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The Effect of Glucagon on Net Splanchnic Balances of Glucose, Amino Acid Nitrogen, Urea, Ketones, and Oxygen in Man *

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The demonstration by Unger, Eisentraut, McCall, and Madison (1) that glucagon is present in the pancreatic vein of dogs and that its concentration varies in response to changes in blood sugar suggests that glucagon is a hormone with a significant role in blood glucose regulation.

The intravenous administration of glucagon characteristically produces a sharp rise in the systemic blood sugar. For some time the liver has been known to play a primary role in the mediation of this effect (2, 3). More recently, Shoemaker, Van Itallie, and Walker (4, 5) demonstrated a marked increase in hepatic glucose output in dogs after the administration of glucagon. Similar studies have not been made in man, since direct sampling from the portal vein is not feasible in human subjects. The enhanced glucose output from the liver induced by glucagon is presumably due to an augmentation of hepatic glycogenolysis. An increased glycogenolysis in liver slices exposed to glucagon has been observed *in vitro* (6). In the intact dog, Cahill, Zottu, and Earle (7) found that a 4-hour infusion of glucagon severely depleted the trichloroacetic acid-soluble glycogen content of the liver. The demonstration by Sutherland and Cori (8) that glucagon activates liver phosphorylase elucidates the mechanism of this effect.

A number of studies have shown that glucagon also increases gluconeogenesis (9–16). Kalant (9) observed in fasted rats that after the initial depletion of hepatic glycogen from a single injection of glucagon, the incorporation of C^{14}-labeled glycine into glycogen was enhanced. With repeated injections of glucagon, less C^{14} went into glycogen, and increased amounts of labeled CO_2 appeared, suggesting an increased rate of oxidation. Kalant suggested that these findings can be interpreted either as a mass action effect resulting from the accelerated glycogenolysis or as a primary effect of glucagon on intermediary metabolism at some point other than at the level of the phosphorylase enzyme. The latter alternative is supported by the work of Best and associates and Izzo and Glasser, who found in both the rat (10, 15, 16) and in man (13, 14) a gluconeogenic effect of glucagon that appeared to be independent of the effect on glycogen metabolism.

Reports of the effect of glucagon on ketone metabolism and peripheral glucose uptake have been conflicting. Ketonemia in animals and ketone production by rat liver slices have been reported to be depressed (17), enhanced (18–20), and unchanged (21, 22). Similar discrepancies have been reported in man. Bondy and Cardillo (23) found no change in arterial blood ketone levels in man during a 2-hour infusion of glucagon. Ezrin, Salter, Ogryzlo, and Best (13), on the other hand, found ketosis in six patients receiving long-term iv glucagon therapy, an effect seemingly independent of the level of blood sugar and occurring before the appearance of glycosuria. In fact, one patient developed ketosis without a rise in blood sugar. Salter, Ezrin, Laidlaw, and Gornall (24) reported similar results in human subjects on long-term glucagon administration. Drury, Wick, and Sherrill (25) found that glucagon decreased the uptake of a constant infusion of glucose in the eviscerated and nephrectomized rabbit. Van Itallie, Morgan, and Dotti (26) and

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Bondy and Cardillo (23), on the other hand, have reported that the arteriovenous glucose differences across the arm in human subjects during glucagon administration were similar to the differences encountered with a comparable degree of hyperglycemia induced by glucose administration. Similar studies by Elrick, Hlad, and Witten (27) have shown an enhancement of peripheral glucose uptake by glucagon.

Further delineation of the physiologic and metabolic effects of glucagon is, therefore, apparently desirable. In our study, the effects of a single injection and a continuous infusion of glucagon on net splanchnic glucose production in man were determined. In man, net splanchnic glucose production closely approximates true hepatic glucose production in the fasting state (28). Concurrently, hepatic blood flow, Bromsulphalein clearance, and splanchnic oxygen consumption were measured. In some subjects splanchnic balances of urea, amino acids, and ketones were determined, and in others the arteriovenous glucose differences across the leg and brain were measured.

Methods

The glucagon used in these studies came from three different lots of crude amorphous material, Lilly lot numbers 208-158B-288, 11049, and 208-158B-197.[1] Each lot had been cysteine-treated to destroy insulin, and the manufacturer's assay of lot 208-158B-197 showed approximately 50% glucagon. The relative potency of the lots was determined by comparing the effect of a given dose on blood glucose or net splanchnic glucose production (NSGP), utilizing the patients reported in this study, as well as other human subjects, for this purpose. We found, for example, that 5 mg of lot 208-158B-197 produces the same rise in blood glucose or NSGP as 3 mg of lot 208-158B-288. Similarly, 10 mg of lot 208-158B-197 produces changes in these parameters equivalent to 6 mg of lot 208-158B-288. Therefore, we expressed all dose levels in terms of the amount of lot no. 208-158B-288 producing an equivalent response. Since the same subjects were not used for many of these comparative studies, however, dose levels must be considered rough approximations only.

