least two oral tests, and extensive clinical and laboratory studies failed

FIGURE 1 Gel filtration of plasma obtained after stimulation with arginine (left) and tolbutamide (right). The arrows at the left and right denote the location of the albumin-¹²⁵I and iodide-¹²⁵I markers. "Little" insulin is the peak of insulin that is about midway between the markers. "Big" insulin is the peak of insulin about midway between the albumin marker and little insulin (6).

FIGURE 2 A. Standard oral glucose tolerance tests in thin normal subjects. For each patient the blood glucose (open circles) and the total plasma insulin concentration (solid circles) in the upper panels, and the per cent big (hatched bars) and the big insulin concentration (solid triangles), in the lower panels, are plotted as a function of time after the oral glucose was administered. B. Starvation glucose tolerance tests in thin normal subjects. After 48 hr of starvation, the oral glucose tolerance test was repeated.

to uncover an etiology for the glucose intolerance. None had complications characteristic of diabetes mellitus or a family history of the disease. In all three, the total insulin concentrations after oral glucose exceeded 4 m μ g/ml, and in two exceeded 8 m μ g/ml. In these two patients big insulin responses were indistinguishable from those in the normal subjects; per cent big increased from 0 to 5% and from 1 to 6% during the course of the tolerance test. In the other patient per cent big was 4% both early and late after oral glucose (Fig. 3).

Thin subjects, starvation glucose tolerance test. To examine further the effects of glucose intolerance and altered patterns of insulin secretion on big insulin, four of the normal subjects were tested again after 48 hr of starvation. Typically, starvation of this duration impairs glucose tolerance (12); total plasma insulin concentrations are higher and the peak occurs much later than under standard fed conditions (13). In the patient with idiopathic glucose intolerance who was retested after starvation, the glucose tolerance, total plasma insulin concentrations, and time of occurrence of the peak of plasma insulin changed in the same way as in the four normal subjects.

The general pattern of big insulin after starvation was similar to that observed in the standard test (Figs. 2 and 3). At 15 min after oral glucose, per cent big was low. In four of five, it was 4% or less and in the other was 7%. By 120 min after oral glucose, per cent big had increased substantially (14–27%). On comparing an individual's response to the standard test with his starvation test, we found: (a) in the three subjects in whom per cent big at 2 hr during the standard test was only modestly increased (6, 7, and 8%), per cent big at 2 hr during the starvation test was clearly higher (15, 20, and 14%, respectively); (b) in the two subjects in whom per cent big at 2 hr during the standard test was quite high (29% and 20%), the per cent big at 2 hr during the starvation test was again quite high (19% and 27%, respectively); interestingly, in these two subjects, the only ones in whom analyses were performed on specimens from both tests at both 15 and at 30 min, the per cent big was low and unchanged at 30 min during the standard test, whereas the per cent big had clearly risen by 30 min in the starvation test.

Obese subjects, oral standard glucose tolerance test. Typically, obesity is accompanied by an impaired re-

sponse to exogenous insulin (14), a high incidence of glucose intolerance (15, 16), and higher than normal total plasma insulin concentrations, both after an overnight fast (17, 18) and after oral glucose administration (17, 19, 20). Our ten obese subjects ranged in age from 14-62 yr and in weight from 113 to 292% of ideal. Eight were glucose-intolerant (2 hr p.c. glucose 142-530 mg/100 ml) of whom two (MIT and CAI) had retinal microaneurysms, typical of diabetes mellitus (Table I). All eight had a peak of total plasma insulin con-

centration that was 3.8 m μ g/ml or more, seven had 4.0 m μ g/ml or more, and five had 8.0 m μ g/ml or more (Fig. 4).

As in the thin subjects, the per cent big insulin in individual obese subjects was lower early (15-30 min) than late (90-120 min), after oral glucose administration. However, the per cent big early was higher in the obese than in normal subjects (Fig. 5). Whereas none of the eight thin subjects exceeded 4% big at 15 min or 30 min during the standard glucose tolerance

FIGURE 3 A. Standard oral glucose tolerance tests in thin subjects with idiopathic glucose intolerance. B. Starvation oral glucose tolerance test in thin subject with idiopathic glucose intolerance.

