

# Insulin Responses to Glucose: Evidence for a Two Pool System in Man

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**ABSTRACT** Four rapid glucose injections of 5 g each were administered to normal young adult subjects before, during, and after an infusion of glucose. After the first glucose pulse, insulin responses measured immunologically reached a peak between 3 and 5 min and rapidly returned to base line. A short glucose infusion of 300 mg/min decreased the rapid insulin response to a second glucose pulse ( $-58\%$ ), but after a longer infusion (20 hr) the acute insulin response to a third pulse was restored to normal. Stopping the infusion was followed by return of glucose and insulin levels to pre-study base line within 1 hr, but a fourth glucose pulse was followed by a supernormal acute insulin response ( $+200\%$ ). Other observations during these studies showed that a short glucose infusion of either 100 mg/min or 300 mg/min produced a parallel rise in glucose and insulin, but continuation of the infusion for 20 hr was associated with a "paradoxical" fall in glucose and continued rise in insulin. These observations are considered incompatible with a simple linear model often used to describe the relation between plasma glucose and serum insulin. Instead, a two pool system—one for acute insulin release, and the other a time-dependent compartment for long term insulin responses—is suggested.

## INTRODUCTION

Glucose is known to stimulate insulin release directly by the pancreas perfused in vitro (1), incubated in vitro

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(2-4), or perfused in vivo (5). The mechanism for this stimulation is unknown but it has been suggested that a metabolite may be involved rather than glucose itself (1). Although in general it has been found that the more glucose available to the islet, the greater the insulin release, the precise quantitative relationship between glucose concentration and insulin release rate has not been completely clarified. In some studies it appeared that there was a complex power function relationship between glucose concentration and insulin release (5), but in others (6) a linear relationship between a change in glucose concentration and a change in insulin release was assumed. The present investigation was designed to test the assumption that there was a fixed relationship between blood glucose and serum insulin concentration. Several alternate hypotheses were considered; that insulin release rate was related to the absolute glucose concentration, to the change in glucose concentration over a unit period of time, or to the relative change in glucose concentration. The results indicate that none of these simple models is adequate to explain insulin levels observed in man. Therefore a more complex multicompartmental release system has been suggested.

## METHODS

All studies were performed on apparently healthy normal men and women between the ages of 21 and 40 yr. None had a sibling, parent, grandparent, aunt, or uncle with a history of diabetes mellitus. All were requested to eat a weight-maintaining diet of normal caloric balance. Subjects were admitted to the research ward after an overnight fast of at least 12 hr and were not permitted to smoke on the day of the test or during the study. Blood samples were withdrawn through an intravenous scalp vein needle placed in an antecubital vein and prevented from clotting by slow infusion of 0.85% sodium chloride. Glucose was injected as a rapid bolus (pulse) of 50% glucose in water into the same vein. It was injected over a period of 15 sec. The beginning of the injection was taken as zero time and subsequent blood samples were taken from 10 sec before to 10 sec after the

TABLE I  
Plasma Glucose

Subject	1st pulse (5.0 g glucose)				2nd pulse (5.0 g glucose)	
	Infusion rate during pulse 2 and 3	Basal*	3-5' $\Delta$	10-30' $K_d$	Glucose infusion 45'	3-5' $\Delta$
	mg/min	mg/100 ml	mg/100 ml	%/min	mg/100 ml	mg/100 ml
A	300	85	18	-1.22	119	33
B	300	83.4	38.3	-1.89	154.5	46.7
C	300	84.2	26.8	-1.65	118	32.3
D	300	97.7	28.3	-1.58	139.5	29.2
E	300	83.8	38.2	-1.90	156	47
F	300	85.5	27.2	-1.93	118	29
G	300	100	34.7	-1.62	146	32.7
H	300	83.8	22.5	-2.24	116	36.3
I	300	74.5	36	-1.63	132	31.3
J	300	81.4	28.3	-2.45	113.5	75.5
K	300	83.3	49.3	-1.93	149	38
L	300	99	30.3	-1.71	153	30.7
Mean (A-H)		88 $\pm$ 7	29.2 $\pm$ 7	-1.75 $\pm$ .3	133 $\pm$ 17	35.8 $\pm$ 7
Mean (A-L)		86.8 $\pm$ 8	31.5 $\pm$ 8	-1.81 $\pm$ .3	134 $\pm$ 17	38.5 $\pm$ 13
M	100	92.6	21.9	-0.91	105	19.3
N	100	78.7	12.6	-1.79	93	7
O	100	89.5	27.8	-1.93	97	23.7
P	100	84	37	-1.73	101	39.6
Q	100	94.5	27.5	-1.32	99	25.5
R	100	90.3	36.7	-1.59	100	37
Mean (M-R)		88.3 $\pm$ 6	27 $\pm$ 9	-1.55 $\pm$ .37	99.2 $\pm$ 4	25.3 $\pm$ 12
Grand mean (A-R)		87.3 $\pm$ 7	30 $\pm$ 8.6	-1.72 $\pm$ .35		

