

THE INTERACTION OF GLUCAGON AND INSULIN ON BLOOD GLUCOSE

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In previous publications from this laboratory (1, 2) evidence was presented which indicates that glucagon enhances the peripheral utilization of glucose¹ in normal men and dogs as well as in the depancreatized dog. The purpose of this paper is to report experiments on the combined effect of glucagon and insulin on arterial and venous glucose levels in normal men, and to compare the magnitude of this effect with that observed in subjects given similar amounts of each hormone separately.

METHODS

Fifty-six men (ages 21 to 47) considered normal with respect to carbohydrate metabolism and in good general health and nutritional state were studied. Controlled basal conditions were obtained in the following manner: (a) Subjects were hospitalized and placed on a high carbohydrate (360 gm.), high caloric diet (3500 calories) for at least three days before being studied; (b) a 14-hour fast preceded each study; (c) experiments were begun at 8 a.m. following a 30 to 60-minute period of bed rest in the test bed in a special room; (d) venous blood samples were obtained from an indwelling needle inserted (using local Procaine anesthesia) five minutes before the experiment was begun; (e) every effort was made to keep the subjects motionless, relaxed and comfortable during the experiments.

Ten per cent glucose was infused intravenously at a constant rate (250 to 370 mg. per min. in different subjects) throughout the test period by means of a Bowman constant infusion pump. The initial 30 to 60 minutes of the infusion period served as a control period, following which glucagon² and/or insulin² was added to the infusion bottle. The glucagon dosage was 0.7 to 1.0 mg. administered by constant intravenous infusion over periods

ranging from 60 to 100 minutes. Insulin dosage ranged from 0.054 to 0.128 units per min. by constant intravenous infusion for periods ranging from 65 to 110 minutes. Mean insulin dosage for the subjects given insulin alone was .076 units per minute, for the subjects given insulin plus glucagon it was .071 units per minute. The dosage of insulin was arrived at by trial and error; the criteria used were that the dose be sufficient to cause a definite drop in blood sugar, but not so large as to result in hypoglycemic values. Data in which arterial blood sugar fell below 65 mg. per cent were discarded. In the subjects who received both insulin and glucagon, the insulin infusion was begun 15 to 25 minutes before the glucagon in order to obtain the maximum effect of each hormone (on blood sugar level) at approximately the same time.

Venous and capillary (finger) blood specimens were drawn at five or ten-minute intervals, a total of 30 to 40 samples during each experiment. Venous samples were obtained from the antecubital vein which carries mixed blood from the deep and superficial tissue of the forearm and hand (3, 4). Capillary blood contains the same glucose concentration as arterial blood (5). Samples were analyzed for glucose content by the Nelson-Somogyi method (6). Sodium fluoride and immediate refrigeration were used to prevent glycolysis in the samples. Capillary samples were analyzed singly, venous samples in duplicate. In approximately 900 duplicate venous samples the maximum difference between any pair was 10 per cent, and the average was 2 per cent.

The A-V/A data were subjected to a statistical analysis by means of a non-parametric median test (7); i.e., using the medians as a cutting point. The data were placed in a 2 × 2 contingency table, and then subjected to a chi-square analysis.

RESULTS

The data on the 12 subjects who received insulin following an initial control period are given in Table I. The data from one of these experiments are shown in Figure 1. In 11 of the 12 subjects insulin resulted in a fall in arterial and venous blood glucose levels to or below fasting levels in spite of the constant glucose infusion.

¹ Glucose utilization is used to mean the disappearance of blood glucose.

² Generously supplied by Drs. O. K. Behrens and W. R. Kirtley of Eli Lilly Company. The glucagon (Lot 208-158B-214A) contains 50 per cent of the potency of the crystalline standard and an insulin content of 0.05 to 0.005 units per milligram. The insulin (Lot No. T-3206) was a preparation free of glucagon.

