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Use of glucagon to terminate insulin induced hypoglycemic reaction in diabetic patients

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THE USE OF GLUCAGON TO TERMINATE INSULIN INDUCED HYPOGLYCEMIC REACTIONS IN DIABETIC PATIENTS

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History of Glucagon:

The repeated finding that insulin therapy could lower liver glycogen even in the absence of hypoglycemia was, for a time a source of considerable confusion in the literature on carbohydrate physiology. Many workers found it difficult to reconcile a glycogenolytic effect of insulin with its known capacity to restore liver glycogen in the depancreatized animal. Probably many of the findings concerning the effect of insulin on liver glycogen resulted from the presence of glucagon as a contaminant in insulin preparations.[1]

In 1926 Abel crystallized insulin and no hyperglycemic glycogenolytic effect was noted with this preparation.[2]

In 1934 Scott described another method of producing insulin which was subsequently taken up by manufacturers in the United States.[3] This insulin possessed the previously noted hyperglycemic effect which was still thought to be insulin induced.

In 1935 Burger suggested that a glycogenolytic contaminant was present in insulin and de Duve demonstrated the presence of a hyperglycemic glycogenolytic factor, termed glucagon, in American commercial insulins.[4]

Chemistry of Glucagon:

The complete structure analysis of the glucagon molecule has been completed by Bromer, et al.[5] The amino acid sequence has been established by a series of enzymatic digestions and chromatographic analysis of peptide fragments and their diphosphopyridine nucleotide derivatives. The molecule consists of a straight chain of twenty-nine amino acids with histidine as the N-terminal and threonine as the C-terminal residue. The structure analysis revealed a minimal molecular weight of 3,482. The amino acid sequence provides final evidence that insulin and glucagon are two different entities. In addition to differences in amino acid content there is no structural similarity between the two molecules.

Site of Formation of Glucagon:

Glucagon is produced by the alpha cells of the pancreas. The absence of alpha cells in the uncinate process developmentally related to the ventral pancreas and the fact that extracts of this area of the pancreas are without hyperglycemic effects coupled with the fact that alpha cells are present in the body and tail of the pancreas with extracts of the latter areas producing a hyperglycemic effect supports the view that there is a difference between the potentialities of these two anlage.[6]

Evidence of extrapancreatic foci of glucagon production has been shown by Rao and De who report that extracts from abdominal lymph nodes, tongue, and spleen show hyperglycemic glycogenolytic activity.[7]

Relationship of the Hypophysis to Glucagon Secretion:

Laboratory findings do not support the concept of a direct relationship between the anterior pituitary and the alpha cells of the pancreas. The adult rat shows no appreciable changes in blood sugar level or in the morphology of the pancreatic islets following administration of growth hormone.[8] A short phase of hypertrophy of both alpha and beta cells was noted beginning after three weeks of administration of pituitary growth hormone. However, this was followed by a period of hypoplasia of the islets and degranulation of the alpha and beta cells. No changes in carbohydrate metabolism were observed.

Hyperglycemic substances have been identified in the pancreatic-vein blood of animals treated with growth hormone.[9] However, Best has reported that these substances do not produce hyperglycemia in animals pretreated with dihydroergotamine, an adrenergic blocking agent.[10] The latter evidence suggests that these are epinephrine-like substances.