\* Mean of four samples.

minute. Glucose was also infused into a smaller arm vein through an indwelling plastic catheter in the opposite arm. When glucose was given as a constant infusion, samples were withdrawn at 15-min intervals. Serum or plasma samples were treated as previously described (7) and analyzed for immunoreactive insulin (IRI) and glucose concentration. Insulin responses to either 2.5 or 5 g of glucose were estimated as the area of the total insulin increment above base line (microunits per milliliter  $\times$  minutes) and as the acute change in concentration from the base line to the peak insulin response quantitated as the mean of the 3, 4, and 5 min samples ( $\Delta 3-5'$   $\mu$ U/ml). Preliminary studies had indicated that there was no change in insulin concentration until after a 2 min sample, but a rapid peak was reached between 3 and 5 min. Therefore in most studies blood samples were taken at 3, 4, 5, 7, 10, 15, 30, 45, and 60 min. In all subjects, insulin levels returned to the base line within 30 min unless a continuous infusion of glucose was being given. Glucose disappearance rate ( $K_d$ ) was calculated from the slope of the linear regression (least-squares analysis) of log plasma glucose with time from 10 to 30 min after glucose injection.

## RESULTS

### A. Pulse studies. Experimental design

4 glucose pulses of 5 g each were given to most subjects except as noted. 1 hr after the 1st glucose pulse a constant infusion of glucose of either 100 mg/min or 300 mg/min was begun. The 2nd glucose pulse was given after 45 min of glucose infusion, the 3rd after 20 hr of infusion. 1 hr after stopping the infusion the 4th glucose pulse was given.

1. *The effect of a rapid glucose injection on acute insulin responses (pulse 1).* After the rapid injection of 5 g of glucose in 16 subjects (A-R, Table I), plasma glucose levels rapidly reached a plateau at 2 min which was maintained for at least the next 3 min, and usually for the next 8 min. From 10 until 30 min blood glucose declined exponentially until it returned to the base line between 30 and 60 min (mean  $K_d$  -1.72%/min, Table

Concentration

2nd pulse (5.0 g glucose)		3rd pulse (5.0 g glucose)			4th pulse (5.0 g glucose)	
10-30' K <sub>g</sub>	Glucose* inf. 20 hr	3-5' Δ	10-30' K <sub>g</sub>	60' post- infusion	3-5' Δ	10-30' K <sub>g</sub>
%/min	mg/100 ml	mg/100 ml	%/min	mg/100 ml	mg/100 ml	%/min
+ .55	104.7	25.3	-1.76	81	28.3	-2.26
+ .54	131.5	56.3	- .43	64.5	37.7	-3.55
+ .43	141	20	- .51	89	25.3	-1.24
- .12	118.7	38.5	-2.10	76.5	38.2	-2.66
- .18	112.3	40.0	-3.35	69	45.7	-2.31
-1.17	106	29.7	-2.60	70	29.7	-1.36
+ .01	115.3	31.4	-2.07	80	38	-2.17
+ .17	94.3	31	-1.77	83	36	-1.67
+ .06						
-1.77						
- .13						
- .11						
+ .03 ±.6	115.5 ±15	34 ±11	-1.82 ±1	76.6 ±8	34.9 ±7	-2.15 ±.8
- .14 ±.7						
- .68	95.9	16.3	-1.74	96	13.7	- .86
+ .15	81	18.3	-4.25	78	38.6	-4.23
-1.08	97.8	17.9	-1.34	94	18.3	-1.48
- .76	96	38	-1.21	85	33	-1.72
-1.15	95	22	-1.70	84	36.7	-1.84
- .86	97.5	35.5	-2.08	95	46.8	-2.11
- .73 ±5	93.9 ±6	24.7 ±9.6	-2.05 ±1.1	88.7 ±7	31.2 ±13	-2.04 ±1.2

I). In three subjects a repeat injection at 60 min produced the same response. Immunoreactive insulin (IRI) in these same subjects remained at basal levels at the 2 min sample in the six subjects in whom it was measured. There was a rapid rise in insulin level between 3 and 5 min with a peak achieved in any one of these samples and then a subsequent decline with return to base line within 15 min in the great majority of subjects and by 30 min in all subjects (mean peak Δ3-5' IRI = 42.1 ±25 μU/ml, Table II). Since the plasma glucose remained relatively stable for the first 5 min and the insulin response was so rapid, it was apparent that the insulin increments achieved during this period of time were due almost entirely to insulin release from the pancreatic beta cell in response to the pulse. An estimate of the response during this period of time minimized any effects of peripheral removal of glucose or insulin which might alter the height of this response.

Therefore it was used as an index of the acute insulin response to a glucose pulse.

