Impaired Glucose Tolerance and Glucosuria Induced in Man by Repeated Injections of Glucagon

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When glucagon is given in a single injection or by infusion it induces a transient hyperglycemia by stimulating hepatic glycogenolysis.[1-3] Under such circumstances, inhibition of peripheral glucose uptake does not occur.[4-7] Thus, despite earlier opinion to the contrary,[8-9] glucagon does not appear to act directly as an insulin antagonist.

On the other hand, it has been reported that when repeated injections of glucagon are given to force-fed rats,[10] cats and dogs,[11] and patients with rheumatoid arthritis,[12,13] glucosuria, hyperglycemia, ketosis and a "protein-catabolic" state may ensue.

Ezrin and associates[12] have attributed the hyperglycemia induced by repeated injections or infusions of glucagon to an increased production of new glucose from amino acids as well as increased hepatic glycogenolysis. They believe increased gluconeogenesis from protein to play the most important role in the genesis of the diabetic-like state induced by glucagon. Elrick, Rachiele, and Head[11] have suggested that glucagon-induced diabetes should be placed in the category of conditions such as occur in force feeding, in which transient hyperglycemia and glucosuria are produced without impairment of glucose utilization.

In an attempt to obtain more information about the metabolic consequences of prolonged administration of glucagon and, in particular, about its effect on carbohydrate homeostasis, glucagon was administered by repeated injection to a series of normal human subjects maintained on constant diets while on a metabolic ward. The results show that, in man, repeated injections of glucagon can induce a transient diabetic-like state in which the glucosuria results from impaired tolerance to ingested carbohydrate. In several respects, glucagon-diabetes resembles the diabetic-like state classically induced by carbohydrate deprivation.

PROCEDURE

The glucagon* used was a crystalline, insulin-free preparation made by the method of Staub et al.[14] It was administered at six-hourly intervals in amounts of 1.3 to 4.0 mg. for periods of two to five days.† Usually the glucagon was administered intramuscularly, but in one experiment (J.O'B.), the subject was given three morning doses and two afternoon doses intravenously.

Three young men, aged 20, 22 and 27, and one young woman, aged twenty-five, served as volunteer subjects. All the subjects were in good health. They were studied on the Metabolic Ward of the Peter Bent Brigham Hospital, with weighed constant diets and quantitative collections of urine. Feces were not collected.

The experimental periods involving glucagon administration were preceded by a glucose tolerance test and by a control period of four to five days during which a constant diet containing at least 250 gm. of carbohydrate per day and calories adequate to maintain body weight was consumed. Control blood and urinary collections were made during this same period.

During the experimental period which lasted two to five days, the subject remained on his constant diet; however, in addition, glucagon was administered every six hours in amounts of 1.3 to 4.0 mg. After completion of the experimental period, the glucose tolerance test was repeated.

One subject (J.O'B.) had a three-day postexperimental control period during which intake remained the

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† Three of the subjects presented in varying degrees one or more of the following symptoms during the period of glucagon administration: anorexia, nausea, bloating and, in one instance, vomiting.

same as in the first two periods.

In two subjects (one of the subjects previously described and an additional young man, aged twenty-eight, not maintained on a strict metabolic balance regimen), measurements of maximal tubular reabsorptive capacity for glucose (glucose T_m) were obtained before and after forty-eight and sixty hours of glucagon administration respectively at a rate of 4 mg. every six hours.

Three subjects had fractional urine collections during at least one twenty-four-hour interval of the experimental period.

Glucose tolerance tests were made in the morning approximately fourteen hours after the previous meal. In two experiments, 0.5 gm. of glucose per kilogram body weight was given intravenously over a period of thirty minutes and capillary (finger tip) and antecubital venous blood sugar levels were measured at fifteen-minute intervals for two hours. Control blood sugar levels also were determined.

In one experiment, glucose was administered intravenously by constant (Bowman pump) infusion at a rate of 0.5 gm. per kilogram body weight per hour for a total of eight hours. Following a control determination, venous blood sugar levels and urinary glucose excretion were measured at hourly intervals during the infusion.

In one experiment, 1.3 mg. of glucagon was administered intravenously (1.0 mg. of glucagon per ml. of diluent) before breakfast and in the early afternoon before luncheon on the first and third day of the experimental period, and before breakfast only during the fifth day of the experimental period. Capillary glucose and antecubital venous glucose and ketone levels were measured at thirty and forty-five minutes after each injection and control blood samples also were obtained.