The subjects of these experiments were adult patients without metabolic or hepatic disorders. They were adequately nourished and had been eating well of the routine hospital diet. They were fasted 6 to 14 hours before study, and in some instances 90 mg phenobarbital was given orally 1 hour before the experiment. Catheterization of the hepatic vein was accomplished under fluoroscopic guidance, and a Cournand-type needle was inserted into the femoral artery. After a stabilization period of 10 to 15 minutes, paired, simultaneous blood samples were collected at 5- to 10-minute intervals from the femoral artery and hepatic vein. Glucose, Bromsulphalein (BSP), oxygen, and, in certain instances, amino acid nitrogen, urea, and ketones were determined. A weighed amount of glucagon, dissolved in 2 ml of physiological saline and acidified with acetic acid, was then either administered as a single, rapid iv injection or further diluted in normal saline and infused through a calibrated Murphy drip at a constant rate for 1 to 2 hours. The injection or infusion was accomplished without further distress to the patient by utilizing a Cournand-type needle that had been inserted in the antecubital vein during the initial part of the study. Blood samples were again collected for the appropriate determinations at 5- to 10-minute intervals after the single injection of glucagon or during the constant infusion of glucagon.

In five subjects a Cournand-type needle was also inserted into the femoral vein of the opposite leg. Samples were drawn from the two leg needles as simultaneously as possible[2] every 10 minutes during a constant 1-hour infusion of glucagon. In four other subjects blood samples were obtained from the femoral artery and jugular bulb. In these latter subjects the hepatic vein was not catheterized.

Hepatic blood flow (HBF), by the technique of hepatic venous catheterization and the BSP method (28, 29), was determined in six subjects before and after administration of glucagon. The results obtained in these subjects (Table I) showed that glucagon has no effect on HBF or splanchnic oxygen consumption. Therefore, in the remaining subjects HBF was determined only during the control period. Hepatic arteriovenous (a-v) oxygen differences, however, were measured in both the control and experimental periods. Since HBF and hepatic a-v oxygen differences have a reciprocal relationship when splanchnic oxygen consumption is constant, that these differences did not change significantly (Table III) supports the assumption that HBF was stable throughout in these subjects. Net splanchnic balances of oxygen, glucose, urea, amino acid nitrogen, and ketones were estimated from the product of their respective hepatic venous-arterial (hv-a) difference and the HBF.

Blood oxygen was determined by the spectrophotometric method of Hickam and Frayser (30), blood glucose by the method of Somogyi as modified by Nelson (31), blood urea by the colorimetric method described by Archibald (32), blood amino acid nitrogen by the colorimetric method of Frame, Russell, and Wilhelmi (33), and blood ketones by the method of Werk and associates (34).

[1] Eli Lilly, Indianapolis, Ind.

[2] Blood was withdrawn from the arterial needle first and immediately thereafter from the venous needle.

TABLE I

The effect of a single rapid iv injection of glucagon on splanchnic circulation in six subjects, with comparative data for epinephrine

	Hepatic blood flow		Splanchnic oxygen consumption		Splanchnic glucose production	
	Glucagon*	Epinephrine†	Glucagon	Epinephrine	Glucagon	Epinephrine
	ml/min/m²		*ml/min/m²*		*mg/min/m²*	
Control	753±34‡	820±80	34±2.9	40±6	48±5.1	51±7
After	723±47	1,345±136	35±3.0	65±8	175±9.9	150±19
% Change	−0.5	+64§	+3	+60§	+260§	+194§

* Six patients: 3 received 3 mg and 3 received 6 mg.
† Ten patients: 0.05 ml of a 1:1,000 dilution intramuscularly.
‡ Standard errors.
§ $p < 0.01$.

Results

Figure 1 shows the effect of a single iv injection of glucagon on the arterial and hepatic venous blood glucose levels. In six subjects (group A), 3 mg of glucagon produced a mean maximal rise in arterial blood glucose of 21 mg per 100 ml, 10 minutes after injection; in six other control subjects (group B), 6 mg gave a mean maximal rise of 36 mg per 100 ml at 15 minutes. The elevation of hepatic venous blood glucose in each group was even more marked and resulted in a striking increase in the hv-a glucose differences (Figure 1, shaded area). In group A the mean, control hv-a difference of 6 ± 1 [3] mg per 100 ml rose at 2½ minutes to a mean, maximal value of 23 ± 1 mg per 100 ml. Comparable values in group B were 7 ± 1 and 28 ± 4 mg per 100 ml at 5 minutes. The p values for these changes were less than 0.01. Since glucagon did not alter HBF (Table I; see also Methods), these increases in hv-a glucose difference indicate fourfold increases

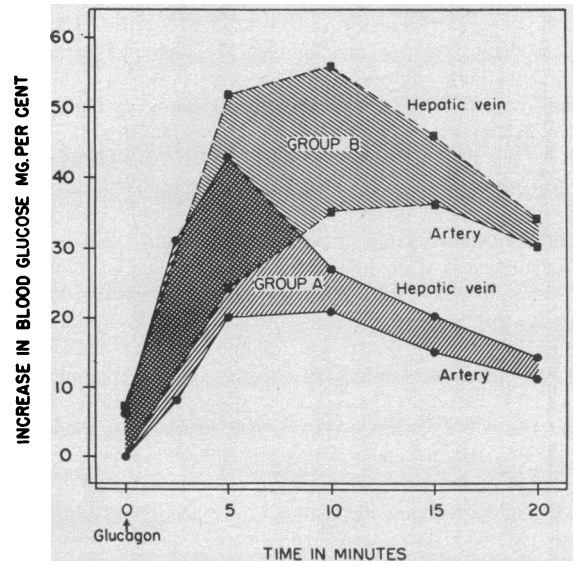

FIG. 1. MEAN INCREASES IN ARTERIAL AND HEPATIC VENOUS GLUCOSE CONCENTRATIONS AFTER A SINGLE RAPID IV INJECTION OF 3 MG (group A, six subjects) AND 6 MG (group B, six subjects) OF GLUCAGON. The values of 6 and 7 mg per 100 ml for the hepatic venous glucose concentration at 0 time represent the mean control levels of hv-a glucose differences. The shaded areas represent the hv-a glucose differences after glucagon.

