Studies of the Physiologic Role of Glucagon

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SUMMARY

By means of an exquisitely sensitive and highly specific radioimmunoassay, glucagon has been measured in the plasma of dogs and man for the first time. Its identification in the effluent plasma of the pancreas and the demonstration of alterations in its secretion induced by changes in blood glucose concentration support the view that it is a true hormone with a major role in blood glucose regulation. Glucagon secretion has been shown to rise during all forms of glucose need; this rise is suppressed by glucose refeeding. This favors the concept of glucagon as a hormone of glucose need, the function of which is to maximize hepatic glucose production when food is not available, thereby serving to maintain the flow of glucose to the brain.

The current status of efforts to identify a disorder of glucagon secretion in man is reviewed briefly.

Recent advances in methodology in the field of immunology[1] have made possible the development of highly specific and sensitive technics for the identification of various peptide hormones and for their measurement in plasma.[2-7] Glucagon has been a prime target for the application of these methods because of longstanding uncertainty as to its hormonal status. It is the purpose of the following review to summarize briefly the evidence, obtained over the past five years by means of a radioimmunochemical assay,[8-10] of the role of glucagon as a regulatory hormone of blood glucose homeostasis, and to examine the initial results of efforts to identify disorders of glucagon secretion.

RADIOIMMUNOASSAY FOR GLUCAGON

In 1956, Berson, Yalow, Bauman, Rothschild, and Newerly[1] characterized the reaction between insulin-I-131 and insulin antibodies and thereby set the stage for the subsequent development of assays, based on inhibition of radioantigen-antibody reaction. These methods far surpass in sensitivity, specificity, and reproducibility the earlier hemagglutination inhibition technics.

Glucagon, like insulin, was found to be antigenic in rabbits, and glucagon antibodies were found to share most of the characteristics of insulin antibodies. The ability of unlabeled glucagon to compete with glucagon-I-131 for antibody in relation to its concentration provided the basis for a radioimmunoassay for glucagon.[4,5] Figure 1 reveals the typical standard curve obtained with the lot of rabbit antiserum currently in use. This antiserum permits the measurement of as little as 150 to 200 $\mu\mu$g./ml. of glucagon with confidence, and with a reproducibility approaching ± 2 per cent. Specificity is considered to be high, and no protein other than glucagon has been found to compete in the system.

Because beef and pork glucagon are the only purified glucagon preparations now available, the assay system employs rabbit antibodies to beef-pork glucagon, beef-pork glucagon-I-131, and beef-pork glucagon standards. Fortunately, the crude pancreatic extracts of all mammalian species thus far checked, including man, dog, mouse, and rat, compete for anti-beef-pork glucagon antibodies, thus permitting a choice of experimental animals. However, because of the possibility of decreased reactivity of the glucagon of these species, results are expressed as "$\mu\mu$g. equivalents," rather than $\mu\mu$g., to indicate the possibility that they may not be truly quantitative.

Plasma obtained from the pancreatic venous effluent of normal fasting dogs was found to inhibit the reaction between glucagon-I-131 and antibodies,[8] indicating the presence of a substance immunologically indistinguishable from glucagon. Like glucagon, this substance in plasma was found to have an affinity for cellulose, as determined by assaying specimens of plasma before and after passage through a cellulose column.[8] Furthermore, its concentration in pancreaticoduodenal venous plasma was higher than in inferior vena caval plasma, as would be expected of a sub-

