THE ENHANCEMENT OF PERIPHERAL GLUCOSE UTILIZATION BY GLUCAGON

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During the past 30 years evidence has been accumulating which suggests that glucagon (the hyperglycemic-glycogenolytic factor of the pancreas) is a true hormone and that it is produced in the Islets of Langerhans (1, 2). Its purification and crystallization were finally accomplished in 1953 by Staub, Sinn, and Behrens (3). The crystalline material is a simple protein with a molecular weight of approximately 4,200 and an amino acid composition which differs in important ways from that of insulin (4). This establishes glucagon as an entity distinct from insulin.

Crystalline glucagon is an extremely active substance. In the cat, 0.05 γ per Kg. body weight causes a significant rise in blood sugar which lasts 25 minutes (4). Sutherland, Cori, Wosilait, and Rall (5, 6) have shown that glucagon stimulates the synthesis of active phosphorylase in the liver. This enzyme catalyzes the rate-limiting reaction (glycogen $\rightleftharpoons$ glucose-1-phosphate) in the transformation of glycogen to glucose. The effect of glucagon on liver phosphorylase adequately explains its well-known hyperglycemic-glycogenolytic action.

With respect to its role in carbohydrate metabolism, some investigators look upon glucagon as an insulin antagonist (1, 2, 7) while others consider it an insulin synergist (8, 9). Still others are skeptical of its hormonal nature (10, 11). In the present paper experiments are reported which indicate that glucagon, in addition to mobilizing liver glycogen, increases the peripheral utilization of glucose.¹

METHODS

Human experiments. Thirty-two male subjects (ages 21 to 47) considered normal with respect to carbohydrate metabolism were studied. All were hospital patients on high caloric and carbohydrate intakes for at least three

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

days before being studied. The test procedure was begun in the morning after a 14-hour fast. Soon after awakening the subjects were placed at bed rest for 30 to 60 minutes in the testing room before the experiment was begun. A 10 per cent solution of glucose was infused intravenously at a constant rate (250 to 370 mg. per min. in different subjects) by means of a constant infusion pump (Bowman) for periods of 120 to 145 minutes. In 12 subjects glucagon² (0.7 to 1.0 mg.) was added to the perfusate following an initial period of 40 to 60 minutes with glucose alone. In 12 subjects the glucose infusion rate was doubled after 50 minutes and after 90 minutes the initial rate was resumed in order to simulate the hyperglycemia caused by glucagon. Two subjects received glucose at a constant rate for 2 hours. The initial control data on 8 other subjects who subsequently received other hormones were also used in the analysis of data.

Glucose determinations (Nelson-Somogyi Method) (12) were done on venous (indwelling needle in ante-cubital vein) and capillary (finger) blood from the same arm at 5 or 10-minute intervals, using 0.2-ml. samples (30 to 40 samples from each subject). Venous samples were drawn without stasis and analyzed in duplicate. (The average difference between duplicate determinations was 2 per cent.) The antecubital vein carries venous drainage from the deep and superficial tissues of the forearm (13-15), while capillary blood has the same glucose content as arterial blood (16-18).

Sodium space was determined in 8 subjects receiving glucagon and 12 subjects receiving glucose alone using radiosodium (Na^{24}) which was added to the infusion fluid. Plasma samples (drawn along with samples for glucose determination) were assayed for radioactivity in a R-C Scientific scintillation well type counter. The method used to calculate sodium space by this technique will be described elsewhere (19).

Dog experiments. The effect of glucagon on arterio-venous glucose differences in the dog was also studied. Six experiments were done in five normal dogs, and nine experiments in three depancreatized dogs. The normal animals were studied under Pentobarbital Sodium anes-

² Preparations No. 208-158B-214 and No. 208-158B-214A kindly supplied by Drs. O. K. Behrens and W. R. Kirtley of the Eli Lilly Company. These preparations have a glucagon potency of 50 per cent of the crystalline standard and an insulin content of between 0.05 to 0.005 unit per mg.

thetia, after a fast of 16 hours. The depancreatized dogs were tested (under anesthesia) 4 to 16 hours after their last dose of regular insulin and food. Glucagon in doses of 0.072 to 1.5 mg. was administered intravenously, either as a single injection or by continuous infusion. Blood samples were drawn without stasis from indwelling needles in the femoral artery and vein. Glucose determinations were done on 1-ml. (normal dogs) or 0.5-ml. (depancreatized dogs) samples in duplicate.