Dietary constituents were determined by means of standard tables and analyses of representative portions. Calorie intake was calculated from weighed portions and refusals on the basis of appropriate tables.

Urinary glucose was measured enzymatically, as described by Froesch and Renold.[15] Glucose was measured in whole blood by Somogyi's method[16] as modified by Nelson.[17] All determinations were done in duplicate on 0.2 ml. samples. Blood ketones were determined by the method of Michaels et al.[18]

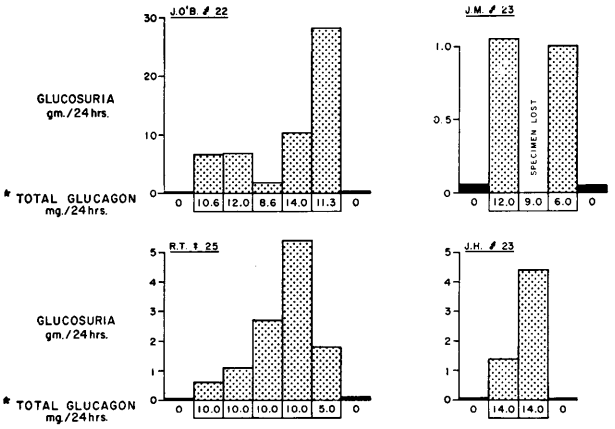
Glucose T_m was determined according to the method of Smith et al.[19] as modified by Froesch and associates,[20] while glomerular filtration rate was measured by means of insulin clearance.[21]

Capillary blood was obtained by direct pipetting from a finger tip after cutaneous puncture.

RESULTS

All four subjects displayed a significant degree of glucosuria while glucagon was being given (figure 1). There was considerable individual variation in the glucosuric response, the maximal glucose output for each subject per twenty-four hours falling within the range of 1.1 to 28.2 gm. (normal <0.25 gm.).

The hyperglycemic response to glucagon given intravenously was measured in one subject as the experimental period progressed (figure 2). At the same time, blood ketones were determined. It will be noted that the blood sugar never rose to levels higher than 130 mg./100 ml. after glucagon, whether this substance was administered intravenously or intramuscularly.* The



*Given in four equal six-hourly injections

FIG. 1. Glucosuria in four subjects during treatment with repeated injections of glucagon. Each vertical bar represents a twenty-four-hour collection period.

pattern of response to glucagon changed as the experimental period progressed and also varied with the time of day. On the third and fifth days of the experimental period (figure 2B and 2C), the fasting blood glucose level was substantially lower than on the first day (figure 2A). At the same time, the total glucose increment was greater on the mornings of the third and fifth days, the capillary-venous (C-V) glucose differences became smaller, and the slopes of the disappearance curves after the hyperglycemic peaks were reached were more gradual. Blood ketone levels on the mornings of the third and fifth days were appreciably higher than on the first day. In contrast, the early afternoon responses to glucagon (pre-luncheon) on the

* Blood sugar levels were determined at hourly intervals for three hours following intramuscular injection of 4.0 mg. of glucagon in subject J. O'B. and did not exceed 118 mg./100 ml.

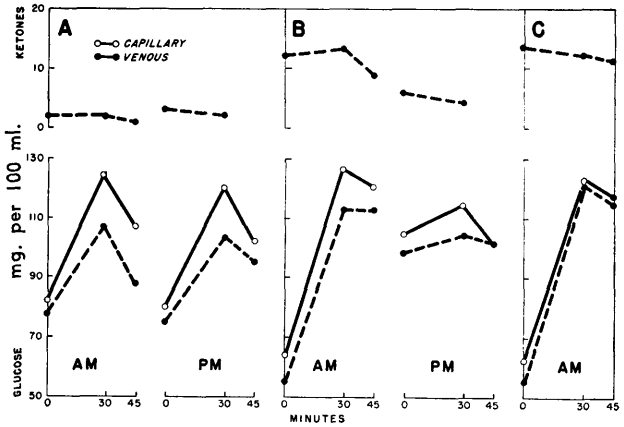


FIG. 2. (Subject J. O'B.) Changes in concentration of blood ketones and glucose after intravenous glucagon (1.3 mg.) on different days. A. First day of glucagon treatment. B. Third day of glucagon treatment. C. Morning of fifth day of glucagon treatment. Morning and afternoon injections at zero time were given before breakfast and luncheon respectively.