2. *The effect of short glucose infusions on the acute insulin response (pulse 2).* 60 min after pulse 1, a glucose infusion of 300 mg/min was begun in 12 subjects (A-L, Tables I and II). After 45 min of glucose infusion, at which time previous experience in 10 subjects had indicated steady levels of glucose and insulin could be expected for at least the next 15 min (8), the rapid injection of 5 g of glucose was repeated (2nd glucose pulse). In all cases the initial 3-5 min insulin incremental response was less than the initial insulin response to the identical glucose stimulus given 105 min previously (ΔIRI pulse 2 less than ΔIRI pulse 1,  $P < 0.01$ ; Table II, Fig. 1). This was associated with a markedly slowed disappearance rate of glucose ( $P < 0.001$ ) which in most instances approximated a plateau during the next 60 min (K<sub>g</sub> for pulse 2 = + 0.03%/min, Table I). The

TABLE II  
Plasma Immunoreactive

Subject	Glucose infusion rate during pulse 2 and 3	1st pulse (5.0 g glucose)			2nd pulse (5.0 g glucose)	
		Basal*	3-5' $\Delta$	Area	Glucose infusion 45'	3-5' $\Delta$
		mg/min	$\mu\text{U/ml}$	$\mu\text{U/ml}$	$\mu\text{U/ml}\cdot\text{min}$	$\mu\text{U/ml}$
A	300	8.3	11	154	16.5	4.5
B	300	5.0	25	187	13	7.5
C	300	9.4	21.6	215	20.5	12.8
D	300	11.3	23.7	274	20	6.7
E	300	3.5	13.3	142	21	1.2
F	300	17.5	92.5	633	55	29
G	300	14.1	21.6	158	25	6.5
H	300	16.7	49.7	399	33	53
I	300	7.6	12.6	134	22	0.8
J	300	7.9	16.8	138	15	5.2
K	300	3.5	30.8	192	14.5	8.8
L	300	11.4	9.6	67.4	21	1.0
Mean (A-H)		10.7 $\pm$ 5	32.3 $\pm$ 27	270 $\pm$ 169	25.5 $\pm$ 13	15.1 $\pm$ 17
Mean (A-L)		9.7 $\pm$ 4.7	27.3 $\pm$ 23	244 $\pm$ 153	23 $\pm$ 11	11.4 $\pm$ 15
M	100	13.4	55.6	397	24	45
N	100	5.5	9.5	193	7.5	9.3
O	100	11.1	52.1	357	14	53.3
P	100	10.5	48.3	401	13	42.2
Q	100	23	34.7	303	25	46.3
R	100	12	37.7	289	8	16
Mean (M-R)		12.6 $\pm$ 6	39.7 $\pm$ 17	323 $\pm$ 79	15.3 $\pm$ 7.6	35.3 $\pm$ 18
Grand mean (A-R)		10.6 $\pm$ 5	42.1 $\pm$ 25	257 $\pm$ 139		

\* Mean of four samples.

smaller initial increments in insulin were followed by a return almost to preinjection level within 10-15 min, and then a subsequent delayed rise. This response was such that most subjects showed a rising insulin level between 30 and 60 min after pulse 2 despite an almost constant plasma glucose. A composite of the mean of these results is shown in Fig. 1 with quantitative evaluations in Tables I and II.

To determine the effect of infusion rate on this response, six other subjects (M-R, Tables I and II) were infused with glucose 100 mg/min 60 min after the acute injection of 5 g of glucose following the same protocol. As in the previous study the acute injection of glucose was repeated 45 min after beginning the 100 mg/min infusion. These responses were somewhat more variable. One subject had a decrease in the acute insulin response to this glucose pulse ( $\Delta$  3-5', subject R, Table II) during a 100 mg/min infusion but the others did not, and there-

fore the mean insulin changes were small and overall were not statistically different compared to preinfusion responses ( $P > 0.4$  Fig. 2). The glucose disappearance rate ( $K_e$ , pulse 2, during this 100 mg/min infusion) was reduced in the six subjects studied in Table I ( $P < 0.05$ ) although not to the degree noted with the faster rate of glucose infusion. The insulin level did not always return to the base line within 15 min but a shoulder developed in the insulin response curve in some patients so that insulin levels were higher than the preinjection values at 30 min (Fig. 2). Total insulin incremental areas, however, were not statistically different (Table II).