TABLE I
*Insulin cases **

Patient Wt. (Kg.)	Age	Glucose mg./ min.	Insulin units/ min.		Time in minutes													
					0	10	20	30	40	50	60	70	80	90	100	110	120	130
W. Y. 77.8	22	344	.128	A↑ V†	89 87	108 105	108 105	125 123	130 128	134 132	↑ 135	136 130	136 127	127 118	118 111	106 99	92 85	
E. J. 79	25	360	.088	A V	86 78	105 100	114 108	120 112	124 115	127 116	↑ 117	129 117	130 114	128 114	117 105	99 93	85 82	77 74
R. B. 81.6	29	360	.113	A V	97 92	109 104	120 115	129 122	134 127	138 131	↑ 133	140 127	135 116	122 116	106 104	90 91	76 78	
C. J. 70.7	26	370	.061	A V	87 82	102 98	116 109	126 117	134 123	140 127	↑ 130	144 132	146 133	146 133	141 132	135 132	127 125	122 117
R. G. 69.7	36	360	.082	A V	89 88	109 105	120 114	127 121	131 125	133 128	↑ 134	134 130	135 131	135 127	122 113	109 101	97 90	87 81
J. C. 70.5	32	295	.069	A V	93 91	123 113	138 126	149 136	↑ 150	139 138	118 113	98 92	82 80					
P. W. 67	21	270	.073	A V	100 95	117 109	128 118	134 123	↑ 134	129 111	120 94	108 85	97 80	88 75	79 71	72 68	70 66	
J. H. 61.3	21	270	.061	A V	96 92	111 105	122 115	130 122	↑ 134	134 122	132 110	126 101	118 92	108 84	100 76	94 69		
A. M. 88	27	250	.054	A V	92 83	100 92	106 98	110 102	↑ 110	109 99	106 94	101 88	97 83	93 78	88 74	83 69	77 65	
R. S. 71	32	300	.059	A V	94 93	117 110	133 124	142 134	↑ 146	146 139	146 139	146 139	146 136	142 130	136 123	128 112	118 98	108 82
W. H. 58.6	24	300	.061	A V	85 82	112 101	128 116	137 126	↑ 142	141 134	137 126	129 113	119 104	108 88	95 78	84 71	76 63	
K. W. 74	38	260	.069	A V	84 85	105 101	113 108	118 108	↑ 122	125 98	124 90	121 83	115 77	108 71	102 66	97 61	92 58	88 55

* Blood sugar values at 10-minute intervals taken from curves constructed from determinations on samples (30 to 40 per subject) drawn at staggered time intervals which varied in the different subjects.

† A—Capillary blood sugar (mg. per cent). V—Antecubital vein sugar (mg. per cent).

‡ Beginning of insulin infusion.

A-V/A values ³ with insulin were significantly increased over control values ($P = .01$).

The data on the 12 subjects who received a combined infusion of insulin and glucagon after an initial control period are shown in Table II and one of the experiments is plotted in Figure 2. In eight subjects of this group there was a marked arterial hyperglycemia (over 200 mg. per cent) when glucagon was added to the glucose-insulin infusion mixture; in three there was little or no increase in arterial blood sugar over control values. In 11 of the 12 subjects a marked increase in A-V glucose differences occurred (see Table II). The

³ A-V/A = arterio-venous glucose difference divided by arterial glucose concentration. This value has been shown to be proportional to the specific rate constant of peripheral glucose utilization under the special conditions of the experiments (2).

three subjects who showed little or no arterial hyperglycemia after the addition of glucagon were, nevertheless, among those who exhibited marked increases in A-V glucose differences.

A comparison of the data of the insulin plus glucagon group with the groups who received glucagon (previously reported [2]) or insulin alone revealed the following noteworthy differences: (a) the A-V/A values for the insulin plus glucagon group were significantly greater ($P = .001$) than those for either of the other two groups (Figure 3); (b) the arterial hyperglycemia of the insulin plus glucagon group was often greater than that of the glucagon group despite the fact that the mean glucose infusion rates ⁴ of the former

⁴ The mean glucose infusion rates (mg. per minute) for the three groups were: glucagon 321; insulin 312; and insulin plus glucagon 296.

were slightly lower than those of the latter, and (c) the fall in blood sugar below initial fasting levels so commonly observed in the insulin group did not occur ⁵ in the insulin plus glucagon group.

DISCUSSION

That insulin enhances the peripheral utilization of glucose is now widely accepted. The recent demonstration (2) that glucagon has a similar action which is independent of a secondary release of insulin has already been cited. The experiments reported here indicate that when glucagon and insulin are administered together peripheral glu-

⁵ The only exceptions are the last two venous values on subject T. H.

cose utilization is increased beyond that with either hormone alone. In addition, the opposing effect of the two on arterial blood sugar may result, at times, in a virtually unchanged level. The greater arterial hyperglycemia often observed with glucagon plus insulin as compared with glucagon alone may have resulted from higher liver glycogen levels induced by the insulin which was administered 15 to 25 minutes before the glucagon in the subjects who received both hormones.