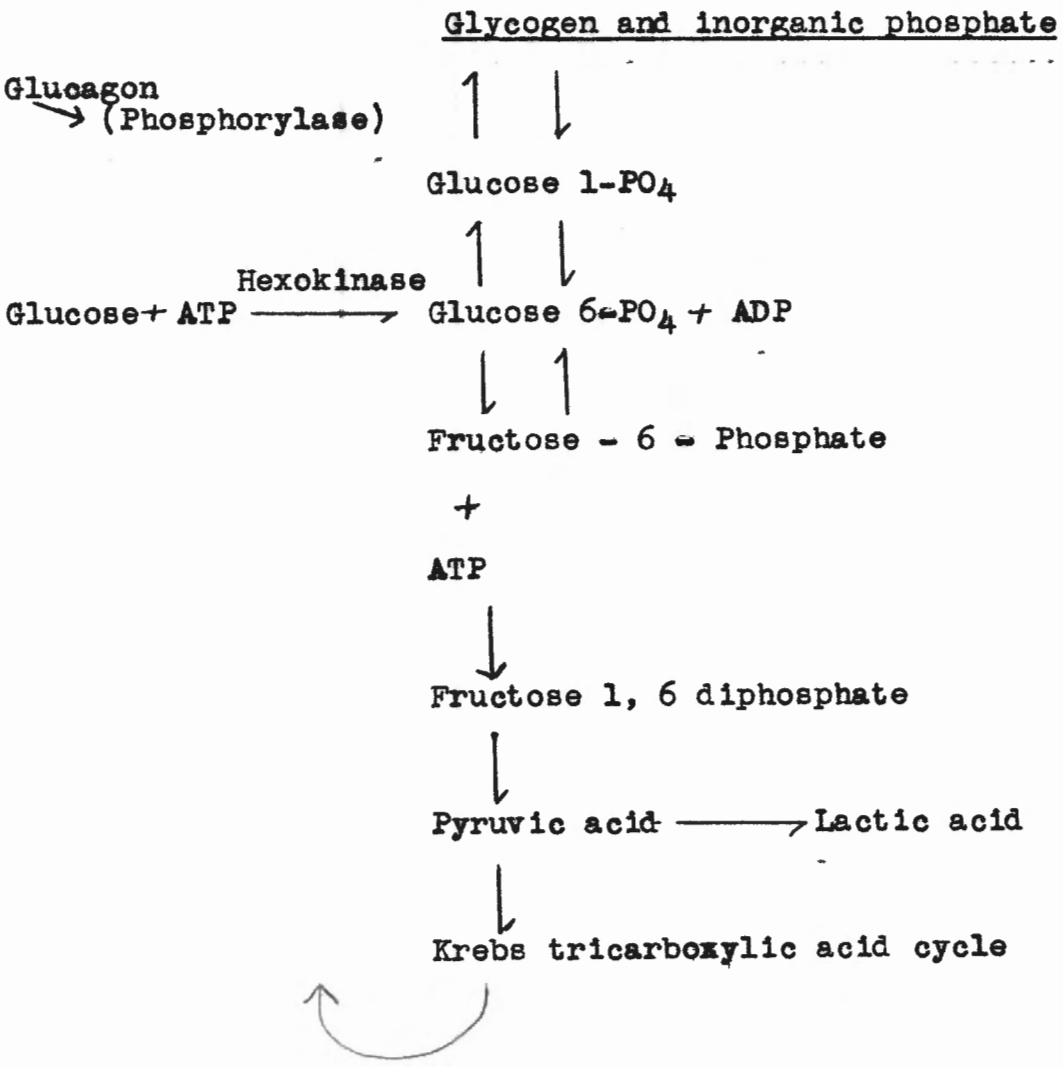
It is interesting to note that Simard reported that alpha cells disappeared almost entirely from a subcutaneous pancreas transplant which was totally deprived of its extrensic innervation.[11]

Mechanism of Action of Glucagon:

Hepatic Action:

Specific glucagon action is thought to be mediated by increased phosphorylase activity which renders more glucose 1-PO_4 available for glucose production.[12] This scheme of action is illustrated in Figure I. Sutherland has shown that the concentration of active phosphorylase in liver tissue is the result of a balance between two opposing reactions:[13] the inactivation of the enzyme by phosphorylase phosphatase (IE) and the reactivation of the resulting dephosphphosphorylase by the phosphokinase system. Sodium fluoride has been shown to decrease the rate of formation of dephosphophosphorylase by inhibiting phosphorylase phosphatase, thereby allowing the constantly operating phosphokinase system to bring about a net increase in liver phosphorylase concentration. Epinephrine and glucagon may stimulate some portion of the phosphokinase system and thus cause a net increase in liver phosphorylase concentration.

Figure I: Mechanism of Action of Glucagon



Schulman, J. L.; and Greben, S. E.: The Effect of Glucagon on the Blood Glucose Level and the Clinical State in the Presence of Marked Insulin Hypoglycemia. J. Clin. Investigation, 36: 74, 1957.