To evaluate the changes at 100 mg/min more completely and to eliminate any effect of the order of study, eight other subjects, in a modified protocol, were given glucose. Four of them received a glucose infusion of 100 mg/min with the simultaneous injections of 2.5 and

*Insulin (IRI) Concentration*

2nd pulse (5.0 g glucose)			3rd pulse (5.0 g glucose)		4th pulse (5.0 g glucose)	
30' area	Glucose inf.* 20 hr	3-5' Δ	Area	60' post-infusion	3-5' Δ	Area
$\mu\text{U}/\text{ml}\cdot\text{min}$	$\mu\text{U}/\text{ml}$	$\mu\text{U}/\text{ml}$	$\mu\text{U}/\text{ml}\cdot\text{min}$	$\mu\text{U}/\text{ml}$	$\mu\text{U}/\text{ml}$	$\mu\text{U}/\text{ml}\cdot\text{min}$
132	23.6	14.4	334	15	25.5	217
200	22.5	72.7	905	10.5	55.2	393
62.2	21.3	20.7	1025	16	57.7	345
192	32	38.7	688	16	49.7	308
5.8	29.4	30.9	662	18	131.8	850
397	63.5	58.2	609	25	95.7	675
234	38.4	12.3	418	19	30.3	323
406	29.1	57.7	450	21	76.7	456
10.9						
158						
179						
149						
203 ±143	32.5 ±14	38.2 ±22	636 ±239	17.6 ±4	65.3 ±35	446 ±212
177 ±127						
242	33.6	89.9	657	22.5	34.3	244
323	12.5	22.8	343	13	57.2	405
461	23.2	20.1	253	17.5	26.7	291
384	16.9	40.9	397	10	24.8	331
459	29.4	57.1	482	23	66	497
262	15.5	28.2	340	12	32.3	304
328 ±16	21.8 ±8	43.2 ±27	412 ±142	16.3 ±5.5	40.2 ±17	345 ±91

5 g of glucose first, and four received the injections first followed by the infusion plus glucose injection. Since the responses were similar, the results are combined in Table III. Some subjects again had a decreased acute response to the larger glucose pulse during the infusion and these changes were statistically significant with 5 g glucose pulse at the 0.05 level but not at all with the 2.5 g pulse (Table III). In general, then, the acute insulin response to a glucose pulse was diminished by a short 300 mg/min infusion but not always during the 100 mg/min infusion.

3. *The effect of continuous glucose infusions for 20 hr on steady-state insulin levels.* The continuous infusion of glucose was begun after the first glucose pulse. The infusion was continued for 20 hr at a rate of 300 mg/min in eight subjects (Fig. 3) and these eight plus two more subjects were similarly given a prolonged glucose infusion (Fig. 4). The infusion was also continued for

20 hr at a rate of 100 mg/min in six subjects (Figs. 2 and 4). Subjects were allowed to eat lunch and dinner

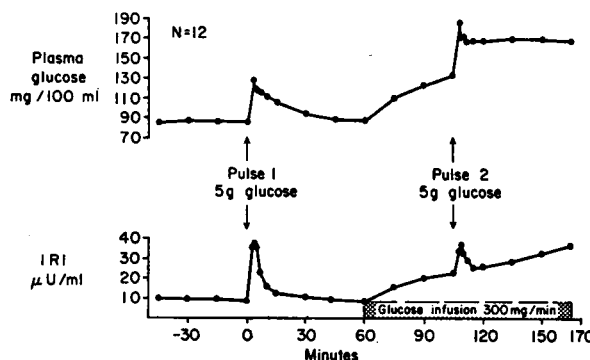


FIGURE 1 The effect of a short (45 min) 300 mg/min glucose infusion on mean insulin and glucose responses to a rapid glucose pulse in 12 normal subjects.

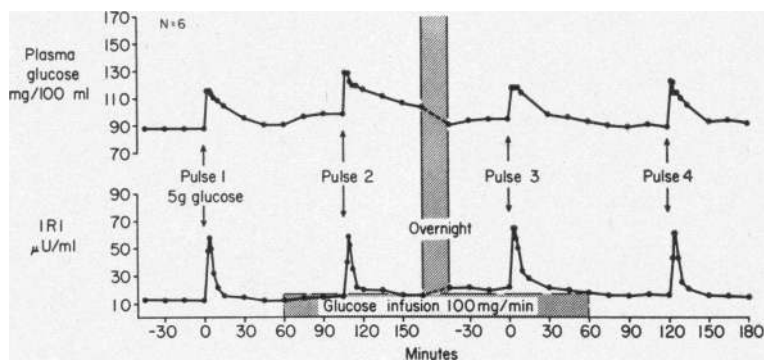


FIGURE 2 The effect of a short (45 min) and prolonged (20 hr) 100 mg/min glucose infusion on the mean insulin and glucose responses to a glucose pulse in six normal subjects.