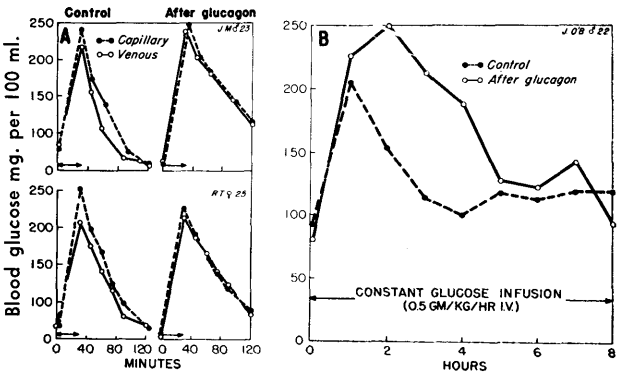


FIG. 3. A. Intravenous glucose tolerance tests in two subjects before and after glucagon treatment. Capillary and venous glucose concentrations were measured. One-half gram glucose per kilogram body weight infused over a thirty-minute period.
B. (Subject J. O'B.) Changes in blood glucose concentration during prolonged constant glucose infusion, before and after glucagon treatment.

first and third days showed small rises in blood glucose concentration, appreciable C-V differences, and a rapid decrement, while blood ketone levels in the afternoon of the third day were considerably lower than on the morning of that day.

Values for glucose T_m were measured in two normal subjects before and after forty-eight and sixty hours of repeated glucagon injections. In the first instance the T_m (mean of four periods) decreased by 6 per cent after glucagon, and in the second instance the T_m (mean of four periods) increased by 11 per cent after glucagon. These changes were not considered to be significant.

The effect of repeated injections of glucagon on tolerance to intravenously administered glucose is shown in figure 3. During the short intravenous glucose tolerance tests (Part A), C-V glucose differences were measured in order to determine the effect of glucagon treatment on peripheral glucose uptake. In each instance, C-V glucose differences diminished in the postglucagon tolerance test, while at the same time the slopes of the disappearance curves became more gradual.

During the eight-hour glucose infusion after glucagon treatment (Part B of figure 3), 56.1 gm. of glucose were lost in the urine while only 7.6 gm. of glucose appeared in the urine during the preglucagon eight-hour infusion.

During glucagon treatment, fasting blood glucose levels tended to remain normal or fall below their control levels, as shown in table 1.

The time at which maximal glucosuria occurred was determined by obtaining four six-hour urine collections over a twenty-four-hour period during glucagon treatment. The results are shown in figure 4. In every instance, the glucosuria occurred principally in the morning after breakfast. Losses of glucose in the urine were negligible at other times.

TABLE 1

Fasting venous blood glucose levels (milligrams/100 ml.) before and during glucagon treatment (previous dose of glucagon six or more hours before blood sampling)

	R.T.	J.O'B.	J.M.	J.M.
Before glucagon	82	92		56
treatment	79	93	70	58
	86	79	73	60
During glucagon	52	78	56	46
treatment	45	52	70	42
		55		
		60		
		55		

At the same time as the maximal glucosuria occurred, there was a marked impairment of glucose tolerance. This is shown in figure 5, where the responses in venous glucose level to a measured, identical breakfast containing 63 gm. of carbohydrates before and after glucagon are compared. Before glucagon administration, there was virtually no rise in venous glucose after the breakfast; after three days of glucagon treatment venous glucose rose to diabetic levels following ingestion of breakfast, and appreciable glucosuria occurred simultaneously.

DISCUSSION

In attempting to account for the glucosuria during glucagon treatment, several possible explanations were

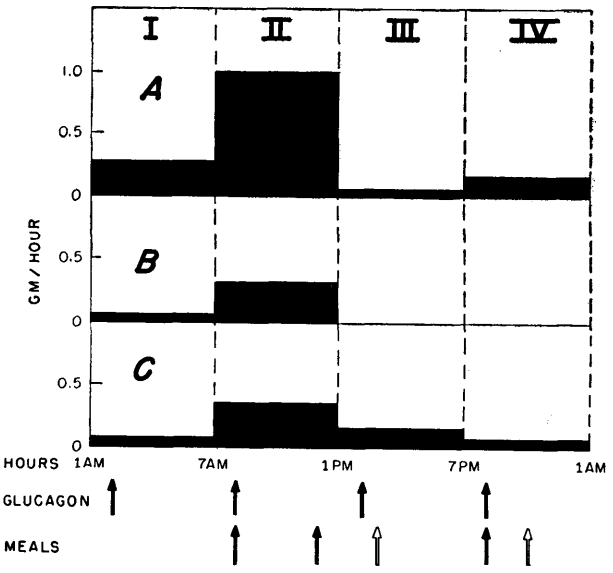


FIG. 4. Quantity of glucose in fractional urine collections from three normal subjects (A, B, and C) given equal injections of glucagon every six hours. Solid black arrows represent regular meals and light arrows snacks.