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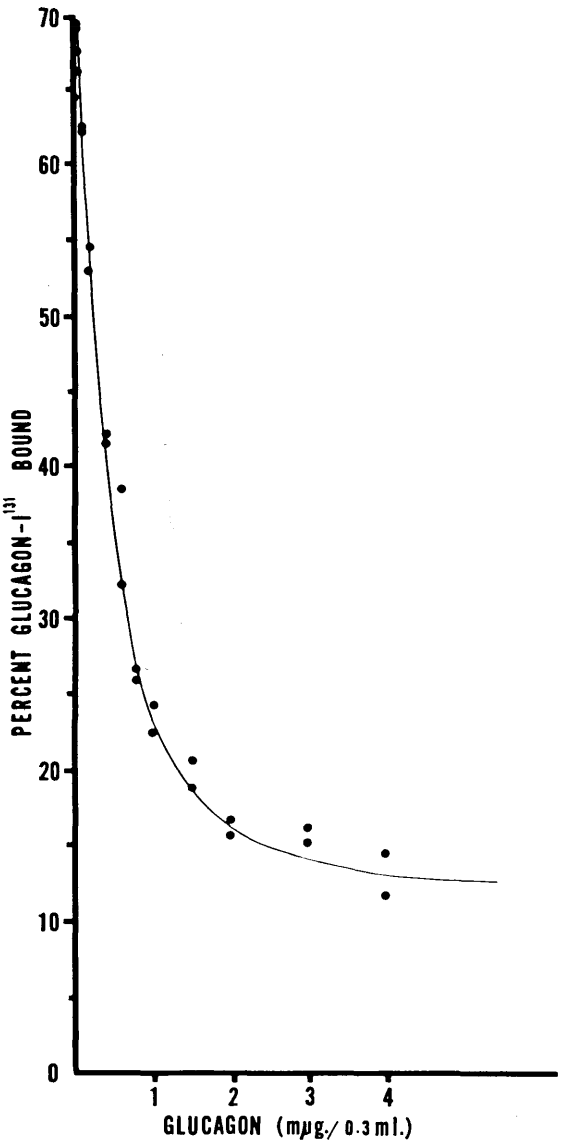

FIG. 1. A representative standard curve for the glucagon assay.

stance which is secreted by the pancreas and bound to hepatic tissue on its initial circulation through the liver.[8] It was, therefore, concluded that this substance was endogenous canine glucagon. The measurement of circulating glucagon in man presented a more formidable problem for a variety of reasons, among them the highly destructive effect of human plasma upon the glucagon-I-131.[9] Although whole human plasma can be assayed for glucagon, the sensitivity and reproducibility is considerably reduced and only major changes in concentration could be measured with confidence. For this reason, acid-alcohol extraction of plasma is now employed routinely prior to assaying glucagon in human plasma, thereby enhancing reproducibility and sensitivity.

STUDIES OF GLUCAGON SECRETION

The hyperglycemic, glycogenolytic, and gluconeogenic properties of glucagon would lead one to predict that need for glucagon would be greatest in time of glucose need; the studies of Foà and co-workers[10] did, in fact, demonstrate a hyperglycemic factor in the pancreatic effluent of dogs made hypoglycemic with insulin. For these reasons, the possible role of glucagon in maintaining blood glucose homeostasis was examined in the following experiments by measuring its secretion during various forms of glucose need.

Dogs, made acutely hypoglycemic by the rapid administration of 0.8 U./kg. of glucagon-free insulin, showed a gradual rise in glucagon secretion which became statistically significant at two hours and reached strikingly high levels at three hours after the injection.[8] When insulin was administered by continuous infusion at a rate of 0.01 U./min., the rise in glucagon secretion was more uniform and began earlier. While there is little doubt that insulin-induced hypoglycemia is followed by a substantial rise in glucagon secretion, the effect is not an immediate one, and varies in intensity from animal to animal. It appears to be correlated with both the depth and duration of the hypoglycemia.

Severe chronic hypoglycemia induced by the administration of phloridzin to a group of nine dogs was accompanied by a more striking and consistent rise in glucagon secretion. The mean pancreaticoduodenal venous glucagon level in the hypoglycemic group was approximately four times that of the control group, and a statistically significant inverse correlation between glucagon and glucose concentration was observed in these experiments.[8]

In man, chronic glucose need induced by total starvation was associated with a threefold rise in mean peripheral venous glucagon concentration after seventy-two hours of fasting (figure 2).[9]

Thus, it would appear that glucose lack, no matter how induced, is followed by an increase in glucagon secretion. The only other stimulus to glucagon release thus far observed is cobaltous chloride; its administration to rats was found to induce a sudden, short-lived rise in the portal venous concentration of glucagon forty-five minutes after its injection.[11]