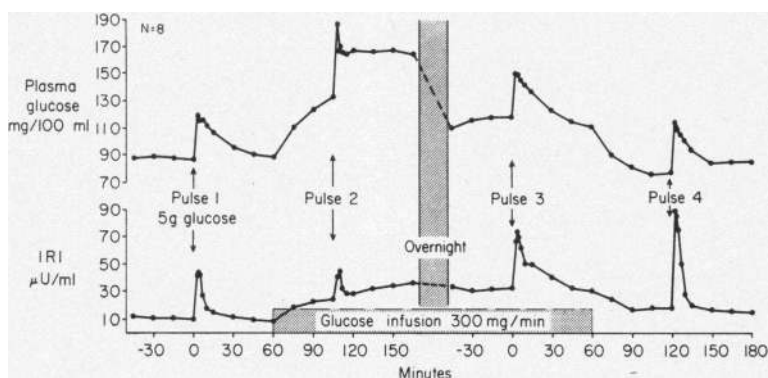


FIGURE 3 The effect of a 300 mg/min glucose infusion of the insulin and glucose responses to a glucose pulse in eight normal subjects.

as usual and then were required to fast overnight for 12 hr prior to repeat measurements the next morning. After this period of 20 hr of a 300 mg/min infusion, steady-state glucose levels remained somewhat above basal ( $P < 0.01$ ) and steady-state insulin levels were markedly increased ( $P < 0.001$ , Tables I and II). Elevated steady-state insulin levels were also observed at a rate of 100 mg/min, although in subject Q, venous glu-

cose levels were no more than 1 mg/100 ml higher than they had been in the original basal state. Thus the response to glucose infusion was a parallel increase in glucose and insulin during the first 45 min of infusion but this parallelism was lost after 20 hr, and there was a continued increase in insulin levels ( $P < 0.02$  for both 100 and 300 mg/min infusions) with a decrease in plasma glucose ( $P < 0.05$ ). These divergent responses

TABLE III

*The Effect of a 100 mg/min Glucose Infusion on Glucose and Acute Insulin Responses to a Glucose Pulse*

Glucose pulse dose	3-5' Δ IRI no infusion	3-5' Δ IRI during infusion*	3-5' plasma glucose no infusion	3-5' plasma glucose during infusion*	3-5' Δ glucose no infusion	3-5' Δ glucose during infusion*
g	μU/ml	μU/ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml
2.5	29.2 ± 9	23.1 ± 10	107 ± 11	121 ± 6†	17.9 ± 5	19.4 ± 2.7
5.0	49.9 ± 22	30.1 ± 15§	126 ± 11	142 ± 16†	35.7 ± 7	33.4 ± 10

\* All subjects given four glucose pulse injections. Five subjects given two pulse injections without infusion first, four subjects given two pulse injections during infusion first.

†  $P < 0.01$ .

§  $P < 0.05$  comparing response during infusion with response without infusion.

THE EFFECTS OF TIME ON IRI DURING GLUCOSE INFUSION

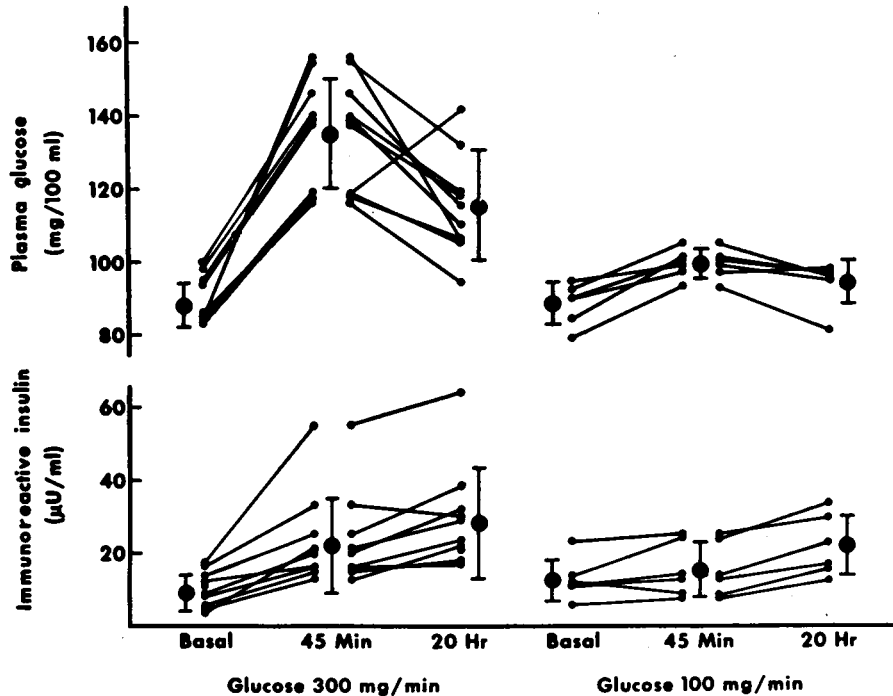


FIGURE 4 Insulin and glucose levels during short (45 min) and long (20 hr) glucose infusions. Mean  $\pm$ sd in 10 normal subjects at 300 mg/min, and six normal subjects at 100 mg/min. Mean of four basal and 20-hr samples compared with a single value after 45 min of glucose infusion.

are shown diagrammatically in Fig. 4 in which a mean of four base line insulin values is compared with a single value 45 min after glucose infusion and with the mean of four steady-state glucose and insulin values after 20 hr of glucose infusion.