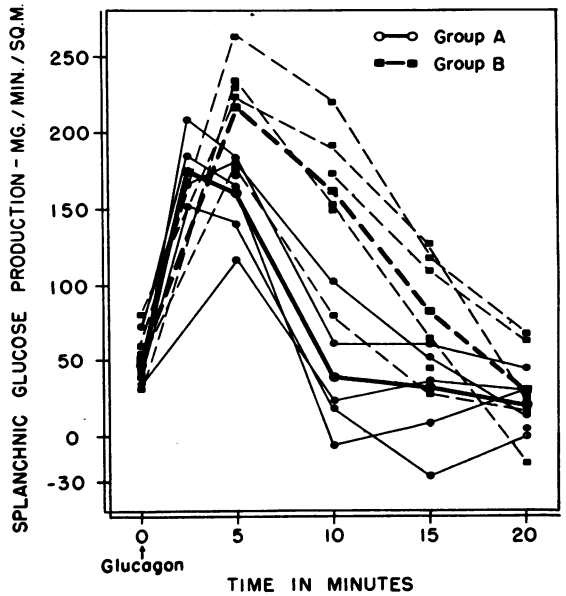

FIG. 2. NET SPLANCHNIC GLUCOSE PRODUCTION AFTER A SINGLE RAPID IV INJECTION OF GLUCAGON (mean and individual data on subjects of Figure 1). The mean curves for each group are indicated by the heavy lines.

[3] Standard error.

TABLE II

The effect of a single rapid iv injection of glucagon on arterial blood glucose, hepatic venous-arterial glucose differences, and splanchnic glucose production

Time	Change in arterial blood glucose		Hepatic venous-arterial glucose difference		Splanchnic glucose production	
	Group A*	Group B†	Group A	Group B	Group A	Group B
min	*mg/100 ml*		*mg/100 ml*		*mg/min/m²*	
0	(78)‡	(89)	6±0.6	7±1.1	48±5.1	52±6.8
			After glucagon			
2.5	+8±1§		23±1‖		175±10‖	
5	+20±1	+24±2	23±1‖	28±4‖	160±11‖	217±14‖
10	+21±2	+35±2	6±2	21±3‖	39±16	161±19‖
15	+15±2	+36±3	5±2	10±2	32±14	82±17
20	+11±2	+30±4	3±1	4±2	20±7	30±13

* Group A: six patients, 3 mg glucagon each.
† Group B: six patients, 6 mg glucagon each.
‡ Figures in parentheses represent absolute values for the fasting blood glucose.
§ Standard errors.
‖ $p < 0.01$.

in NSGP at 2½ and 5 minutes, respectively. Figure 2 illustrates the individual and mean responses of the NSGP for both groups of control subjects. After 5 minutes the NSGP fell rapidly in all patients and reached control levels or below in the next 5 to 15 minutes. The reduction in NSGP below control levels in some subjects may have resulted from the suppression of NSGP by the persistence of hyperglycemia (28) at a time when the glycogenolytic effect of glucagon had subsided.

The total, mean increases in NSGP after a single injection of glucagon can be calculated from the areas beneath the mean curves of Figure 2. These increments are 700 and 1,400 mg glucose per square meter of body surface for groups A and B, respectively. These seem adequate to account for the accompanying 21 and 36 mg per 100 ml maximal, mean elevation in arterial blood glucose. It is also apparent that the relative magnitude of these total increases in NSGP is directly related to the difference in the two doses of glucagon. The mean data of Figures 1 and 2 are presented in Table II.

The effects of a continuous infusion of glucagon in six patients are shown in Figures 3 through 6. No mean data for this group have

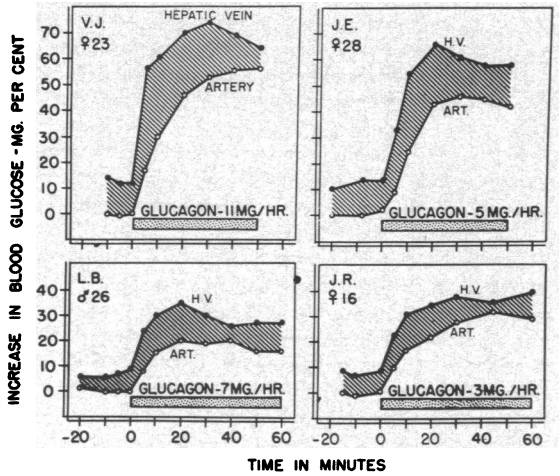

FIG. 3. INCREASES IN ARTERIAL AND HEPATIC VENOUS GLUCOSE CONCENTRATIONS DURING A CONTINUOUS, 1-HOUR, IV INFUSION OF GLUCAGON IN FOUR CONTROL SUBJECTS. Shaded areas represent hv-a glucose differences.