FIGURE 4 Standard oral glucose tolerance tests in obese subjects.

test, 8 of 10 obese subjects exceeded 4% big. The elevation in per cent big insulin at early times after oral glucose was roughly proportional to the increase in per cent ideal body weight (Fig. 6). By late times, the per cent big in the obese was in the same wide range as in the thin subjects. Thus, the difference in the per cent big insulin between early and late specimens is often less than in a thin subject. The per cent big insulin observed at different times during the test did not correlate with glucose tolerance, with total plasma insulin concentrations, or with the time of occurrence of the peak insulin concentration.

Acromegaly, standard oral glucose tolerance test. Acromegaly, like obesity, is frequently accompanied by insensitivity to intravenous insulin (21), impaired glucose tolerance (22, 23), and high concentrations of total plasma insulin both after an overnight fast and after oral glucose administration (17, 24). We studied three acromegalics, all of whom had moderately elevated plasma growth hormone concentrations (20–30 $\mu\text{g}/\text{ml}$) (Table I); two had glucose intolerance and in all three, the peak concentrations of total plasma insulin were in the upper part of the normal range (Table II).

In the two acromegalics in whom analyses were performed on samples obtained early after oral glucose, the per cent big was elevated (11 and 22%). In three acromegalics, the per cent big at 2 hr (10, 17, and 27%) was in the same range as in thin and obese subjects.

In obesity and in acromegaly, both the total plasma insulin concentrations after an overnight fast and the per cent big early after glucose were elevated (Fig. 5). In the 20 standard glucose tolerance tests on 8 thin, 10 obese, and 2 acromegalic subjects, we found that the per cent big early was closely related to the fasting total plasma insulin concentration. Of 12 subjects (7 thin, 5 obese) with fasting plasma insulin of 1.0 $\mu\text{g}/\text{ml}$ or less, 10 (7 thin, 3 obese) had per cent big at early times of 5% or less. Of the eight subjects (one thin, five obese, two acromegalic) in whom fasting plasma insulin exceeded 1.0 $\mu\text{g}/\text{ml}$, six (four obese, two acromegalic) had per cent big early in excess of 5%.

Big insulin response in islet cell carcinoma. Two patients with histologically proven islet cell carcinoma, metastatic to the liver, were studied (Table I). Both patients had severe hypoglycemia on fasting, accompanied by hyperinsulinemia. Patient DRE demonstrated 35% big insulin in the plasma 120 min after oral glucose and 18% at 200 min after a small meal, while patient HAN demonstrated 37% big insulin at 200 min after a small meal. The greatest total plasma insulin rise was seen in patient HAN after tolbutamide administration, but big insulin constituted only 3% of the total insulin (Table III).

Response to arginine and tolbutamide. To determine whether stimuli of insulin secretion other than glucose would cause fluctuations in plasma big insulin, three pa-

FIGURE 5 Per cent big early and late after oral glucose. Each point represents the mean $\pm$ SEM for each group. Closed circles represent standard oral glucose tolerance tests, and open circles represent starvation tests. The mean for each group at 15-30 min was derived from a single value on each patient in the group. For most patients, the single value represents the mean of per cent big at 15 min and at 30 min. When specimens from only one of the two times were analyzed, the single determination of per cent big for that patient was used in the calculation of the mean for the group. The data for 90-120 min were derived in the same way.

tients were infused with arginine (25), two patients with sodium tolbutamide (26), and two with sodium tolbutamide 120 min after having received 100 g of glucose orally; pretreatment with glucose augments the plasma insulin response to tolbutamide (27).

Arginine in obese patient ACA caused a prompt rise in total plasma insulin and blood glucose concentration. 15 min after the start of the infusion, big insulin was

not detected, but by 60 min, big represented 23% of the insulin (Fig. 1). Patient CAI had negligible big insulin both at 15 and at 60 min after arginine. Patient NOY had none at 15 min but had 8% big insulin by 60 min.