These findings suggest that insulin and glucagon are capable of functioning jointly to enhance the peripheral utilization of glucose and at the same time, of counteracting the opposing effect of each other on the blood sugar. Judging from the variation in responses observed in different subjects

TABLE II
*Insulin + glucagon cases **

Patient Wt. (Kg.)	Age	Glucose mg./ min.	Insulin units/ min.		Time in minutes																
					0	10	20	30	40	50	60	70	80	90	100	110	120	130	140		
H. C. 71.8	33	370	.086	A† V†	86 83	103 98	114 109	121 116	126 121	130 124	133 127	↑ ↑	134 127	136 128	↑↑ ↑↑	158 141	164 137	163 133	151 129		
D. D.† 68.1	39	316	.111	A V		130 124	136 129	141 133	↑ ↑	145 136	138 126	127 106	↑↑ ↑↑	128 105	140 110	133 110	126 105	119 99	114 96		
M. R.† 66.4	27	333	.065	A V		149 132	150 134	153 136	↑ ↑	161 141	162 145	↑↑ ↑↑	175 138	200 153	212 159	209 155	189 147	174 137	165 126		
T. D. 68.4	37	265	.062	A V	106 100	128 118	143 132	150 140	↑ ↑	155 145	157 140	↑↑ ↑↑	184 142	208 145	220 142	222 138	217 136	208 138	197 145	185 138	172 110
J. G. 53.8	26	275	.084	A V	100 95	108 100	121 112	130 121	↑ ↑	135 127	140 132	↑↑ ↑↑	163 145	189 171	201 186	197 184	183 171	167 160	157 152	149 144	134 128
E. H. 73.4	37	270	.058	A V	93 91	110 104	121 113	126 118	↑ ↑	129 120	129 120	↑↑ ↑↑	136 123	163 141	172 143	172 137	159 128	145 119	132 110	122 103	113 100
A. O. 73.6	43	320	.064	A V	88 85	100 96	110 105	119 114	↑ ↑	125 119	135 124	↑↑ ↑↑	166 136	193 155	213 167	219 174	212 171	205 154	202 148	197 153	176 147
C. R. 71	23	310	.054	A V	82 82	101 101	115 109	124 113	↑ ↑	129 114	130 115	↑↑ ↑↑	161 110	198 136	213 140	201 137	192 122	184 116	177 121	170 120	163 116
R. R. 75	29	290	.062	A V	95 92	113 106	121 114	127 120	↑ ↑	131 124	135 128	↑↑ ↑↑	146 116	185 163	199 187	203 197	185 181	170 167	160 156	151 147	137 134
J. C. 80.5	34	290	.061	A V	90 90	108 101	121 112	129 121	↑ ↑	133 127	135 121	↑↑ ↑↑	163 133	189 145	202 150	197 151	190 150	184 149	180 147	177 147	176 151
T. H. 75.5	38	250	.065	A V	92 92	111 104	119 112	123 117	↑ ↑	126 119	128 121	↑↑ ↑↑	149 122	167 136	166 140	151 123	138 113	126 105	118 97	107 85	91 71
J. S. 79	38	300	.077	A V	98 92	122 113	141 128	154 137	↑ ↑	161 141	158 141	↑↑ ↑↑	179 154	213 170	227 174	230 173	229 173	224 180	212 184	199 185	198 183

* Blood sugar values at 10-minute intervals taken from curves constructed from determinations on samples (30 to 40 per subject) drawn at staggered time intervals which varied in the different subjects.

† Control period data not used for statistical analysis because a loading dose of glucose was used.

‡ A—Capillary blood sugar (mg. per cent). V—Antecubital vein sugar (mg. per cent).

↑ Beginning of insulin infusion. ↑↑ Beginning of glucagon infusion.