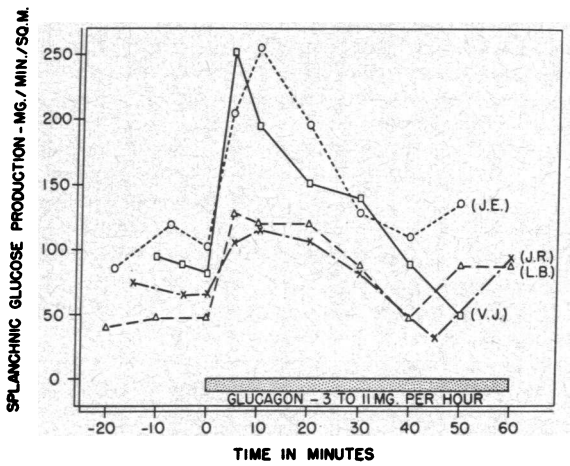

FIG. 4. NET SPLANCHNIC GLUCOSE PRODUCTION DURING THE IV INFUSION OF GLUCAGON IN THE SUBJECTS OF FIGURE 3.

TABLE III

The effect of a continuous iv infusion of glucagon on the arterial blood glucose, hepatic venous-arterial glucose difference, splanchnic glucose production, and arterial-hepatic venous oxygen difference

	Change in arterial blood glucose						Hepatic venous-arterial glucose difference						Splanchnic glucose production						Arterial-hepatic venous oxygen difference					
Time	V.J.*	J.E.	L.B.	J.R.	M.D.	C.S.	V.J.	J.E.	L.B.	J.R.	M.D.	C.S.	V.J.	J.E.	L.B.	J.R.	M.D.	C.S.	V.J.	J.E.	L.B.	J.R.	M.D.	C.S.
min			*mg/100 ml*							*mg/100 ml*						*mg/min/m²*						*vol/100 ml*		
−15	(79)†	(97)	(83)	(83)	(83)	(77)	14	10	6	9	12	13	94	85	40	74	82	76	4.0	3.3	4.2	4.0	4.7	5.4
−7.5	−1	0	0	−1	−1	0	13	14	7	8	11	11	88	119	48	66	75	64						
0	0	2	0	1	−1	0	12	12	9	8	9	10	82	102	48	66	61	58	4.0	3.2	4.8	4.3	5.0	6.0
											After glucagon													
5	+17	+9	+8	+10			40	24	16	13			252	204	128	107								
10	+30	+25	+15	+17	+47	+28	31	30	15	14	43	65	195	255	120	115	230	331						
20	+46	+43	+20	+22	+89	+52	24	23	15	13	39	40	151	196	120	107	254	234						
30	+53	+46	+19	+28	+103	+71	22	15	11	10	37	28	139	128	88	82	240	164	4.2	3.4	4.4	4.8		
40	+55	+45	+20		+111	+67	14	13	6		39	30	88	110	48		254	176						
45				+32						4						33								
50	+56	+42	+16			+72	8	16	11			25	50	136	88			146						
60			+16	+29	+111	+71			11	11	39	19			88	94	254	111	4.2	3.4	4.6	4.0	5.0	5.9
70					+96	+66					53	22					344	129						
80					+92	+66					45	8					292	47						
90					+90	+62					33	10					214	59						
100					+77	+58					39	8					254	47						
110					+64	+50					32	14					208	82					5.2	5.3

* V.J., 11 mg glucagon per hour; J.E., 5 mg; L.B., 7 mg; J.R., 3 mg; M.D., 15 mg; C.S., 15 mg.

† Figures in parentheses represent absolute values for the fasting blood glucose.

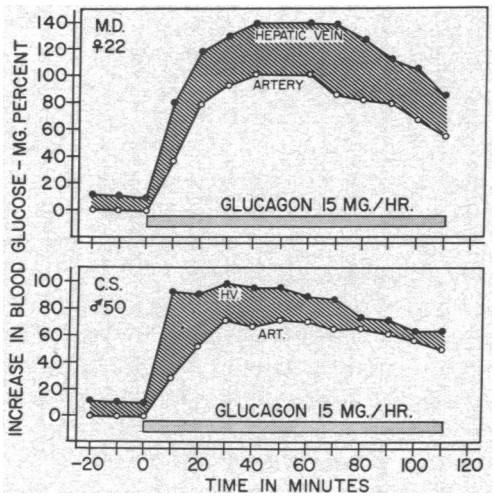

FIG. 5. INCREASES IN ARTERIAL AND HEPATIC VENOUS GLUCOSE CONCENTRATIONS DURING A CONTINUOUS, 110-MINUTE, IV INFUSION OF GLUCAGON IN TWO CONTROL SUBJECTS. Shaded areas represent hv-a glucose differences.

been calculated because of the wide range of dose used. Figures 3 and 5 are comparable to Figure 1. They show that a brisk rise in arterial blood glucose is accompanied by an even sharper elevation of the hepatic venous blood glucose, so that the hv-a glucose differences increase significantly. The curves for NSGP in Figures 4 and 6 have been calculated from the product of these hv-a glucose differences and the control HBF (see Methods). In all subjects the major increment in NSGP occurred during the first 10 minutes of the infusion. With one exception, the NSGP then gradually fell to control or slightly below control levels; in three subjects there was a subsequent modest rise. In subject M.D. (Figure 6) the NSGP remained elevated throughout

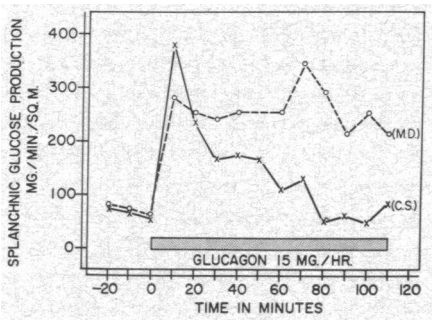

FIG. 6. NET SPLANCHNIC GLUCOSE PRODUCTION DURING THE IV INFUSION OF GLUCAGON IN THE SUBJECTS OF FIGURE 5.