Tolbutamide in patient ACA produced a prompt rise in total plasma insulin and a slow decline in the blood glucose concentration. Big insulin was 25% at 10 min and 45% at 120 min (Fig. 1). In patient CAI, big insulin was 5% at 10 min and 7% at 120 min after tolbutamide.

Patients VOL and WHI were given tolbutamide 120 min after oral glucose (Fig. 3 and 4). Within 5 min of the tolbutamide infusion, total plasma insulin rose 2- to 3-fold but no significant alterations in per cent big were noted at 10 min or at 30 min after the infusion. Thus it would appear that the fluctuations in big insulin, after arginine and tolbutamide, are similar to the responses after glucose; the per cent big insulin increases with time after stimulation and is independent of the total plasma insulin concentration.

FIGURE 6 Per cent big insulin in relation to body weight. Standard glucose tolerance tests are represented by closed circles and starvation tests by open triangles. For each patient, the per cent big at 15-30 min or at 90-120 min is plotted as a function of his per cent ideal body weight. All thin and obese subjects regardless of glucose tolerance are included. Patients with acromegaly or islet-cell carcinomas were excluded. For the standard test at 15-30 min, $r = 0.61$ and 90-120 min, $r = 0.25$.

DISCUSSION

Before discussing our conclusions, three shortcomings of the study should be emphasized: (a) The number of patients that have been studied is small because each big insulin determination requires so many assays. (b) The per cent big, as determined, is a semiquantitative estimate because our antiserum distinguishes between big and little insulin, and to a smaller extent because

TABLE II
"Big" Insulin Response in Acromegaly

		Minutes						
Subject		0	15	30	60	90	120	180
CAS	Glucose, <i>mg/100 ml</i>	104			170		130	142
	Total insulin, <i>mμg/ml</i>	2.5			12.0		5.2	10.2
	Big, %*	21			8		27	22
	Concentration big, <i>mμg/ml</i>	0.5			1.0		1.4	2.2
HOU	Glucose, <i>mg/100 ml</i>	72	146	130	144	132	111	
	Total insulin, <i>mμg/ml</i>	1.2	8.2	7.5	8.9	6.6	4.4	
	Big, %		22				10	
	Concentration big, <i>mμg/ml</i>		1.8				0.4	
MUL	Glucose, <i>mg/100 ml</i>	120	156	211	202	184	216	224
	Total insulin, <i>mμg/ml</i>	1.5	3.6	5.2	3.3	3.9	7.4	13.5
	Big, %		11			16	17	
	Concentration big, <i>mμg/100 ml</i>		0.4			0.6	1.3	

* Big, % = per cent of plasma insulin that was big insulin.

of the range of recoveries of insulin after filtration. (c) Big insulin may represent more than one substance; the gel filtration method for identifying big insulin would not distinguish among the three major and multiple minor insulin-like components that have been found in peak b of commercial insulin (3). Thus, important differences between groups of subjects may be masked by including all of the immunoassayable material that is in the less retarded peak as big insulin.

We have tentatively concluded that a rise in the per cent big largely reflects release of big insulin from the pancreas (8). This is based on the observations that big and little usually do not interconvert spontaneously in vitro, that there is an arteriovenous difference across the pancreas for both big and little insulin (6), and that big and little insulin disappear from plasma at roughly the same rate since we found that in patients in whom the total plasma insulin concentration fell rapidly, the big insulin concentration fell about as rapidly.

The major determinant of per cent big in plasma in all cases appears to be the time interval after the commencement of insulin secretion. Almost invariably, the per cent big late (90–120 min) was greater than early (15–30 min) after stimulation regardless of glucose tolerance, carbohydrate intake preceding the test, adiposity, plasma growth hormone concentration, or the type of stimulus used. In individual subjects, the per cent big both early and late was modified by other factors. Thus the per cent big early after glucose in obese subjects increased with an increase in the per cent ideal body weight and in obese and in acromegalic subjects with an increase in the fasting total insulin concentration. It is not surprising that in obese subjects the per cent

big early correlates with both per cent ideal body weight and the fasting insulin, since the latter two have previously been found to correlate reasonably well (18). It is as yet uncertain whether in obese and acromegalic subjects the per cent big early, in response to other stimuli, is higher than in normal subjects and whether in other diseases in which the fasting insulin is elevated, the per cent big early is as high as in obesity and acromegaly.