The effect of rapid glucose loading upon hyperglu-

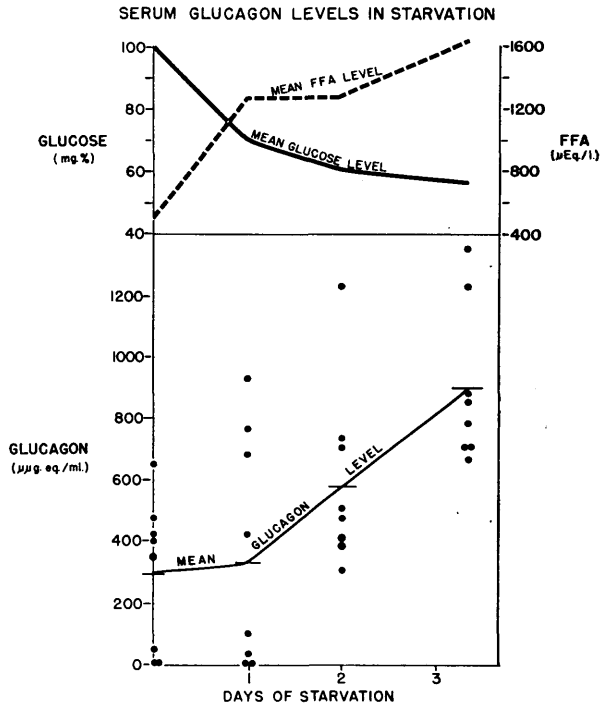

FIG. 2. After three days of total starvation the plasma glucagon level rose in all subjects to an average of 893 μμg./ml. from a prestarvation mean of 292 μμg. Eq./ml. (Reprinted with permission of "The Journal of Clinical Investigation," vol. 42, 1963.)

cagonemia induced by glucose need was evaluated by the rapid intravenous administration of 25 gm. of glucose to dogs made hypoglycemic by insulin infusion or by phloridzin. In all cases, hyperglycemia was accompanied by a rapid fall in glucagon to baseline levels; this suppression of hyperglucagonemia persisted until the blood glucose concentration had returned to a near-normal level, following which a rebound usually occurred.[8]

The results of the foregoing experiments reveal the presence of endogenous glucagon in pancreatic venous effluent and indicate that glucagon secretion is enhanced during glucose need, irrespective of cause, and that sudden glucose replenishment abruptly abolishes this hyperglucagonemia. These findings provide inferential support for the concept of glucagon as one of the hormones of glucose need, one probably concerned with maintaining the maximal hepatic glucose output. The obvious beneficiary of this effect would be vital glucose-dependent tissues such as the brain.

The concept of the islets of Langerhans as a bihormonal organ, regulating the disposition of glucose to the tissues of the body according to glucose availability, is an attractive one. Insulin is the hormone of glucose abundance (figure 3A) concerned with storage of ingested glucose in fat, muscle and liver cells, tissues which, without insulin, do not admit or retain glucose; glucagon secretion would be minimal under these circumstances. During glucose need, however, glucagon secretion rises, thus maintaining the maximal possible flow of new glucose to the brain, a task which is aided by a concomitant decline in insulin secretion and a rise in growth hormone secretion, thus minimizing unnecessary diversion of glucose to insulin-dependent storage sites (figure 3B).

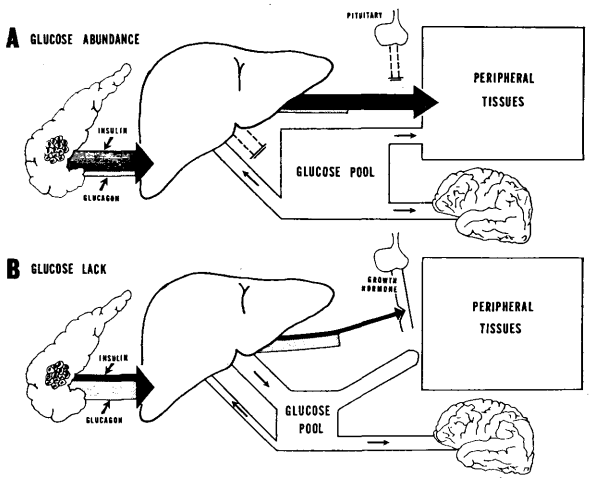

FIG. 3. A schematic representation of the functional interrelationship of insulin, glucagon, and growth hormone under varying extremes of glucose availability. During glucose abundance (A), insulin is secreted to enable storage of glucose as glycogen or fat. During glucose lack (B), the "anti-storage" hormones, glucagon and growth hormone, serve to maintain maximal flow of glucose to the brain.