Extrahepatic Action:

Renal: Staub has observed that the renal excretion of sodium, chloride, potassium, inorganic phosphate and I^{131} is increased 200 to 500 percent by glucagon.[14] Elrick suggests that "this action is not due to increased glomerular filtration, hyperglycemia or osmotic diuresis but it is due to a direct tubular effect of glucagon."[15]

Peripheral Utilization of Glucose: DeMeio and Pincus incubated rat hemidiaphragms in Warburg flasks in the presence of glucose, labeled glucose, and, except for the controls, insulin or insulin plus glucagon.[16] In addition to phosphate buffer each flask contained enough labeled glucose to give 100,000 counts per ml./min. Insulin was present in a concentration of .5 units per ml. and glucagon (Lilly 208 - 158B - 197) when present was in a concentration of .1 mg./ml. At the conclusion of the incubation period the muscle was removed, washed in cold buffer containing 500 mg. per cent glucose, placed in 3 ml. of hot potassium hydroxide and the glycogen subsequently precipitated with alcohol. Total glycogen values were determined using anthrone. An aliquot of the glycogen was further refined and plated for C^{14} determination. Results showed that the addition

of glucagon invariably resulted in smaller "insulin effect" than when insulin was present. However, Elrick states that "these workers used large doses of relatively crude glucagon preparations (10 and 50 per cent purity) and that other workers have shown that glucagon stimulated the uptake of glucagon by the rat diaphragm.[16]

Elrick, Hlad and Witten have presented evidence that glucagon, in addition to mobilizing liver glycogen, increases the perpheral utilization of glucose.[17] Twenty-four male subjects which were considered normal with respect to carbohydrate metabolism were studied. The test procedure was began in the morning after a fourteen hour fast. A 10 per cent solution of glucose was infused intravenously at a constant rate for periods of 120 to 145 minutes. In twelve subjects glucagon (17 mg. to 1.0 mg.) was added to the perfusate following an initial period with glucose alone. In twelve subjects the glucose infusion rate was doubled after 50 minutes and after 90 minutes the initial rate was resumed, in order to stimulate the hyperglycemia caused by glucagon. Glucose determinations were done venous (indwelling needle in antecubital vein) and capillary (finger) blood from the same arm at 5 and 10 minute intervals. Two types of response to glucagon were observed. In eleven subjects

the blood sugar fell after reaching a peak level, despite the constant infusion of glucagon. 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 the arteriovenous glucose differences. In the twelve 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 arteriovenous glucose differences were much less. These results were interpreted by Elrick as evidence that glucagon causes an increased peripheral utilization of glucose. These workers have further shown that the administration of glucagon and insulin together increase the peripheral utilization of glucose significantly more than either hormone does alone.[18] Elrick states that "these findings indicate that glucagon has a dual, integrated action on carbohydrate metabolism which consists of mobilization of liver glycogen and enhancement of peripheral glucose utilization." He suggests that "the body has a mechanism (glucagon and insulin) whereby it can markedly increase glucose utilization and at the same time

maintain greater constancy of blood sugar level and liver glycogen content than is possible with either hormone alone." This concept is shown below in Figure II.

It has also been suggested that it may be possible to augment the effect of insulin on peripheral glucose utilization with glucagon in diabetic patients.[18]