RESULTS

Human experiments

Tables I, II, and III contain the arteriovenous glucose data from the 32 subjects. Two types of response to glucagon were observed. In 11 subjects, blood sugar fell after reaching a peak level, despite the constant infusion of glucagon

(Figure 1-A). In one subject the peak glucose levels persisted throughout the test period. In all subjects glucagon, in addition to producing a sharp rise in blood sugar levels resulted in a marked increase in arteriovenous (A-V) glucose differences. In the 12 subjects in whom the glucose infusion was doubled following the control period, the arterial glucose concentrations closely simulated those observed in the subjects receiving glucagon. However, the A-V glucose differences were much less (Figure 1-B). The A-V/A^{*} values of the 12 glucagon periods were compared statistically with those of the 32 control glucose

^{*} A-V/A = arteriovenous glucose difference/arterial glucose concentration. This serves as an index of the peripheral utilization constant of glucose (see appendix for derivation).

TABLE I
Glucagon cases *

			Time in minutes															
Patient	Age		0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	
B. D.	30	A†	86	98	107	112	115	117	118	↑	137	172	187	178	162			
		V†	79	88	95	100	102	105	106	↑	107	144	150	146	133			
W. D.	26	A	85	104	118	129	137	142	↑	147	173	197	200	195	188			
		V	85	101	111	118	124	127	↑	129	152	167	174	164	130			
K. R.	21	A	78	101	111	118	124	129	↑	142	167	183	183	177	169	165		
		V	77	93	103	110	116	121	↑	125	146	158	160	157	149	137		
C. C.	46	A	90	97	102	107	110	↑	114	136	151	160	167	171	175	177	176	
		V	89	95	100	105	108	↑	112	125	139	147	155	159	164	167	165	
R. L.	24	A	80	89	95	99	↑	101	150	184	207	222	230	211	193	177	164	
		V	77	86	92	96	↑	98	135	170	195	207	212	192	175	158	134	
J. D.	36	A	85	96	103	107	109	↑	126	153	169	176	172	156	144	133	122	112
		V	82	94	100	102	103	↑	111	139	157	158	150	142	133	122	113	106
R. B.	47	A	87	98	106	113	118	↑	127	147	164	172	165	161	157	153	148	143
		V	82	92	98	94	106	↑	108	125	139	141	135	120	118	116	114	114
R. R.	29	A	99	109	116	120	125	↑	141	167	190	198	203	207	219	187	178	173
		V	97	106	113	117	122	↑	133	154	174	189	194	192	188	149	143	139
F. R.	34	A	80	93	99	101	102	↑	119	155	179	194	189					
		V	80	91	95	96	96	↑	105	137	161	177	174					
G. N.	35	A	110	130	142	149	155	↑	158	164	209	231	243	228	213	202	198	
		V	105	123	136	144	150	↑	153	157	194	214	215	196	189	183	182	
R. T.	30	A	91	105	113	119	122	↑	138	168	186	191	178	161	145	131	125	122
		V	87	102	108	112	114	↑	124	148	159	163	149	136	123	112	104	99
G. W.	32	A	78	89	99	108	114	↑	131	166	198	214	217	206	184	170	161	156
		V	76	87	97	106	112	↑	117	147	167	175	178	172	157	144	135	131

* 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 glucagon infusion.