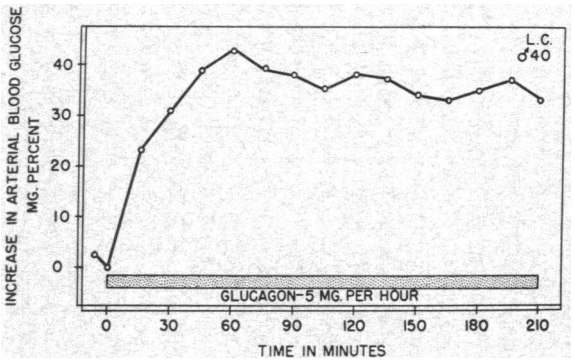

FIG. 7. INCREASE IN ARTERIAL GLUCOSE CONCENTRATION DURING A 3½-HOUR IV INFUSION OF GLUCAGON IN A CONTROL SUBJECT.

the entire 110-minute period of infusion. Arterial hyperglycemia was reasonably well maintained in all subjects during the infusion. Figure 7 shows that a fairly constant hyperglycemia can be maintained by an infusion of glucagon for as long as 3½ hours. The individual data for Figures 3 through 6 are presented in Table III.

Tables IV through VI demonstrate the effect of glucagon on blood amino acid nitrogen (Table IV), urea (Table V), and ketones (Table VI). Each table includes data for both a single injection and a continuous 1-hour infusion of glucagon. Arterial levels and hv-a differences only are recorded; for the sake of brevity, splanchnic balances are not included. Data have already been

TABLE IV

The effect of glucagon on the arterial concentration and arterial-hepatic venous difference of amino acid nitrogen

	Arterial blood amino acid nitrogen				Arterial-hepatic venous amino acid nitrogen difference			
Time	R.B.*	E.S.	C.W.	K.S.	R.B.	E.S.	C.W.	K.S.
min	*mg/100 ml*				*mg/100 ml*			
−20	4.3	4.9	4.6	4.9	0.3	0.4	0.1	0.4
−15	4.2	4.8	4.6	4.9	0.2	0.5	0.1	0.4
−10	4.3	4.7	4.4	4.8	0.2	0.3	0.2	0.4
− 5	4.4	5.0	4.6	4.9	0.3	0.4	0.2	0.4
			After glucagon					
5	4.6	4.6	4.1		0.7	0.7	0.7	
10	4.5†		4.3		0.6		0.5	
15	4.3	4.8†	4.4†	5.0	0.3	0.7	0.6	0.7
20	4.1	4.4	4.4		0.1	0.5	0.6	
30		4.2	4.7	5.0		0.2	0.9	0.8
45				4.9†				0.5
60				4.7				0.3

* Single injection of glucagon: R.B., 3 mg; E.S., 3 mg; C.W., 6 mg; K.S., 7 mg glucagon per hour.
† Time of maximal arterial blood glucose rise.

TABLE V

The effect of glucagon on arterial urea concentrations and hepatic venous-arterial urea differences

Time	Arterial blood urea							Hepatic venous-arterial urea difference						
	M.W.*	A.L.	J.R.	R.J.	V.J.	J.R.	L.B.	M.W.	A.L.	J.R.	R.J.	V.J.	J.R.	L.B.
min				*mg/100 ml*							*mg/100 ml*			
−20	14.4	25.0		48.1		27.2	11.6	0.7	1.3		0.5		1.0	0.5
−15	14.4	25.0	25.8	48.6			11.7	1.1	1.1	0.4	0.3			0.7
−10	14.4	25.0		48.6	16.6	26.8		0.8	1.3		0.5	1.1	1.0	
− 5	14.6	24.9	25.6		16.9			1.1	1.3	0.6		0.8		
						After glucagon								
5	14.2	24.9	25.4			26.3	11.6	0.8	0.8	0.8			0.7	0.6
10		24.0	25.2	46.9	16.2	24.9			1.3	0.5	0.0	0.7	0.9	
15	†	24.9	26.7†						2.3	1.6				
20	14.4	25.0†	26.7	46.6	16.4		11.5†	0.3	0.2	−0.1	0.3	0.7		0.9
30	14.8		26.9	46.1	16.4			0.4		0.2	0.3	1.2		
45				45.6†		24.0†	11.2				0.4		0.5	0.6
60				45.9	†	22.5	11.4				1.1		0.9	1.0

* Single injection of glucagon: M.W., 6 mg; A.L., 6 mg; J.R., 6 mg. Glucagon per hour: R.J., 7 mg; V.J., 11 mg; J.R., 3 mg; L.B., 7 mg.