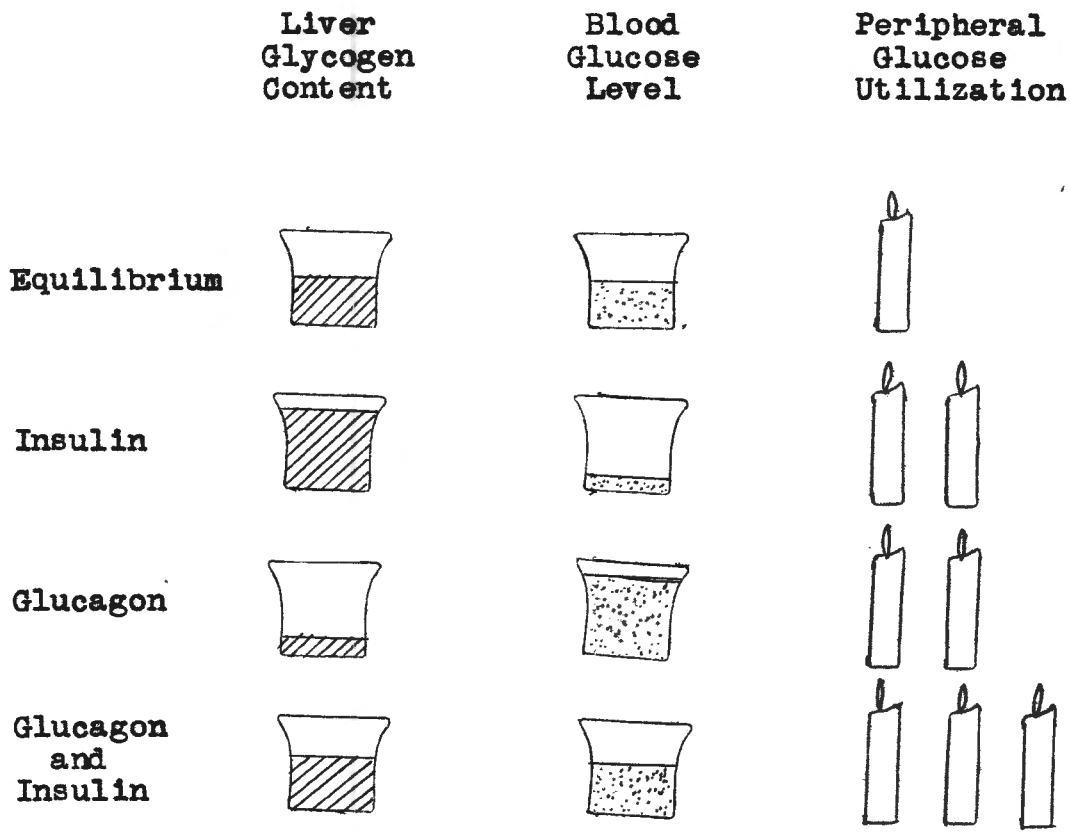
Glucagon as an Etiologic Agent of Diabetes Mellitus:

De Duve suggested in 1953 that the blood glucose level was maintained by an equilibrium between glucagon and insulin.[19] However, he also expressed the view that glucagon was an "insulin antagonist" and that "an excess of it was a factor in diabetes mellitus." However, the above stated role of glucagon in carbohydrate metabolism coupled with the failure to produce permanent diabetes mellitus by the chronic administration of glucagon is strong evidence against the view held by De Duve.[20]

Glucagon as an Etiologic Agent of the Zollinger Ellison Syndrome:

Zollinger and Ellison postulated in 1955 that glucagon might be a factor in the Zollinger Ellison Syndrome in which gastric hypersecretion, peptic ulceration and islet tumors are noted.[21] They postulated the existence

Figure II:



Hypothesis of the Role of Glucagon in Carbohydrate Metabolism. Elrick, H., Staub, A., and Helmut, M. Recent developments in glucagon research. New Eng. J. of Med. 256: 742-746, 1957.

of an "ulcerogenic factor" which had its origin in the islet cell tumor and suggested that glucagon might be this factor. They believed that they had isolated glucagon from the blood of one of their patients but were subsequently unable to confirm this finding. Eiseman and Maynard have been unable to isolate glucagon from the serum of a patient with the Zollinger Ellison Syndrome.[22]

Previous Clinical Use of Glucagon:

Schulman and Gieben have employed glucagon to terminate hypoglycemic coma in schizophrenic patients undergoing insulin shock therapy.[15] The glucagon was given by intravenous, intramuscular, and subcutaneous route. It was used intravenously in ninety-seven patients in dosages ranging from .003 mg./kg. to .3 mg./kg. of body weight. On ninety-five of the ninety-seven cases the coma was successfully terminated. Glucagon was given intramuscularly on twenty-two occasions, the dose varying from .02 mg./kg. to .1 mg./kg. of body weight. Nineteen of the terminations were successful. The three patients who did not awaken responded to intravenous glucose. Glucose was given subcutaneously on eleven occasions with seven awakenings and four failures.