TABLE II
*Glucose hyperglycemia cases **

			Time in minutes															
Patient	Age		0	10	20	30	40	50	60	70	80	90	100	110	120	130		
G. N.	35	A† V†	96 92	112 104	123 114	131 122	137 128	142 133	↑ 151	166 168	183 182	197 182	208 194	↓ 196	199 190	193 187	187 185	
H. C.	33	A V	82 79	94 89	101 96	106 101	109 104	↑ 127	132 138	144 144	152 148	157 148	↓ 141	144 134	137 128	131		
R. W.	27	A V	84 79	96 92	104 101	109 107	115 113	↑ 114	126 128	142 137	151 144	157 144	↓ 148	160 124	136 103	121 95	111	
F. R.	34	A V	87 85	100 96	109 105	116 111	121 116	125 121	↑ 133	144 144	157 155	168 165	↓ 165	177 159	167 153	159 148	153 143	148 143
H. R.	42	A V	92 88	105 101	114 112	121 119	126 125	130 129	↑ 144	151 159	165 170	174 170	↓ 179	181 170	173 162	166 156	160 153	156 153
A. S.	22	A V	86 85	100 98	108 107	114 113	118 117	122 121	↑ 138	142 152	156 152	167 163	↓ 166	176 155	165 145	154 145	144 136	134 129
B. M.	47	A V	93 89	106 103	115 111	121 117	126 121	130 124	↑ 140	156 154	172 162	183 162	↓ 170	193 162	181 162	171 151	162 141	
T. B.	35	A V	84 81	100 95	109 104	113 107	115 107	116 108	↑ 122	135 137	152 149	165 149	↓ 160	173 143	157 127	143 127	133 114	127 108
C. H.	35	A V	81 81	108 104	121 116	129 125	135 131	139 135	↑ 164	169 183	186 183	200 197	↓ 209	212 200	202 192	194 186	187 186	181 180
V. F.	37	A V	81 78	95 90	105 98	112 102	117 106	121 108	↑ 118	145 140	159 153	168 153	↓ 161	174 150	164 141	155 141	146 135	138 133
E. A.	27	A V	84 83	98 92	109 99	116 104	121 108	124 110	↑ 128	143 139	154 147	164 147	↓ 154	172 133	150 119	135 112	127 112	122 109
V. B.	31	A V	89 87	112 107	123 118	130 126	135 131	139 134	↑ 157	165 171	180 171	192 183	↓ 193	202 178	184 167	171 161	164 161	160 157

* 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 increased rate of glucose infusion. ↓—Resumption of initial speed of glucose infusion.

TABLE III
*Miscellaneous control cases **

Patient	Age		Time in minutes												
			0	10	20	30	40	50	60	70	80	90	100	110	120
C. F.	45	A†	102	121	135	145	154	161	167	171	174	176	178	179	180
		V†	100	118	132	142	151	158	164	168	170	172	173	174	175
P. H.	30	A	95	112	124	133	140	146	150	154	156	159	162	164	
		V	88	104	115	125	132	139	143	147	149	151	154	156	
G. M.	22	A	86	110	128	141	150	157	161						
		V	83	107	124	136	145	152	156						
H. C.	33	A	86	103	114	121	126	130	133						
		V	83	98	109	116	121	124	127						
D. Z.	24	A	87	104	116	125	131	135	137						
		V	85	100	110	117	123	127	131						
R. G.	36	A	87	109	121	127	131	133							
		V	85	104	115	121	125	128							
R. B.	29	A	97	109	120	129	134	138							
		V	92	104	114	122	127	131							
C. J.	26	A	87	102	116	126	134	139							
		V	82	97	109	117	123	127							

* These subjects received glucose infusion alone at one constant rate. Data treated as in Tables I and II.

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

FIG. 1. COMPARISON BETWEEN THE EFFECTS OF (A) GLUCAGON AND (B) HYPERGLYCEMIA DUE TO GLUCOSE ON ARTERIAL AND VENOUS GLUCOSE CONCENTRATIONS IN MAN

periods. The data were subjected to a median test employing a 2×2 contingency table (20). The resulting χ^2 values showed that the increase in A-V/A values during the glucagon periods was highly significant ($P = .001$). A similar statistical analysis was applied to the differences between the control and experimental periods for both the glucagon and glucose hyperglycemia cases. This revealed that glucagon caused a significantly greater ($P = .001$) increase in A-V/A than did glucose hyperglycemia. A comparison between the three groups of experimental periods is shown in Figure 2. These experiments demonstrate that the increased A-V glucose differences observed with glucagon cannot be attributed to

the increase in blood glucose levels *per se*. Further, it should be noted that the increased A-V/A values caused by glucagon persist throughout the test period despite the fall of blood sugar toward pre-glucagon levels in 11 of the 12 subjects.

Sodium space did not change following glucagon administration or when the rate of the glucose infusion was doubled. The mean value for the 20 subjects tested was 21.35 ± 3.35 per cent of body weight.

Dog experiments

Tables IV and V contain the data from the animal experiments. Glucagon causes a marked

THE EFFECT OF GLUCAGON ON THE PERIPHERAL UTILIZATION OF GLUCOSE

FIG. 2. A SUMMARY OF CONTINUOUS INFUSION EXPERIMENTS IN MAN SHOWING THE DIFFERENCE BETWEEN THE EFFECT OF GLUCAGON AND OF GLUCOSE HYPERGLYCEMIA ON THE PERIPHERAL UTILIZATION OF GLUCOSE (A-V/A)

Each point of the control period represents the median value of 32 cases, whereas each point of the other two groups represents the median of 12 cases.

increase in A-V glucose difference in the normal dog when given as a single injection or by continuous infusion (Figure 3). Similar results were observed in the depancreatized animal (Figure 4). As in the human, the increased A-V glucose differences persist after the disappearance of the hyperglycemia.