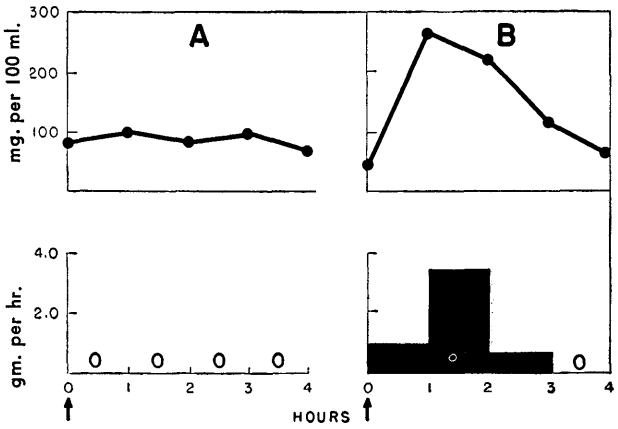


FIG. 5. Changes in blood glucose (mg./100 ml.) and glucosuria (gm./hr.) in a normal subject after breakfast. A. Before glucagon treatment. B. During glucagon treatment. Arrows represent ingestion of an identical breakfast containing 63.0 gm. of carbohydrate.

considered. It was shown that the glucosuria could not have been caused by an excessive hyperglycemia directly induced by glucagon since the blood sugar after glucagon administration never exceeded 130 mg./100 ml., considerably below the renal threshold for glucose. Root[22] has described an increasingly hyperglycemic response to glucagon associated with increased concentration of glycogen in liver in rabbits given repeated injections of glucagon. In the human subject studied here from the standpoint of responsiveness of blood glucose to glucagon, there was only a slight increase in the height of the glucagon-induced hyperglycemia after five days of glucagon treatment.

The possibility of an altered renal threshold to glucose also was considered. Elrick and associates[23] have reported on changes in renal clearance of sodium, chloride, potassium, and inorganic phosphate, although they have not described an effect of glucagon on excretion of glucose by the kidney. The two subjects in whom glucose T_m was measured before and during glucagon treatment did not show any significant change in maximal tubular reabsorptive capacity for glucose; hence, it was believed that a renal effect of glucagon to account for the glucosuria was unlikely.

Cahill et al.[24] have demonstrated increased hepatic glucose-6-phosphatase activity secondary to the increased phosphorylase activity following glucagon administration in the dog. The possibility was entertained that impaired tolerance to dietary carbohydrate might have occurred because of temporary inability of the liver to retain glucose removed from blood. Although there is no evidence to rule out such an effect as a contributing factor, it seems more reasonable to account for the impaired tolerance to carbohydrate induced by glucagon treatment by a diminished rate of glucose removal by the extrahepatic tissues. The decreased peripheral C-V glucose differences measured during postglucagon glucose tolerance tests suggest that the principal site of impaired tolerance may have been in the periphery.

Thus, it would appear that glucagon affects carbohydrate metabolism by at least two mechanisms. The first mechanism is well understood and entails a rapid glycogenolytic response in the liver with consequent transient hyperglycemia. During this time, impairment of peripheral glucose utilization does not occur. The second mechanism is a delayed one in which impaired tolerance to glucose is demonstrable within less than eight hours after a single injection of glucagon.[25] The degree of impairment of glucose tolerance is particularly marked after an overnight fast during which an injection of glucagon has been administered. Conversely, ingestion of food alleviates the impaired glucose tolerance, at least in part.

The diabetic-like state induced by glucagon differs from spontaneous diabetes mellitus, steroid diabetes and metahypophyseal diabetes in that the fasting blood sugar is normal or low. It most closely resembles so-called hunger or starvation diabetes in that the fasting blood sugar remains normal or low, and liver glycogen is depleted.

The mechanism causing hunger diabetes still is not well understood.[26] Impaired glucose tolerance in hunger diabetes presumably represents an attempt by the body to conserve carbohydrate so that its own stores of protein will not have to be raided to provide indispensable glucose needed by the organism. When glucagon is given, the only mobilizable stores of carbohydrate also are depleted (although there need be no net loss of carbohydrate from the body). It seems likely that glucagon-induced "diabetes" may result from the body's attempt to maintain the blood sugar at a normal level in spite of drastic depletion of liver glycogen.