The average termination time of the hypoglycemic comas with intravenous glucagon was 13.5 minutes while with intramuscular administration it was 16.9 minutes. No time was given for subcutaneous administration. At no time was any adverse effect noted following administration of glucagon, either locally or generally. The average blood sugar level just prior to the administration of glucagon was 15 mg. per cent. When glucagon was given intravenously the average time of maximal blood sugar elevation was 25.2 minutes with a range of 17 to 40 minutes with an average blood sugar elevation of 32.6 mg. per cent. When given intramuscularly the average time of maximal blood sugar response was 31.6 minutes with a range of 15 to 56 minutes and an average elevation of blood sugar of 22.2 mg. per cent. The above author advocates the intravenous route because of the greated rise in blood sugar.

Use of Glucagon to Terminate Insulin Induced Hypoglycemic Reactions:

This report concerns experience with glucagon used to treat mild insulin reactions on a pediatric ward, medicine ward, and in a summer camp for diabetic children. Glucagon (Lilly, 1 mg. per ml.) was given subcutaneously

in a dosage of 0.03 mgm/kg. body weight in cases I, II, III, and VI and in a dose of 1 mg. in cases IV and V. For purposes of the present observations, the attendants refrained from administering carbohydrate for one hour following the administration of the glucagon. Capillary blood was used for patient R. F. Venous blood was obtained from the remainder of the patients. Blood sugar was determined by the Benedict method after preparation of a tungstic acid filtrate.

Case I:

R. F. was a 25 month old boy with diabetes of one week known duration when admitted to the University of Nebraska Hospital on 4/27/57. He was receiving a mixture of Semi-Lente and Ultra-Lente insulins in a ratio of 3 to 1. His total daily insulin dosage was given before breakfast, and ranged from 10 to 30 units, adjusted by a plan of flexible insulin dosage.[23] His diet contained approximately 1200 calories and included between-meal and bedtime snacks. On 5/21, his hypoglycemia was manifested by unconsciousness; on 5/28, he was merely drowsy and irritable.

Case II:

A. K. was a 10 year old girl with diabetes complicated by hyperthyroidism, who was admitted to the University of Nebraska Hospital on 5/14/57. Her diabetes was of three years duration. During these studies, the patient was receiving propylthiouracil in preparation for subtotal thyroidectomy. On the first four tests, hypoglycemia was suspected because of dizziness. Total daily insulin dosage of ordinary Lente was given before breakfast. Insulin dosage ranged from 24 to 44 units. Diet contained approximately 2500 calories. The study on 8/3 was two days post-thyroidectomy. At that time, she was having some respiratory difficulty and complained of dizziness. This was interpreted clinically as hypoglycemia, but the blood test later showed that her blood sugar was actually elevated. The glucagon caused further rise. See Table I.

TABLE I

EFFECT OF SUBCUTANEOUS GLUCAGON ON BLOOD SUGAR

PATIENT	DATE	BLOOD SUGAR mgm/100 ml.				COMMENT
		Before Glucagon	15 min.	30 min.	60 min.	
R. F.	5/51	20	41	64	54	Responded clinically within 8 min.
	5/28	32	127	82	80	Responded within 10 min.
A. K.	5/29	55	79	-	110	Responded within 10 min.
	5/31	72	140	190	187	
	7/24	41	111	155	123	Felt better within 10 min.
	7/29	40	112	170	160	Felt better within 10 min.
	8/3	170	268	300	268	No improvement in sympt.

Case III:

This was a 10 year old girl with a history of diabetes of 2 year duration who was attending summer camp. Shortly before the noon meal she complained of weakness and within several minutes became somewhat disoriented and was able to sit only with assistance. She was given glucagon in a dosage of .03 mg. per kilogram of body weight subcutaneously. Three minutes after the injection she appeared well oriented and stated that she was feeling stronger. Five minutes after the injection she expressed the desire to walk back to her tent from the dining hall (approximately one quarter mile) stating that she felt quite well but that she was no longer hungry. Due to technical difficulties, blood sugar determinations were not obtained in this case.