DISCUSSION

Previous work from this laboratory, using a single glucose injection technique, suggested that glucagon causes a marked increase in A-V glucose differences in man (21). Recently, Van Itallie, Morgan, and Dotti (22) have reported experiments which support this finding. Both of these studies depended on the assumption that blood flow and A-V glucose differences remain constant from day to day in a given individual. Actually, blood flow and A-V glucose differences may vary considerably in a given individual from day to day (23, 24). The present studies were designed to minimize these variables. Thus, both the control and hormone test periods were run in sequence within a 2½-hour period under stand-

ardized basal conditions. Under these experimental conditions, the variations in A-V/A values observed during the control periods were not significant.⁴ This is good evidence that blood flow was essentially constant. Glucagon itself (unlike adrenalin) has no cardiovascular effects (1, 2). Thus the A-V glucose differences observed in the present experiments may be considered proportional to glucose utilization in the forearm and hand.

The relationship of glucose utilization to the height of the arterial blood sugar is pertinent to this study, since glucagon causes a marked rise in arterial glucose concentration. The glucose infusion experiments indicate that peripheral utilization of glucose is proportional to arterial glucose levels in the range between 80 and 210 mg. per cent in agreement with previous work (16-18). Glucagon results in significantly greater glucose utilization at comparably elevated glucose levels.

⁴ A Rank Sign Test (25) of the significance of the variation in successive 10-minute time periods revealed no significant variation beyond the first 10-min. interval. This appears obvious from Figure 2.

TABLE IV
Effect of glucagon on blood sugar * of normal dogs

Dog number		Time in minutes															
		0	5	10	15	20	25	30	35	40	45	50	60	70	80	90	100
St 564	A	81	82	82	84	↑ 116	146	160	167	159	146	134	112	97	92		
	V	80	80	81	82	↑ 107	125	140	148	146	133	122	101	88	83		
C 580	A	87	88	89		89	↑	102		142	148	143	129	115	105	96	
	V	85	89	87		88	↑	95		120	138	132	116	99	82	79	
S 435b	A	115	108		109	↑ 146	171		195		188						
	V	103	103		103	↑ 129	151		172		162						
C 563	A	102	102	101		101		102	↑	146		190	202	187	171	157	144
	V	101	100	100		100		100	↑	125		165	164	156	148	138	128
S 529†	A	92	86	85	88	↑ 124	145	157	165	163	156	149					
	V	85	83	85	86	↑ 102	124	132	139	139	137	134					
S 529§	A	88	91	88	89	↑ 115	125	128	126	123	120	115					
	V	82	86	83	86	↑ 93	100	104	107	110	111	110					

* Blood sugar values taken from curves which were constructed from values taken at staggered time intervals which varied in the different animals, and are expressed in mg. per cent. ↑—Beginning of glucagon infusion.

† S—Single injection of glucagon, C—Continuous infusion of glucagon.

‡ 48-hr. fast.

§ 86-hr. fast.

|| A—Femoral artery, V—Femoral vein.

TABLE V
Effect of glucagon on blood sugar * of depancreatized dogs

Dog number		Time in minutes															
		0	5	10	15	20	25	30	35	40	45	50	60	70	80	90	100
St 560†	A¶	362	352	351	357	368	↑ 410	441	454	463	470	473	473	453	433	417	404
	V¶	356	356	351	353	357	↑ 386	413	430	440	447	453	454	432	411	396	384
S 435a‡	A	270	272	274	275	277	↑ 316	353	379	394	404	410	418	419	413		
	V	272	279	280	281	282	↑ 288	305	322	337	350	362	381	393	399		
C 435a‡	A	439		431		428	427	427	425	↑ 473	499	519	565	600	584	560	544
	V	423		416		410	412	415	423	↑ 431	450	473	506	526	529	525	518
S 560†	A	310		316		322		328	↑ 345	374	390	400	414	420	309	412	400
	V	302		306		309		311	↑ 314	338	355	365	383	390	393	392	388
C 543†	A	235	230	233	234	230	↑	286	310	323	332	337	334	329	323	316	309
	V	232	233	234	234	230	↑	252	283	304	315	324	324	322	317	310	303
C 560§	A	75		79		82	85	↑ 128	175	199	215	226	239	248	253	257	260
	V	72		74		76	82	↑ 96	129	157	174	185	203	215	225	231	234
S 435a	A	62	60	56	58	60	↑ 128	200	255	280	300	317	338				
	V	60	58	53	51	48	↑ 95	147	190	217	229	241	257				
S 435a	A	87	90	89	89	↑ 119	139	152	162	168	173	178	184				
	V	80	72	78	84	↑ 99	112	124	131	142	148	152	157				
S 435a	A	69	78	89	73	↑ 114	143	158	167	177	184	189	194				
	V	69	72	75	68	↑ 94	113	127	136	143	149	153	157				