Another possibility is that glucagon administration directly inhibits insulin formation or release.[27] In any event, the impaired tolerance to glucose induced by glucagon would seem to be due to (1) a decrease in circulating insulin, (2) an increase in the secretion of insulin antagonists, or (3) a decreased tissue sensitivity to insulin.

No evidence is obtained in the present studies that appreciable hyperglycemia is present except after ingestion of carbohydrate. The fractional urine collections correlated with blood sugar determinations, as illustrated in figure 5, suggest that the glucosuria induced by repeated injections of glucagon in this series was largely, if not entirely, alimentary in origin.

SUMMARY AND CONCLUSIONS

Crystalline glucagon was administered by repeated injection to four normal human subjects maintained on constant diets. The glucagon was given in amounts of 1.3 to 4.0 mg. intramuscularly every six hours for two to five days.

All four subjects developed significant glucosuria, ranging up to 28 gm. per twenty-four hours, during glucagon treatment. Fractional urine collections showed the glucosuria to occur principally after the morning meal. During the period of glucagon administration, fasting blood sugar values were normal or below their usual levels, but tolerance to ingested and intravenously administered glucose became impaired. Decreased capillary-venous glucose differences during intravenous glucose tolerance tests and during glucagon-induced hyperglycemia suggested that the rate of glucose assimilation by peripheral tissues had been reduced by glucagon treatment.

In one subject who displayed marked glucosuria, glucagon treatment also was associated with morning hyperketonemia.

Glucose T_m determinations in two subjects before and after glucagon administration disclosed no significant change in maximal tubular reabsorptive capacity for glucose.

It is concluded that, in man, repeated injections of glucagon can induce a transient diabetic-like state in which the glucosuria results from impaired tolerance to ingested carbohydrate. A possible relationship of this phenomenon to the "diabetes" caused by carbohydrate deprivation is discussed.

SUMMARIO IN INTERLINGUA

Derogation Del Tolerantia De Glucosa E Induction De Glucosuria In Humanos Per Repetite Injectiones De Glucagon

Glucagon in forma crystallin esseva administrate per repetite injectiones a quatro normal subjectos human qui esseva mantenite con dietas constante. Le glucagon esseva administrate in quantitates de inter 1,3 e 4,0 mg per via intramuscular a intervallos de sex horas durante periodos de inter duo e cinque dies.

Omne le quatro subjectos disveloppava grados significative de glucosuria, in concentrationes de usque a 28 g per vinti-quatro horas, in le curso del tractamento con glucagon. Fractional collectiones de urina monstrava que le glucosuria occurreva principalmente post le repasto de matino. Durante le periodo del administration de glucagon, le valores pro sucro sanguinee in stato jejun esseva normal o infra lor nivellos usual, sed le tolerantia pro glucosa administrate per via oral o intravenose deveniva derogate. Reducite differentias de glucosa inter capillares e venas durante tests de tolerantia pro glucosa intravenose e durante hyperglycemia inducite per glucagon suggereva que le assimilation de glucosa per le histos peripheric habeva essite relentate durante le tractamento con glucagon.

In un subjecto, qui monstrava grados marcate de glucosuria, le tractamento con glucagon esseva etiam associate con hypercetonemia matinal.

Determinationes de T_m pro glucosa in duo subjectos ante e post le administration de glucagon revelava nulle significative alteration del maximal tubular capacitate reabsorptive pro glucosa.

Es concludite que repetite injectiones de glucagon in humanos pote inducer un transiente stato diabetoide in que le glucosuria resulta ab le derogate tolerantia pro ingerite hydratos de carbon. Es discutite un relation possibile inter iste phenomeno e le "diabete" causate per privation de hydrato de carbon.

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Although the phosphogluconic acid cycle (in which a number of pentose phosphates occur as intermediates) was first demonstrated in plants and bacteria, there has now accumulated abundant evidence to indicate that similar biochemical processes are present in mammalian tissues. The occurrence of pentosurias in humans associated with congenital defects, drug ingestion, and neurological disease suggests that pentoses are of fundamental biological importance. The papers of Segal, Wyngaarden, and Foley represent one of the first attempts to clarify pentose metabolism in man, and, additionally, to offer supportive evidence obtained in the human of the membrane theory of insulin action.

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