4. *The effect of prolonged glucose infusion on the acute insulin responses to glucose (pulse 3).* A third 5 g of glucose was injected rapidly intravenously in eight subjects given continuous glucose infusion of 300 mg/min for 20 hr. Glucose disappearance rate ( $K_d$ , pulse 3) was now more rapid in all instances than was found in the same subjects after pulse 2, 45 min after the glucose infusion was begun ( $P < 0.001$  Table I) and similar to the glucose disappearance rate noted after pulse 1. Despite the higher starting levels of insulin, the rapid insulin responses, i.e. the mean increment of the 3-5 min increases, were now equivalent to the initial responses prior to beginning the glucose infusion (pulse 1) but much greater than the responses to pulse 2 ( $P < 0.01$ ). The shape of the rest of the insulin response curve was changed, in that beyond 15 min the levels tended to remain elevated thus clearly broadening the descending limb of the insulin response curve so that insulin levels did not reach preinjection base line until 45 min after

the 3rd glucose pulse. Thus, although the acute insulin response was similar, total insulin release (insulin area) was significantly greater when the response to pulse 3 was compared with pulse 1 ( $P < 0.02$ , Table II, and Fig. 3).

In the six subjects given 100 mg/min for 20 hr, there was very little effect on the subsequent rapid insulin response to the glucose pulse with mean 3-5'  $\Delta$  insulin response remaining relatively constant. However, the same tendency for further broadening of the peak was observed. This pulse was associated with an improved glucose disappearance rate ( $K_d$ ) in all subjects when compared with the previous glucose tolerance after 45 min of glucose infusion ( $K_d$  pulse 2 - 0.73%/min,  $K_d$  pulse 3 - 2.05%/min,  $P < 0.05$ , Table I, and Fig. 2).

5. *The effect of continuous glucose infusion on a subsequent acute glucose injection (pulse 4).* In eight of the subjects given glucose infusion of 300 mg/min for 20 hr, a fourth rapid injection of 5 g of glucose was given 1 hr after the glucose infusion. During this hour glucose and insulin levels rapidly returned to near original base line values. Despite this return to what appeared to be the original steady state, the last 5 g of glucose pulse resulted in a doubling of the acute insulin re-

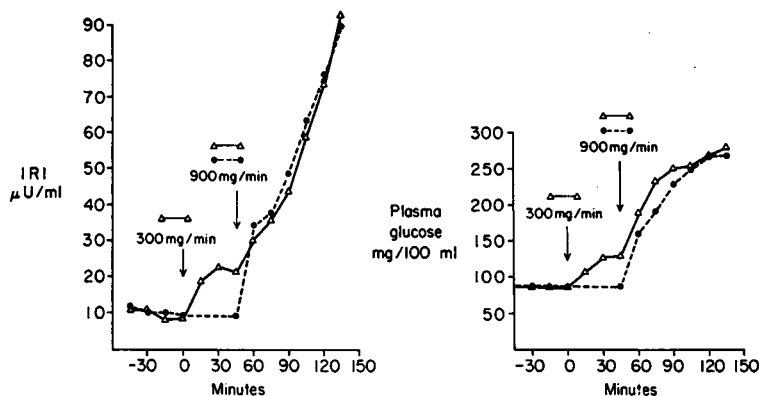


FIGURE 5 The effect of a short (45 min) 300 mg/min glucose infusion on mean glucose and insulin responses to a 900 mg/min glucose infusion for 90 min.

response. The broadening of the pulse that had been observed during glucose infusion (pulse 3) was not observed, and these very high insulin levels rapidly returned to base line within 15 min (Fig. 3). This "supernormal" acute and total insulin response ( $P < 0.05$ , pulse 4 compared with pulse 1) was associated in five of the eight subjects with a more rapid disappearance rate of glucose, but this change was not statistically significant ( $-1.75\%/min$  vs.  $-2.15\%/min$ , Table I). This phenomenon was not observed in the six subjects given 100 mg/min overnight in whom the acute insulin response and glucose disappearance rate was virtually identical with the response observed during the first pulse on the previous day (Tables I, II, and Fig. 2).