In thin subjects, with or without glucose intolerance, starvation appeared to augment the late phase of big insulin output. We have not extended these observations to other inducers of glucose intolerance in normal subjects, e.g. glucocorticoid administration, or to starvation in obese or acromegalic subjects.

TABLE III
"Big" Insulin Response in Islet Cell Tumor

Subject		200 min fast	
Dre		120 min after 100 g oral glucose	
	Glucose, mg/100 ml	296	62
	Total insulin, mμg/ml	6.8	7.0
	Big, %	35	18
	Concentration big, mμg/ml	2.4	1.3
Han		15 min after 1 g sodium tolbutamide i v.	
	Glucose, mg/100 ml	44	43
	Total insulin, mμg/ml	20.4	2.0
	Big, %	3	37
	Concentration big, mμg/ml	0.6	0.7

We have no data on the biological activity of big insulin and have insufficient data to specify the chemical relationship of big insulin to other insulin-like components in the pancreas and in commercial insulin preparations (3, 4). At present our data are consistent with the hypothesis that at least a portion of big insulin is proinsulin or the proinsulin-intermediate. Both proinsulin and big insulin are produced in the pancreas (1, 6). Bovine or porcine proinsulin, unlabeled or labeled with iodine, added to plasma and filtered in our system does not form little insulin but is recovered with big insulin (8). Trypsin converts both proinsulin and a major portion of big insulin into material that is recovered after filtration in the position of little insulin* (8). An antiserum which distinguishes porcine proinsulin from porcine insulin makes a similar distinction between big and little insulins obtained from plasma.*

Tentatively we will assume that big insulin, like the components of peak b, has immunoreactivity greater than biological activity (3), and like proinsulin, when present, reflects recent insulin synthesis (2). The implications of these speculations have been presented in detail elsewhere (8). Briefly, if big insulin has biological activity that is quantitatively reduced but normal in spectrum, then big insulin *cannot* account for the findings in thin diabetics; in these subjects, big insulin responses are indistinguishable from those observed in other thin subjects, and the glucose intolerance with normal or elevated total plasma insulin concentrations remains unexplained. In starvation, obesity, and acromegaly, part, but only a relatively minor part, of the observed hyperinsulinism can be explained by the presence of increased per cent big insulin. If big insulin has an altered spectrum of biological activity or if it acts to block the action of little insulin, these data need to be reinterpreted.

As a final caution, it should be noted that the presence of big insulin in plasma does not appear to alter significantly the measurements of total plasma insulin by routine methods (11). The limited data available suggest that total plasma insulin as measured by immunoassay is, with only rare exception, a good approximation of the little insulin concentration (8).

ACKNOWLEDGMENTS

We are grateful to C. M. Hendricks and M. Lesniak for superior technical assistance, to the nurses and physicians on Ward 8 West, Clinical Center, for care of the patients, to I. Pastan for advice on the studies, and to I. Pastan, R. S. Yalow, R. Perlman, B. M. Sherman, and J. Robbins for critical review of the manuscript.

* Sherman, B. M., J. Roth, and P. Gorden. Unpublished observations.