Figure 4 represents actual measurements of insulin, glucagon, and growth hormone made during a five-hour oral glucose tolerance test in four normal subjects. Although the number of observations are few, there is the suggestion of a fall of glucagon concentration occurring from forty to 240 minutes after glucose ingestion; growth hormone is also depressed during this period as shown previously by Glick, Roth, Yalow and Berson.[12] Suppression of these hormones would favor maximum hepatic and peripheral storage of glucose. At 300 minutes, both glucagon and growth hormone appear to rise, suggesting that the "storage phase" is ended and the "anti-storage" phase begins, so as to conserve glucose for the glucose-dependent tissues of the central nervous system.

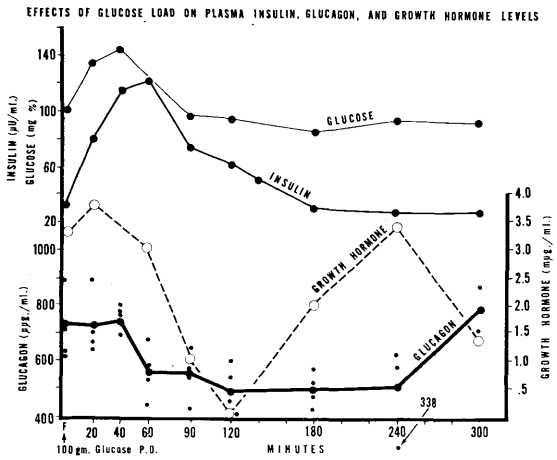

FIG. 4. The mean plasma level of insulin, glucagon, and growth hormone in four normal subjects following the ingestion of 100 gm. of glucose after an overnight fast. Insulin rises rapidly in parallel with the glucose concentration. Glucagon and growth hormone levels decline after forty minutes and remain depressed until the second or third hour, as if to facilitate storage of glucose in liver and fat. After the third hour, when the storage phase is ended, their concentration rises, as if to oppose further glucose storage at the expense of the brain.

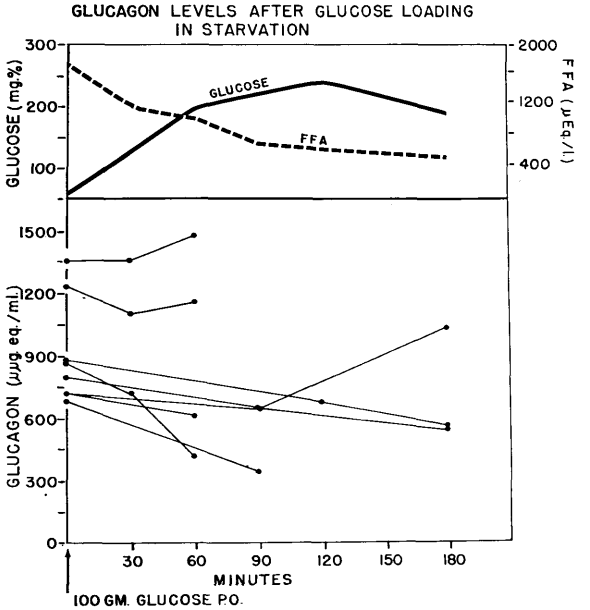

FIG. 5. After three days of starvation, normal subjects were given 100 gm. of glucose orally. Despite marked hyperglycemia, the glucagon levels did not decline rapidly. (Reprinted with permission of "The Journal of Clinical Investigation," vol. 42, 1963.)

DISORDERS OF GLUCAGON SECRETION

If the hormonal interrelationships suggested above are of critical importance in regulating blood glucose concentrations, it follows that disorders of blood glucose regulation might result from absolute or relative changes in these patterns. A disorder in glucagon secretion would be of particular interest, because such a lesion has not been previously identified.

Thus far the search for "glucagon diseases" has not been fruitful, but more extensive screening of the various hypoglycemic and hyperglycemic syndromes will be required before their existence can be excluded. Certain apparent abnormalities have been encountered, however, and these will be summarized.