#### B. The effect of a glucose infusion of 300 mg/min upon insulin and glucose response to a subsequent infusion of 900 mg/min

The results above indicated a diminished insulin response after 45 min of the glucose infusion of 300 mg/min and raised the possibility that the acute insulin response was dependent upon the glucose concentration at the time of challenge which might in some way influence the sensitivity of the islet. To determine whether the sensitivity of the islet was changed to other forms of glucose stimulation, six subjects were made hyperglycemic by infusing them with glucose for 45 min at a rate of 300 mg/min, but instead of a glucose pulse, the infusion was stopped and replaced immediately by another glucose infusion of 900 mg/min for 90 min. The responses to the double infusion were compared with the insulin and glucose responses to a glucose infusion of 900 mg/min alone on a separate day. In contrast to the diminished insulin response to an acute pulse noted in the previous studies, insulin levels were essentially identical throughout the 900 mg/min infusion whether or not

this infusion was preceded by a 300 mg/min infusion (Fig. 5). There was, however, a significantly ( $P < 0.05$ , 0.01, and 0.02) higher plasma glucose for the first 45 min when the larger infusion was preceded by the smaller infusion. This period of time was consistent with previous observations that approximately 45 min is required for glucose levels to return to base line after an hour long 300 mg/min infusion (8). Thus it would appear that a 300 mg/min infusion primarily affected the acute insulin response to a glucose pulse and not the response to a subsequent glucose infusion. It was of interest to note that although insulin levels rose progressively during the 90 min of the 900 mg/min infusion, glucose levels rose rapidly during the first 60 min and less steeply during the last 30 min of infusion showing in a different way dissociation between changes in plasma glucose and serum insulin concentrations.

#### DISCUSSION

The results of this series of investigations indicate clearly that although glucose administration results in an increase in immunoreactive insulin levels, there is not a simple linear relationship between plasma glucose and insulin concentrations. There are numerous examples of anomalous behavior. 5 g of glucose given rapidly during the early phase of a 300 mg/min glucose infusion (pulse 2) resulted in a higher glucose level but a smaller acute insulin response than an identical stimulus produced when given 60 min before the infusion (pulse 1). Yet, 1 hr after the 300 mg/min infusion of glucose had ended, a larger acute insulin response was found after the same 5 g stimulus (pulse 4) despite preinjection glucose and insulin levels which were similar to those preceding pulse 1. Also noted after pulse 2 given during the 300 mg/min infusion were plasma glucose concentrations which were essentially constant at 170



mg/100 ml for 60 min while simultaneous insulin levels slowly rose from 26  $\mu$ U/ml to 37  $\mu$ U/ml. A similar dissociation between glucose levels and insulin responses was observed during the 100 mg/min infusion in which higher absolute glucose levels during pulse 2 were coincident with no change or decrease in the acute insulin response. Any one of these observations would be sufficient to reject a simple model which relates glucose-stimulated insulin secretion solely to blood sugar concentration at any instant in time. The observation that glucose levels decline between 45 min and 20 hr of glucose infusion while insulin levels are rising suggests that there is a slower phase of insulin release which is glucose metabolism or time dependent. The same slow response phase may have been responsible for the rising insulin levels after pulse 2 during the 300 mg/min infusion despite unchanging glucose values and for the slowly rising glucose levels and rapidly rising insulin concentrations during the later phase of the 900 mg/min glucose infusion. The exact quantitative relationships in these delayed insulin responses cannot be defined since arterial glucose levels, which were presumably the stimulus, would be expected to differ significantly from venous glucose levels particularly when insulin concentrations are high. However, it would appear unlikely that there would be any further increase in arterial glucose levels between 45 min and 20 hr of glucose infusion, and this was still associated with a rise in insulin levels at both 300 mg/min ( $P < 0.05$ ) and 100 mg/min ( $P < 0.05$ ).

Two hypotheses were considered to account for the variations in the acute insulin responses. First, a decrease in the sensitivity in the islet cell occurs when it is exposed to hyperglycemia prior to a subsequent acute glucose challenge. This hypothesis seemed to be at odds with physiologic and clinical experience since it has usually been observed that glucose injection induces a state of improved glucose tolerance (9). The only observation which had suggested a decrease in islet cell sensitivity to hyperglycemia was the decreased acute response to pulse 2 during the 300 mg/min infusion. However, several observations are incompatible with this hypothesis. First, the acute response to pulse 3 was normal and the total response increased, yet hyperglycemia was still present after 20 hr of glucose infusion. If the sensitivity of the islet were dependent solely on the glucose level, a decreased acute insulin response would have been observed during prolonged glucose infusion. Furthermore, the acute insulin response to pulse 4 was increased only after the 300 mg/min infusion despite similar glucose and insulin preinjection concentrations. If the level of blood sugar were the only important determinant of the sensitivity of the islet, one would have expected a similar response as was found after the 100 mg/min infusion. Finally, the similar in-

sulin responses to the 900 mg/min infusion, despite starting at two different glucose values, indicates that some types of insulin response are not diminished by hyperglycemia.