REFERENCES

- Steiner, D. F., and P. E. Oyer. 1967. The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. *Proc. Nat. Acad. Sci. U. S.* 57: 473.
- Steiner, D. F., D. Cunningham, L. Spigelman, and B. Aten. 1967. Insulin biosynthesis: evidence for a precursor. *Science (Washington)*. 157: 697.
- Steiner, D. F., O. Hallund, A. Rubenstein, S. Cho, and C. Bayliss. 1968. Isolation and properties of proinsulin, intermediate forms, and other minor components from crystalline bovine insulin. *Diabetes*. 17: 725.
- Chance, R. E., R. M. Ellis, and W. W. Bromer. 1968. Porcine proinsulin: characterization and amino acid sequence. *Science (Washington)*. 161: 165.
- Shaw, W. N., and R. E. Chance. 1968. Effect of porcine proinsulin in vitro on adipose tissue and diaphragm of the normal rat. *Diabetes*. 17: 737.
- Roth, J., P. Gorden, and I. Pastan. 1968. "Big insulin": a new component of plasma insulin detected by immunoassay. *Proc. Nat. Acad. Sci. U. S.* 61: 138.
- Rubenstein, A. H., S. Cho, and D. F. Steiner. 1968. Evidence for proinsulin in human urine and serum. *Lancet*. 1: 1353.
- Gorden, P., and J. Roth. 1969. Circulating insulins: "big" and "little." *Arch. Intern. Med.* 123: 237.
- Darrow, D. C. 1945. Congenital alkalosis with diarrhea. *J. Pediat.* 26: 519.
- Hoffman, W. S. 1937. A rapid photoelectric method for the determination of glucose in blood and urine. *J. Biol. Chem.* 120: 51.
- Yalow, R. S., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* 39: 1157.
- Traugott, K. 1922. Über das verhalten des blutzuckerspiegels bei wiederholter und verschiedener art enteraler zuckerzufuhr und dessen bedeutung für die leberfunktion. *Klin. Wochenschr.* 1: 892.
- Unger, R. H., A. M. Eisentraut, and L. L. Madison. 1963. The effects of total starvation upon the levels of circulating glucagon and insulin in man. *J. Clin. Invest.* 42: 1031.
- Rabinowitz, D., and K. L. Zierler. 1962. Forearm metabolism in obesity and its response to intra-arterial insulin. Characterization of insulin resistance and evidence for adaptive hyperinsulinism. *J. Clin. Invest.* 41: 2173.
- Paullin, J. E., and H. C. Sauls. 1922. A study of the glucose tolerance test in the obese. *S. Med. J.* 15: 249.
- John, H. J. 1929. A summary of the findings in 1100 glucose tolerance estimations. *Endocrinology*. 13: 388.
- Karam, J. H., G. M. Grodsky, and P. H. Forsham. 1963. Excessive insulin response to glucose in obese subjects as measured by immunochemical assay. *Diabetes*. 12: 197.
- Bagdade, J. D., E. L. Bierman, and D. Porte, Jr. 1967. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and non-diabetic subjects. *J. Clin. Invest.* 46: 1549.
- Yalow, R. S., S. M. Glick, J. Roth, and S. A. Berson. 1965. Plasma insulin and growth hormone levels in obesity and diabetes. *Ann. N. Y. Acad. Sci.* 131: 357.
- Perley, M., and D. M. Kipnis. 1966. Plasma insulin responses to glucose and tolbutamide of normal weight

- and obese diabetic and nondiabetic subjects. *Diabetes*. 15: 867.
21. Fraser, R., G. F. Joplin, L. H. Opie, and D. Rabinowitz. 1962. The augmented insulin tolerance test for detecting insulin resistance. *J. Endocrinology*. 25: 299.
 22. Davidoff, L. M., and H. Cushing. 1927. Studies in acromegaly; disturbance of carbohydrate metabolism. *Arch. Intern. Med.* 39: 751.
 23. Coggeshall, C., and H. F. Root. 1940. Acromegaly and diabetes mellitus. *Endocrinology*. 26: 1.
 24. Karam, J. H., F. C. Paulatos, G. M. Grodsky, and P. H. Forsham. 1965. Critical factors in excessive serum-insulin response to glucose. *Lancet*. 1: 286.
 25. Floyd, J. C., S. S. Fajans, J. W. Conn, R. F. Knopf, and J. Rull. 1966. Stimulation of insulin secretion by amino acids. *J. Clin. Invest.* 45: 1487.
 26. Yalow, R. S., H. Black, M. J. Villazon, and S. A. Berson. 1960. Comparison of plasma insulin levels following administration of tolbutamide and glucose. *Diabetes*. 9: 356.
 27. Perley, M., and D. M. Kipnis. 1966. Effect of glucocorticoids on plasma insulin. *N. Engl. J. Med.* 274: 1237.