THE EFFECT OF INSULIN ON BLOOD GLUCOSE

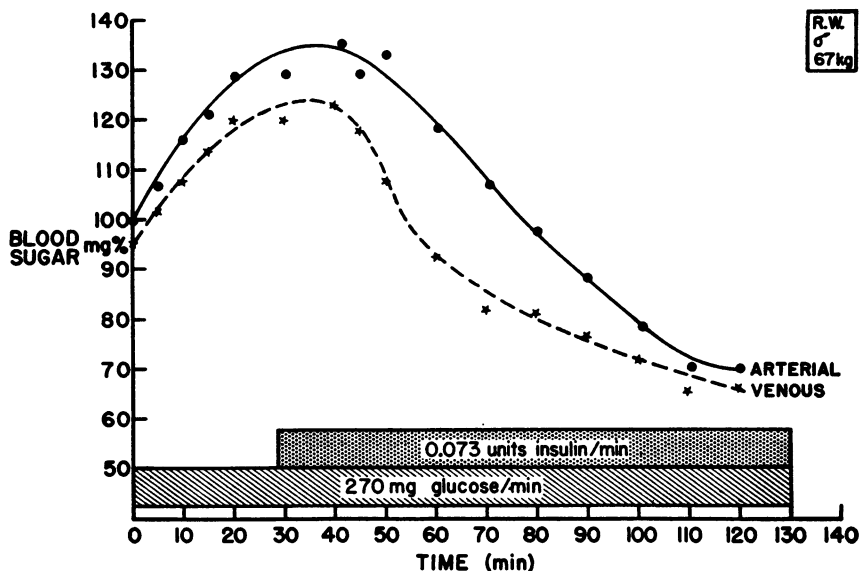


FIG. 1. THE EFFECT OF A CONTINUOUS INTRAVENOUS INFUSION OF INSULIN ON ARTERIAL AND VENOUS BLOOD SUGAR LEVELS IN A NORMAL MAN

THE EFFECT OF INSULIN PLUS GLUCAGON ON BLOOD GLUCOSE

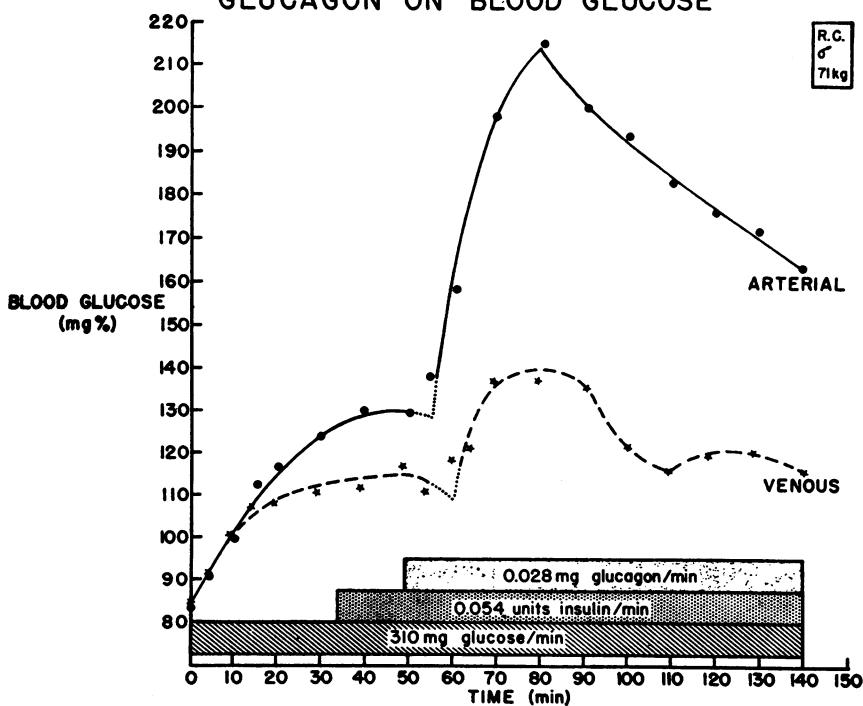


FIG. 2. THE EFFECT OF A CONTINUOUS INTRAVENOUS INFUSION OF GLUCAGON PLUS INSULIN ON ARTERIAL AND VENOUS BLOOD SUGAR LEVELS IN A NORMAL MAN