† Time of maximal arterial blood glucose rise.

presented indicating that HBF did not change, so that the hv-a differences may be considered to reflect directly any change in the splanchnic balances of these substances. It is apparent from Table IV that glucagon had no significant effect on arterial blood amino acid nitrogen levels. There was, however, an approximate twofold increase in the normal, small hepatic a-v differences in the four subjects during the first 15 to 30 minutes after glucagon, so that splanchnic amino acid nitrogen uptake was also doubled, i.e., an increase from an average control value of 2.2 mg per minute per m^2 to a value of 4.5 mg per minute per m^2 after glucagon. There was no corresponding increase in either the arterial level or hv-a differences of urea (Table V). Table VI shows that glucagon did not significantly alter either the arterial level or the hv-a differences of blood ketones. Most of the minor changes observed are within the range of error for the method.

Figures 8 and 9 and Table VII present data relevant to the effect of glucagon on glucose uptake. A single, rapid injection of 10 mg of glucagon had little effect on the cerebral, a-v glucose differences of four control subjects (Figure 8). The only significant change was an increased a-v

TABLE VI

The effect of glucagon on arterial ketone concentrations and hepatic venous-arterial ketone differences

Time	Arterial blood ketones					Hepatic venous-arterial ketone difference				
	C.W.*	R.B.	E.S.	K.S.	L.B.	C.W.	R.B.	E.S.	K.S.	L.B.
min			*mg/100 ml*					*mg/100 ml*		
−20	0.8	2.6	0.7	1.9	1.2	0.1	1.0	0.1	0.7	0.4
−15	0.7	2.4	0.6	1.9	1.2	0.2	1.0	0.2	0.8	0.3
−10	0.8	2.7	0.6	2.1	1.0	0.2	0.6	0.2	0.4	0.5
− 5	0.8	2.3	0.6	1.8	1.1	0.1	1.0	0.0	0.6	0.5
				After glucagon						
5	0.7	1.6	0.4		1.4	0.1	0.9	0.4		0.4
10	0.6	1.6†	0.4		1.2	0.2	1.1	0.3		0.6
15	0.8†	1.8	0.6†	1.7		0.0	1.0	0.0	0.3	
20	0.7	2.0	0.5		1.0†	0.2	0.2	0.2		0.4
30	0.8		0.5	1.5				0.0	0.2	
45				1.4†	0.8				0.2	0.1
60				1.1					0.4	

* Single injection of glucagon: C.W., 6 mg; R.B., 3 mg; E.S., 3 mg. Glucagon per hour: K.S., 7 mg; L.B., 7 mg.

† Time of maximal arterial blood glucose rise.

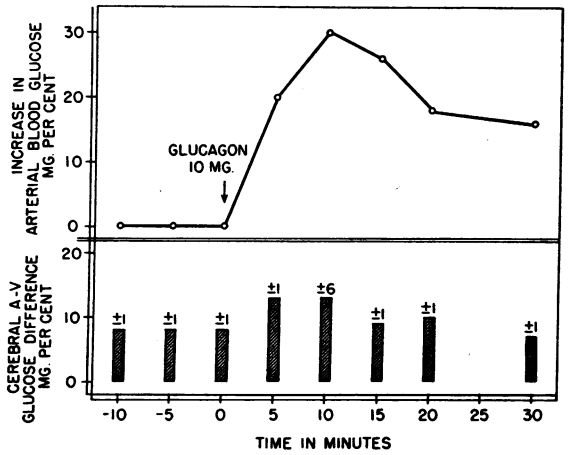

FIG. 8. MEAN DATA ON THE EFFECT OF A SINGLE RAPID IV INJECTION OF GLUCAGON ON THE CEREBRAL A-V GLUCOSE DIFFERENCE OF FOUR CONTROL SUBJECTS. The values above the bars are standard errors.

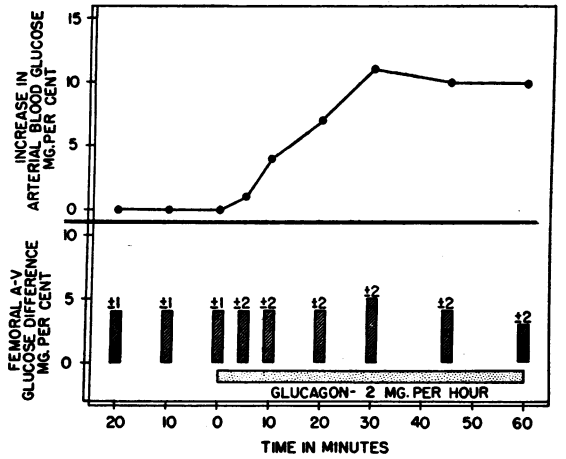

FIG. 9. MEAN DATA ON THE EFFECT OF A CONTINUOUS, 1-HOUR, IV INFUSION OF GLUCAGON ON THE FEMORAL A-V GLUCOSE DIFFERENCES OF FIVE SUBJECTS. The values above the bars represent standard errors.

glucose difference at 5 minutes, and this occurred at a time when the arterial glucose level was rising rapidly. In an unsteady state, such as a rapidly rising concentration of a material in arterial blood, simultaneously collected arterial and venous blood samples are not truly comparable, since the circulation time through the organ will cause the venous concentration to lag behind the arterial. Under these circumstances a falsely high a-v difference will be recorded. The value of 8 mg per 100 ml for the mean, control cerebral a-v glucose difference compares favorably with the value of 10 mg per 100 ml reported by Scheinberg and Stead (35). The mean, cerebral, a-v oxygen difference was 8.33 vol per 100 ml during the control period and 8.57 vol per 100 ml after glucagon. Since there was no apparent anxiety in these patients nor any other reason to suspect a change in cerebral oxygen consumption, these data on a-v oxygen differences suggest that cerebral blood flow and, hence, cerebral glucose uptake were not affected by glucagon.