Post-starvation hyperglucagonemia: The marked hyperglucagonemia present after seventy-two hours of starvation appears to be less readily suppressed by glucose loading than is normally the case.[9] Despite the more intense hyperglycemia of "starvation" diabetes, glucagon concentrations declined slowly, and, in six of eight subjects remained well above the normal fasting values for at least sixty to ninety minutes (figure 5). It is reasonable to wonder if the persistence of the starvation hyperglucagonemia plays an etiologic role in the diabetic glucose tolerance curve, although many plausible alternative explanations come to mind.

Glucagon in tumors: In the search for a "glucagon disease," the glucagon content of several malignant islet cell tumors, considered on histologic grounds to be of alpha cell origin, was determined by radioimmunoassay! Hepatic metastases from such lesions were extracted by the method of Kenny,[13] and were found to contain from .002 to .064 mug. of glucagon per gram of wet tumor weight, while adjacent uninvolved liver tissue contained no measurable glucagon. These findings were initially interpreted as evidence for the existence of glucagon-secreting tumors, "glucagonomas."[14]

Subsequently, four hepatic metastases from patients with nonislet cell malignancies, selected from random autopsies, were assayed for control purposes. One of the metastases, an anaplastic carcinoma of bronchial origin, contained 0.55 ug./gm. of wet tumor weight, almost ten times as much as the maximum found in the alpha cell malignancies.[15] The importance of the quantities of glucagon noted in the alpha cell tumors is, therefore, in doubt, and the existence of glucagon-secreting alpha cell neoplasms has not been proved.

However, the surprising finding of large quantities of glucagon in an hepatic metastasis from an undifferentiated bronchogenic carcinoma was of considerable interest, particularly since insulin immunoassays revealed the presence of insulin as well, in a concentration of approximately 0.3 U./gm. of wet tumor weight. If these hormone concentrations are representative of the

other hepatic metastases in this patient's liver, estimated to weigh approximately 1,000 gm., then 0.5 mg. of glucagon and almost 300 U. of insulin were present in the liver alone.

These startling results in a patient not known to be suffering from a disorder of carbohydrate metabolism prompted further efforts to substantiate the identity of the hormones. Despite the presumably high specificity of radioimmunoassays for both glucagon and insulin, the physicochemical and biologic properties of the so-called tumor hormones were examined.[15] The tumor "glucagon," like pancreatic glucagon, was destroyed by trypsin but not by cysteine, had the identical electrophoretic properties and, on the basis of gel filtration studies, appeared to have a similar molecular size. The tumor "insulin," like pancreatic insulin, was totally destroyed by cysteine, had identical electrophoretic properties and appeared to be similar in molecular size.

Finally, assay of biologic activity, performed in Dr. Holbrooke Seltzer's laboratory by the rat hemidiaphragm technic,[16] revealed the presence of 0.3 U. of insulin-like activity per gm. of wet tumor weight in the extract of this tumor, whereas the other tumor extracts were devoid of activity. This combination of immunochemical, physicochemical and biologic evidence provides powerful support for the contention that this tumor extract did, in fact, contain true glucagon and insulin.

If this is true, the mechanism by which an hepatic metastasis, presumably of nonislet cell origin, contains both islet cell hormones in quantities found previously only in extracts of the pancreas must be explained. Ectopic synthesis in a nonendocrine tumor is a concept which has been invoked to explain the various syndromes of hormone excess encountered in patients with malignancy of nonendocrine origin and is, of course, biologically possible. However, the possibility that these highly disorganized cells of bronchogenic origin could suddenly acquire the highly sophisticated functions of both the alpha and beta cells does not seem likely. An alternative concept would ascribe the high hormone content to a nonspecific "sponge" effect, in which binding of hormones to tumor tissue takes place at a rate which exceeds the ability of the tumor to degrade them, thus resulting in progressive accumulation of hormones. While this might be devoid of physiologic significance in the usual patient, prolonged patient survival might be associated with the development of large, hormone-laden masses. In this event, accelerated breakdown of tumor tissue could result in the release of clinically excessive quantities of hormone. This mechanism might account for certain of the syndromes of hormone excess encountered in the course of massive nonendocrine malignancy.