THE EFFECT OF GLUCAGON AND INSULIN ON PERIPHERAL UTILIZATION OF GLUCOSE

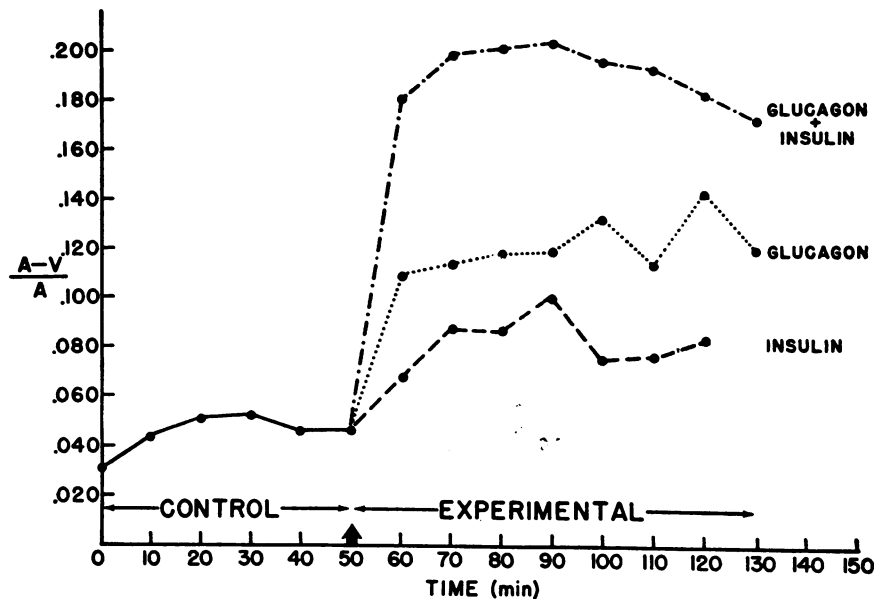


FIG. 3. A SUMMARY OF CONTINUOUS INTRAVENOUS INFUSION EXPERIMENTS IN NORMAL MAN SHOWING THE DIFFERENCE BETWEEN THE EFFECT OF INSULIN, GLUCAGON, AND GLUCAGON PLUS INSULIN ON AN INDEX OF THE PERIPHERAL UTILIZATION OF GLUCOSE ($A-V/A$)

Each point of the control period represents the median value of 54 subjects, whereas each point of the three experimental groups represents the median value of 12 subjects. The data on the glucagon subjects were taken from a previous publication (2).

receiving similar doses, there is considerable individual difference in responsiveness to the two hormones. No correlation of response with body weight was observed.

The fact that glucagon and insulin have opposing actions on blood glucose and liver glycogen levels has led many to postulate that they are antagonists (8, 9). Another concept of glucagon's role has been advanced by Bürger (10) and by Pincus and Rutman (11). These workers suggest that glucagon acts as an adjunct to insulin because it mobilizes liver glycogen for the enhancing action of insulin on glucose utilization in the peripheral tissues.

It has been reported that glucagon inhibits the action of insulin on glucose uptake by the isolated rat diaphragm (12). This was a preliminary report on work done before purified preparations of glucagon were available. Smith in F. G. Young's laboratory was unable to confirm these results (13). Ingle, Nezamis, and Humphrey observed

no effect of glucagon on the disposal of administered glucose in the eviscerated rat (14). Drury, Wick, and Sherill reported that in the eviscerated rabbit glucagon had no effect on glucose oxidation but had a slight inhibiting effect on the disappearance of blood glucose (15). The apparent discrepancy between some of these findings and those reported here might be explained by the studies of Lang, Goldstein, and Levine (16). These workers have presented evidence suggesting that the liver contains a "humoral agent" which enhances peripheral glucose utilization even in the presence of maximal insulin dosage. It is conceivable that glucagon causes the release of this liver factor and that this accounts for the difference between the results in the normal human as compared with the eviscerated animal and the isolated rat diaphragm. In keeping with this hypothesis is the observation that glucagon has no effect on blood sugar levels or arterio-venous glucose differences in the hepatectomized dog (17).

SUMMARY

Studies on the effect of glucagon and insulin on the disappearance of blood glucose in 56 normal men using a constant glucose infusion technique are reported. The results indicate that the two hormones administered together (a) cause a significantly greater increase in peripheral glucose utilization than either one alone, and (b) may result in a neutralization of the opposing action of each other on arterial blood sugar levels. These findings suggest that glucagon and insulin may function jointly to regulate the level of blood glucose and the utilization of carbohydrate by peripheral tissues.

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