* Blood sugar values taken from curves which were constructed from values taken at staggered time intervals which varied in the different animals and are expressed in mg. per cent. ↑—Beginning of glucagon infusion.

† S—Single injection of glucagon, C—Continuous infusion of glucagon.

‡ Experiment started 16 hours after last food and insulin (regular).

§ Experiment started 11 hours after last food and insulin (regular).

|| Experiment started 4 hours after last food and insulin (regular).

¶ A—Femoral artery, V—Femoral vein.

THE EFFECT OF GLUCAGON IN THE NORMAL DOG

FIG. 3. THE EFFECT OF A SINGLE I.V. INJECTION OF GLUCAGON AND OF CONTINUOUS I.V. GLUCAGON INFUSION (IN NORMAL SALINE) ON (FEMORAL) ARTERIAL AND VENOUS GLUCOSE LEVELS IN THE NORMAL DOG

These observations are of considerable importance from the standpoint of glucagon's potential role in the regulation of carbohydrate metabolism. They indicate that glucagon has two integrated actions; namely, the mobilization of liver glycogen and the enhancement of the peripheral utilization of glucose. The persistence of the peripheral effect beyond the disappearance of the hyperglycemia suggests that glucagon has a more prolonged action than hitherto believed. The occurrence of these two effects of glucagon in preliminary experiments in the depancreatized dog indicates that the effects are independent of insulin action.

There is some experimental evidence which suggests that pituitary growth hormone causes the release of glucagon and that part of the diabetogenic action of the former is due to the released glucagon (1, 2). This hypothesis is not supported by the present experiments which show that glucagon increases the peripheral utilization of glucose; whereas there is abundant evidence that growth hormone decreases carbohydrate utilization (26).

The lack of change in Na^{22} space with glucagon

THE EFFECT OF GLUCAGON IN A DEPANCREATIZED DOG

FIG. 4. THE EFFECT OF A SINGLE I.V. INJECTION OF GLUCAGON ON (FEMORAL) ARTERIAL AND VENOUS GLUCOSE LEVELS IN THE DEPANCREATIZED DOG

Experiment was done 16 hours after the last dose of insulin and food.

or glucose infusions at two different infusion rates indicates that no significant alteration in extracellular space has taken place during the experiments.

SUMMARY AND CONCLUSIONS

Studies on the effect of glucagon on blood glucose utilization in normal men using a constant glucose infusion technique have been presented. They show that glucagon causes a highly significant increase in the peripheral utilization of glucose which persists after the disappearance of the hyperglycemia. This increased glucose utilization is significantly greater than that observed with the hyperglycemia produced by glucose alone. The findings suggest that glucagon may fulfil a dual role in carbohydrate metabolism by producing hyperglycemia through mobilization of liver glycogen and concomitantly increasing the peripheral utilization of blood glucose. They also indicate that glucagon has a more prolonged action than previously thought.

Preliminary studies in normal and depancrea-tized dogs are also reported. Both groups of animals respond to glucagon in the same way as the normal human subject. These experiments indicate that the enhancing action of glucagon on the peripheral utilization of glucose does not depend upon a release of insulin.

APPENDIX

In the normal dog (18) it has been shown that the peripheral utilization of glucose (P) is a linear function of arterial glucose concentration (A). Therefore:

$$P \propto A$$

or

$$P = kA, \quad (1)$$

where k is the proportionality constant. At any time t:

$$P = R(A - V), \quad (2)$$

where R is the blood flow and V is the venous blood glucose concentration. If (1) and (2) are equated, it may be shown that:

$$\frac{A - V}{A} = K \text{ (constant)}, \quad (3)$$

where K is a function of the specific rate constant of peripheral glucose utilization, the volume of glucose distribution and peripheral blood flow. If blood flow and glucose distribution remain constant, then $A - V/A$ must remain constant unless the rate constant of peripheral glucose utilization changes.

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