Other possible disorders of glucagon secretion: At present the possibility of glucagon deficiency is being studied in patients with idiopathic hypoglycemic states, and in insulin-sensitive diabetics; evidence of glucagon hypersecretion is being searched for among patients with hyperglycemia of various types. As yet no conclusive evidence for the existence of either hyper- or hypoglucagonism has been obtained.

SUMMARIO IN INTERLINGUA

Studios Concernente le Rolo Physiologic de Glucagon

Per medio de un exquisitemente sensibile e altemente specific radio-immuno-essayo, le mesuration de glucagon ha essite complite pro le prime vice in le plasma de canes e de humanos. Su identification in le effluente plasma del pancreas e le demonstration de alterationes in su secretion inducite per alterationes in le concentration sanguinee de glucosa supporta le conception que glucagon es un ver hormon con un rolo major in le regulation de glucosa sanguinee. Ha essite monstrate que le secretion de glucagon se augmenta durante omne formas de demanda pro glucosa. Iste augmento es supprimite per le alimentation de glucosa. Isto favora le conception que glucagon es un hormon in le economia de glucosa con le function de stimular un maximal production hepatic de glucosa quando nulle alimento es disponibile e assi de assecurar que le fluxo de glucosa al cerebro es mantenite.

Le stato currente del effortios de identificar disordines del secretion de glucagon in humanos es revistate brevemente.

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Treatment of Hypercholesterolemia with Nicotinic Acid

Annoying side effects from nicotinic acid therapy consist of cutaneous flushing, gastrointestinal symptoms, and cutaneous changes. Most patients develop tolerance to the sensation of flushing but in those who failed to do so the substitution of a delayed release preparation lessened or eliminated this symptom. Gastrointestinal distress, consisting of heartburn and, in a few patients, nausea, did not seem to be lessened by using delayed release forms. Seven patients developed activation of peptic ulcer (Parsons, JAMA *173*:1466, 1960). Mild diarrhea occurred in three patients.

Many patients in the author's series complained of dryness of the skin. Four developed localized roughness and pigmentation suggestive of acanthosis nigricans. The author speculated that such alterations might indicate changes in cholesterol metabolism.

Other metabolic effects indicated alterations in hepatic function and impairment of glucose tolerance. In most cases hepatic dysfunction seemed to be reversible and presumably related to enzymatic reactions. During the first year of therapy the author found no significant evidence of hepatic damage. However, beginning in the second year of the study, eight patients showed significant sulfobromophthalein retention in two consecutive tests at least a week apart. Four of those underwent needle biopsy of the liver. The finding in these varied from marked cloudy swelling to fatty metamorphosis, inflammatory infiltration and, in one instance, fibrosis and cholangiolitic involvement. One of these subjects developed diabetes mellitus. The author felt that patients who were given a slowly absorbing form of nicotinic acid were more apt to have hepatic dysfunction, perhaps because of the continuous blood levels of the drug.

The glucose tolerance curve was altered in many patients taking nicotinic acid. Only three of the patients in this series developed frank diabetes mellitus and in these it was easily controlled. The levels of serum uric acid were increased slightly but no clinical symptoms could be attributed to this effect.

The author discussed, in considerable detail, possible mechanisms of action whereby nicotinic acid might alter cholesterol metabolism. They include (1) vitamin effects, (2) direct relationship to cutaneous flushing, (3) competition for methyl groups, (4) competition for glycine, (5) presence of nicotinic acid, (6) decreased fatty acid synthesis and increased cholesterol synthesis in the liver, (7) decreased cholesterol synthesis, (8) increased oxidation of cholesterol, (9) subclinical anorexia, and (10) artifact. The author considered the mechanism to be an open question, but he felt that tenable ideas included alterations in cholesterol synthesis and increased intrahepatic oxidation of cholesterol.

These studies are of great interest to investigators, not only because of potential clinical application, but because through them the chemistry and metabolism of cholesterol can be better understood. It is apparent that this method of treating hypercholesterolemic patients, like all others, is an experiment in which the patient must participate with the investigator. The possibility of inducing permanent diabetes mellitus or permanent hepatic dysfunction must be considered. The author has done an admirable job of collecting data, interpreting it conservatively, and studying his problem in sufficient depth to warn others of potential hazards.

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