Case IV:

A. J. P. This patient was a 24 year old white female with a history of diabetes of 15 years duration. She was being maintained on 50 units of N. P. H. insulin per day and a 2000 calorie diabetic diet. During her period of hospitalization at University Hospital she had several insulin reactions, one of which was treated by the subcutaneous injection of 1 mg. of glucagon. Marked

subjective and objective improvement occured within five minutes after the administration of the glucagon. The blood sugar just prior to glucagon administration was 50 mg. per cent and ten minutes later was 55 mg. per cent. Although the increase in blood sugar was only 5 mg. per cent here the prompt termination of the hypoglycemic reaction was in all probability due to the increased peripheral utilization of glucose as outlined earlier in this report.

Case V:

Mr. L. was a 28 year old white male with a history of diabetes of 14 years duration. He was being maintained on a 2000 calorie diabetic diet and 35 units of N. P. H. insulin and regular insulin per clinitest. On one occasion shortly before supper he became somewhat disoriented, complained of headache and hunger and became comatose. One mg. of glucagon was injected subcutaneously and the patient regained consciousness within 5 minutes and appeared alert and well oriented 7 minutes following the injection. On another occasion the patient complained of hunger, nervousness, and headache and an attempt was made to give glucose orally. Convulsions occurred however and were terminated by the administration intravenously of 50 per cent glucose and water. No blood sugars were obtained during either of the above episodes.

Case VI:

A. F. was a 4 year old white male with a history of diabetes of approximately one months duration and was receiving 12 units of Lente insulin daily. At 11:50 A. M. the patient was noted to be hyperirritable, perspiring excessively, and complaining of hunger. He was given glucagon .03 mg./kg. with complete remission of the above within 5 minutes. The blood sugar values obtained were as follows: before glucagon administered, 20 mg. per cent; 15 minutes following glucagon administration, 45 mg. per cent; 30 minutes following glucagon administration, 50 mg. per cent.

Summary:

Following the discovery of insulin and the beginning of its use in the diabetic patient, hyperglycemic glycogenolytic tendencies were noted in addition to its hypoglycemic effect. This was at first thought to be an action of insulin but later workers discovered that it was due to the presence of a hyperglycemic glycogenolytic substance which is produced by the alpha cells of the pancreas. This contaminant, which was present in many insulin preparations, was called glucagon, a polypeptide entirely different in structure and amino acid content from insulin.

There is no present evidence indicating that the pituitary gland directly influences pancreatic secretion of glucagon, although there is suggestive evidence that the autonomic nervous system plays a role.

The intrahepatic action of glucagon is that of stimulation of some portion of the phosphokinase system which causes an increase in the amount of active phosphorylase an enzyme need in the process of conversion of glycogen to glucose. Glucagon, by a direct tubular effect, also causes an increased renal excretion of certain electrolytes.

Glucagon, in addition to mobilizing liver glycogen, also causes an increased peripheral utilization of glucose.

The balance of the present evidence does not point to glucagon as an etiological agent in diabetes mellitus or the Zollinger Ellison Syndrome.

Glucagon has been used successfully to terminate insulin induced hypoglycemic shock in schizophrenic patients.

In the author's study, glucagon was used successfully for the termination of hypoglycemic reactions occurring in diabetic children and adults. Glucagon was given in doses of .03 mg. per kg. of body weight by

subcutaneous route. It was successful in the termination of hypoglycemic reactions in all of the cases in which it was tried. No local or generalized complications were noted.

Conclusion:

Glucagon, a hormone produced by the alpha cells of the pancreas is a valuable agent in the treatment of hypoglycemic reactions occurring in diabetic children and adults. It possesses the ease of administration and rapidity of action of epinephrine and, in addition, does not possess any of the well known side effects of the latter. It is not an insulin antagonist, produces no local or generalized detrimental effects and is not an etiological factor in any disease state. Although some favor the intravenous route of administration, this writer favors the subcutaneous route because of the excellent clinical results noted using this route and the relative ease of administration in comparison to the intravenous route which may enable parents of diabetic children, in properly selected cases, to use this drug.

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