TABLE VII

The effect of glucagon on cerebral and femoral arteriovenous glucose differences

Time	4 control subjects, 10 mg glucagon (single injection)		5 control subjects 2 mg glucagon (per hour)	
	Change in arterial blood glucose	Cerebral a-v glucose difference	Change in arterial blood glucose	Femoral a-v glucose difference
min	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
−20			(88)	4±1
−10	(103)*	8±1	0	4±1
− 5	0	8±1†		
0	0	8±1	0	4±1
		After glucagon		
5	+20	13±1‡	+ 1	4±2
10	+30	13±6	+ 4	4±1
15	+26	9±1		
20	+18	10±1	+ 7	4±2
30	+16	7±1	+11	5±2
45			+10	4±1
60			+10	3±2

* Figures in parentheses represent absolute values for the fasting blood glucose.
† Standard errors.
‡ $p < 0.02, > 0.01$.

As shown in Figure 9, an infusion of 2 mg of glucagon per hour had no significant effect on the a-v glucose differences across the leg in five control subjects. Again, the only change occurred at a time when the arterial blood glucose was rising rapidly. The small dose of glucagon was given deliberately to produce a degree of hyperglycemia that would not stimulate the release of insulin by the pancreas. The mean, fasting a-v glucose difference of 4 mg per 100 ml agrees closely with the value of 3.4 mg per 100 ml obtained by Bell and Burns (36) by a more accurate method of glucose determination. Although no attempt was made to estimate blood flow through the leg in the present studies, Bondy and Cardillo (23) have observed in two control subjects that glucagon does not affect blood flow through an extremity.

Discussion

In these studies glucagon produced a rapid and striking increase in NSGP in man. Since HBF did not change, the augmented glucose production was due to the increase in hv-a blood glucose differences alone. It is unlikely that a decrease in glucose uptake by the extrahepatic tissues of the splanchnic bed contributed significantly to the increase in these differences in the absence of a demonstrable effect of glucagon on peripheral glucose uptake. The assumption appears justified, therefore, that the changes noted in "net splanchnic glucose production" were in fact changes in hepatic glucose production. Nevertheless, the term "net splanchnic glucose production" rather than "hepatic glucose production" is used throughout this report.

Within the dose range used in these studies, the total increase in NSGP was proportional to the dose of glucagon administered. Calculations based upon the data provided in Figure 2 (see Results) indicate that doubling the amount of glucagon from 3 to 6 mg resulted in a doubling of the total increase in NSGP from 700 to 1,400 mg of glucose per m^2, respectively. The arterial blood glucose response also reflected differences in the dose of glucagon, but these changes were not so directly proportional to dose as were the changes in NSGP. This is not surprising since arterial blood glucose is influenced by peripheral glucose diffusion and utilization as well as by hepatic glucose production. Despite these considerations and the fact that our dose levels were only rough approximations, it is difficult to understand why Loube, Campbell, and Mirsky (37), Helmer and Root (38), and Carson and Koch (39) found no difference in the hyperglycemic response to graded doses of glucagon in either animals or human subjects.

The continuous infusion of glucagon produced a prolonged increase in NSGP. In all subjects the increment in NSGP persisted for 30 minutes, even when the administered dose was quite low. The largest dose of glucagon, 15 mg per hour, resulted in a threefold increase in NSGP for 2 hours in one of two subjects who received this amount. This high level of NSGP continued despite the fact that the arterial blood glucose concentration remained elevated throughout the infusion period. Hyperglycemia of the degree attained here, when produced by an infusion of glucose, is sufficient to abolish the normal hepatic glucose production (28). Clearly, the continuing action of glucagon was sufficient to counter this opposing effect of hyperglycemia on NSGP. Figure 5 shows that a fairly constant level of hyperglycemia may be maintained for as long as 3½ hours during glucagon infusion. These results contrast to the substantial drop in blood glucose noted by Van Itallie and associates (26) during the last 30 minutes of a 1-hour infusion of glucagon in two subjects.

In contrast to epinephrine, which also enhances NSGP in man, glucagon had no significant effect on HBF or splanchnic oxygen consumption (Table I). In dogs, however, Shoemaker and his associates (4, 5) have found that glucagon doubles the HBF during the first 30 minutes after injection. Similar findings in dogs are reported by Landau, Leonards, and Barry (40). Since the method of estimating HBF and the observation periods were the same in the animal and in the present human experiments, this discrepancy may represent a species difference.

Although glucagon had no significant effect on splanchnic balances of urea or oxygen, the doubling of the normally small, fasting splanchnic amino acid nitrogen uptake suggests some stimulation of gluconeogenesis. It is not known what contribution the extrahepatic tissues of the splanchnic bed may have made to the changes