Therefore, we have considered a two pool model for insulin secretion to be a better explanation for the observations. One pool is conceived as a small compartment available for rapid insulin release. The other, a larger pool is conceived for sustained insulin release which is more tightly coupled to insulin synthesis. The 45 min 300 mg/min infusion can be considered analogous to multiple small injections of glucose; and therefore, during the early part of the glucose infusion, there is sufficient discharge of this acutely releasable pool so that there is less insulin available to respond to the second glucose pulse. The same phenomenon was observed in some subjects at 100 mg/min but many subjects apparently did not secrete enough insulin during 45 min of 100 mg/min to make a difference in the size or availability of this acutely releasable pool. Nevertheless, when pulse 2 was given during the 100 mg/min infusion, it was still observed that glucose levels preceding and during pulse 2 were 10 mg/100 ml higher than those during pulse 1 but the delta, or change in glucose concentration during these pulses, was constant. Therefore, the property of glucose which relates to the acutely releasable pool is probably the delta or change in glucose level rather than the absolute glucose concentration.

It was of great interest that when this acute pool was discharged by the 300 mg/min infusion, there was a markedly slowed glucose disappearance rate as measured by the glucose  $K_t$  following pulse 2. This would suggest that the acute pool plays an important role in determining the subsequent rate of glucose disappearance. Similar conclusions would be drawn from the observation that most subjects had a faster rate of glucose disappearance after pulse 4 than pulse 1 at which time the acutely releasable pool was increased by a factor of 1.5 to 2. The simplest explanation for the improved response to pulses 3 and 4 would be that continuous glucose infusion stimulates insulin synthesis thereby refilling the acutely releasable pool as in pulse 3 or actually increasing its size as observed in pulse 4.

The second slower responding pool has been inferred from the observations of a late and delayed rise in insulin levels after pulse 2 and the discrepancy between the rising insulin levels from 45 min to 20 hr of glucose infusion despite a fall in venous glucose concentrations during the same time period. These observations and the refilling of the acutely releasable pool suggest that insulin release from the slower pool is dependent upon glucose metabolism and is probably closely related to insulin synthesis. The pronounced broadening of the insulin response curve after pulse 3 contributed greatly

to the increased total insulin release observed. This tendency toward a broader insulin response during glucose infusions at both 300 mg/min or 100 mg/min indicates that this model may still be an oversimplification with still another pool of stored insulin to be described. Since broadening of the insulin response was only found when the glucose pulse was given during prolonged glucose infusions, release of this additional insulin may be dependent upon the absolute blood glucose concentration.

These observations taken together may explain why it has been so difficult to predict insulin levels in normal subjects after glucose challenges. It would appear that the release from the acute pool is dependent not only upon the glucose dose but is also dependent at least upon carbohydrate tolerance, the basal insulin level (10), and the size of the pool. Thus, in order to predict the insulin release after pulse 4, one would have to know not only basal glucose, basal insulin, carbohydrate tolerance, and the size of the pulse, but also the previous history of the islets exposure to glucose. Factors controlling the slowly responding pool are even less well defined.

Recent studies in the isolated perfused pancreas have shown insulin responses which are also compatible with a multicompartmental model for insulin secretion. In a system where insulin and glucose turnover do not have to be considered and a square wave or constant glucose input can be achieved, insulin release is biphasic with a small fraction released acutely followed by a later rising phase which is partly synthesis-dependent (11). Although these observations were initially considered to be consistent with feedback inhibition of insulin release by high local insulin concentrations (11, 12), more recent studies have indicated a two pool model to be more likely (13). The fact that acute release appeared to be independent of protein synthesis (11) may resolve some of the conflicts in the literature in which there has been controversy over the effects of stimulators or inhibitors of insulin secretion. For example, in the isolated islet system it has been reported that inhibition of glucose oxidation by dinitrophenol is a potent inhibitor of insulin secretion (3), whereas in the isolated perfused system, the acute release was independent of the administration of this drug (14). These and other conflicts may simply be related to the fact that different phases of insulin release were under study.

An analogue computer model to explain insulin release in man, in which pulse injection and glucose infusions were given simultaneously, contains factors which are similar to the pools suggested here (15). In those studies an immediate insulin response has been tentatively identified as separate from a later phase of insulin secretion. Although separate functional pools were not specifically part of the model, the pattern of insulin

release observed and the conclusions reached are similar to those presented in this paper. Attention was focused on the initial response as a concomitant of the diabetic syndrome (16), but there was considerable overlap between normal and abnormal subjects. Clearly, further investigation is required in order to quantitate the relationships between glucose administration and the stimulation of insulin release from these several pools. Whether or not abnormalities in one or another of these pools may better explain pathophysiologic disorders of carbohydrate metabolism remains to be explored. It is of interest that other hormones, particularly catecholamines (17) acetylcholine (18), and antidiuretic hormone (19) which are also stored as granules have been shown to exist in various functional pools within the cell of origin. It seems conceivable that most other cells which store hormones in a granular form will be found to have more than one functional pool for hormonal release.

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