noted in a-hv amino acid nitrogen differences. That these changes were probably hepatic in origin is suggested by the work of Shoemaker and Van Itallie (5), who sampled the portal and hepatic veins directly in dogs and found a fourfold increase in amino acid nitrogen uptake within 45 minutes after a single injection of glucagon. In our study the magnitude of the amino acid nitrogen uptake after glucagon was still of a low order of magnitude, and that gluconeogenesis contributed significantly to the large amounts of glucose released by the liver during this time seems unlikely. We presume, therefore, that glycogenolysis was largely responsible for the increased glucose output from the liver. The small magnitude of the change in amino acid nitrogen uptake without significant alterations in splanchnic balances of urea or oxygen suggests that the changes observed were merely secondary to the effect of glucagon on glycogenolysis. There is considerable evidence, however, from studies on the long-term effects of glucagon in favor of a direct action of glucagon on protein catabolism. Salter, Davidson, and Best (10) demonstrated that intact, fasting rats injected with 400 μg of glucagon every 8 hours excreted 40% more urinary nitrogen than control animals, although the rats showed no hyperglycemia or glycosuria. Izzo and Glasser (15), comparing the effects of glucagon and epinephrine administered to fasted rats over a 5-day period, found that although both cause a similar depletion of liver glycogen, only glucagon brings about an increase in protein catabolism. In a further study, Glasser and Izzo (16) found a gluconeogenic effect of glucagon in the adrenalectomized fasted rat similar to that in the intact, fasted rat without, however, an effect on liver glycogen. During prolonged iv glucagon therapy in patients with rheumatoid arthritis, Ezrin and colleagues (13) had one patient who showed a negative nitrogen balance with normal blood sugar concentrations. Izzo (14) gave repeated im injections of glucagon (1 to 2 mg every 8 hours) for 3 to 9 days to well-regulated diabetic patients and found not only hyperglycemia and glycosuria but also an increase in urinary nitrogen. Comparable or greater degrees of hyperglycemia and glycosuria seen in two such patients while off insulin were accompanied by less nitrogen excretion than that seen with glucagon. In one patient, simultaneous administration of carbutamide virtually abolished the hyperglycemic-glycosuric response to glucagon but had no effect on the increased nitrogen excretion induced by glucagon.

Neither the blood ketone levels nor the ketone output by the liver was altered by glucagon under the conditions of the present experiments; all the subjects were well-nourished and had low fasting blood ketone levels and small hv-a ketone differences. Our results are contrary to the long-term studies in which ketosis has been observed in the absence or independent of hyperglycemia and glycosuria (13, 24).

That glucose differences across the leg and brain did not change significantly except during the period of a rapidly rising arterial blood glucose level suggests that glucagon does not affect peripheral glucose uptake. The advantages of using the leg rather than the forearm for making these observations on glucose utilization in the limbs have been discussed elsewhere (28). However, since our studies were not controlled by observations during a comparable degree of hyperglycemia with infused glucose and since leg and cerebral blood flow were not measured, the data on a-v glucose differences serve only to exclude gross changes. As noted previously, Bondy and Cardillo (23) observed no effect of glucagon on extremity blood flow in two subjects. Henneman and Shoemaker (41), on the other hand, found that glucagon decreases blood flow in the hind limb of the unanesthetized dog. Despite this decrease in blood flow, glucose uptake was enhanced. But from further observations in fasted dogs, these authors concluded that this effect of glucagon on peripheral glucose uptake is secondary to the changes in blood glucose. As already discussed, the dose of glucagon used in the present study of a-v glucose differences across the leg was relatively small in order to minimize the effect of a rise in blood sugar per se in bringing about changes in peripheral glucose uptake. Conceivably, larger doses of glucagon may have a direct effect on peripheral glucose uptake.

Thus, in the present acute experiments in man, glucagon brought about marked increases in NSGP without significant changes in other metabolic and circulatory parameters except for a small increase in net splanchnic amino acid nitrogen uptake. In the light of these findings, the impressive changes

in protein and ketone metabolism reported in the literature with long-term glucagon administration may well be phenomena secondary to the exhaustion of liver glycogen rather than primary effects of glucagon. This interpretation is at variance, however, with the changes, noted above, in protein and ketone metabolism in the absence or independent of changes in glucose metabolism. Our studies have no direct bearing on recent reports that glucagon affects the plasma levels of nonesterified fatty acids (42, 43), total lipids (44, 45), and cholesterol (46). Dreiling and associates (43) consider the changes in plasma nonesterified fatty acids to be independent of the hepatic glycogenolytic effect of glucagon, since the changes were noted in patients with liver disease in whom the hyperglycemic response to glucagon was reduced or absent. A word of caution is justified, however, in the interpretation of this and other studies that are based on blood glucose values alone and propose to show an effect of glucagon in the absence of an effect on carbohydrate metabolism. As previously indicated, the change in blood sugar is probably a less sensitive index of the glycogenolytic action of glucagon than is the change in NSGP.

Summary

Glucagon by intravenous injection or infusion produced a rapid and marked increase in net splanchnic glucose production in the normal fasting human subject. The magnitude of this response seems adequate to account for the degree of hyperglycemia observed. Responses to graded doses of glucagon are better compared on the basis of total increases in net splanchnic glucose production than on increases in blood glucose concentrations alone.

Glucagon had no effect on hepatic blood flow, splanchnic oxygen consumption, or Bromsulphalein clearance. In this respect, it differs from epinephrine.

Glucagon had no appreciable effect on splanchnic balances of urea or oxygen. It caused a doubling of the normally small, amino acid nitrogen uptake by the liver, but the magnitude of this effect does not seem sufficient to account for the marked changes in net splanchnic glucose production. Presumably, therefore, glycogenolysis was largely responsible for the increased glucose output from the liver.

Glucagon had no effect on splanchnic ketone output.

Glucagon did not significantly alter the arteriovenous glucose differences across the leg or brain. We conclude, therefore, that glucagon does not modify peripheral glucose uptake under the conditions of these experiments.

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