Effect of Purified Glucagon (Hyperglycemic-Glycogenolytic Factor, HGF) on Carbohydrate and Corticoid Metabolism in Normal and Diabetic Subjects

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The presence of a substance in commercial insulin which has a blood sugar elevating effect has been known for thirty years. The factor, identified as the hyperglycemic-glycogenolytic factor, should more properly be called "glucagon" since it was first designated as such by Murlin[1] in 1923. Postulated to be a product of the alpha cells of the islets of Langerhans[2], it is carried so precisely through the purification steps used in the commercial preparation of insulin that the richest source of the factor has been commercially available amorphous insulin.

It has been suggested by some investigators that since the exact properties of the material have been so difficult to delineate, it may merely be an artefact, a denatured form of insulin, or a nonspecific substance having an epinephrine-like effect. Others have concluded that the factor is in all probability a hormone[3].

The question of specificity has yet to be answered since a substance having apparently identical properties can be obtained from the gastric mucosa[4]. Cells having some, but not all, of the staining characteristics of pancreatic alpha cells have been found in the gastric mucosa[5], and the two types of cells may be related.

Glucagon can be separated from insulin only with great difficulty. Most of the material previously available for study has been prepared by selective destruction or inactivation of insulin by treatment with either 0.08N potassium hydroxide[4] or with cysteine[6], procedures which leave glucagon relatively unharmed. Some insulin activity could usually be detected, however.

The authors have recently had available for study a highly purified preparation. Utilizing a new method of extraction, glucagon has been isolated by Staub et al[7], of the Lilly Research Laboratories, in crystalline form as illustrated in Figure 1.

Chromatographic analysis has revealed definite differences in the amino acid content of glucagon and insulin. Cysteine, proline and isoleucine are known to be present in insulin and are not found in glucagon. Methionine and tryptophane are both present in glucagon and are not present in insulin. The preparation used in this study was not the crystalline form, although prepared by the same technics and of such purity that one milligram of dry glucagon contained

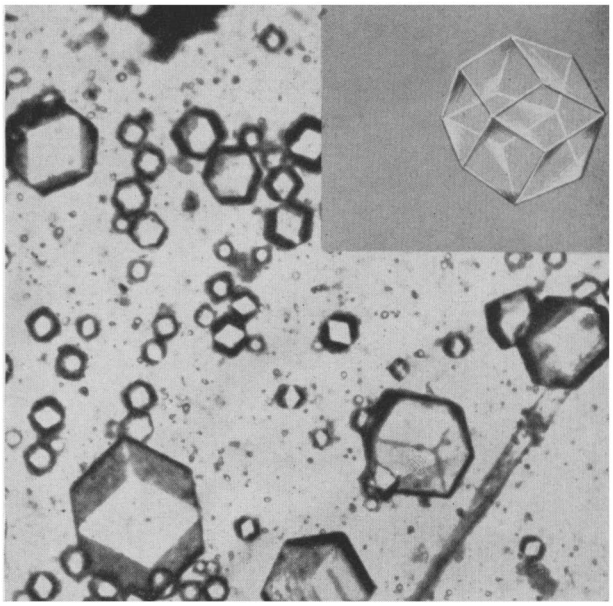

FIGURE 1—Glucagon Crystals. Reproduced courtesy Staub, Sinn and Behrens[7].

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only 0.005 units of insulin as estimated by animal assay.

Hyperglycemic activity also required stardardization, and the following has been proposed tentatively as a unit. In terms of the biological activity, the unit has been defined as that amount of material per kilogram body weight which, on intravenous administration into a twenty-four-hour fasted, anesthetized (Amobarbital Sodium) cat, produces a blood sugar rise of 30 mg. per 100 cc. within twenty-five minutes[7]. The preparation used in this study assayed at such potency that 0.5 microgram equaled one unit.

Most of the experimental work reported to date has consisted of *in vitro* studies, although several reports on the effect of glucagon on the metabolism of the intact experimental animal and the human are available.

Sutherland and others[3,8,9], utilizing liver slice and liver homogenate technics, have shown that of the three enzymatic reactions involved in the conversion of glycogen to glucose, the limiting one is the phosphorylase reaction which depends upon the ratio of active to inactive phosphorylase. See Figure 2. Glucagon and epinephrine act by accelerating the resynthesis of active phosphorylase, and thereby enhance the production of glucose-1-phosphate. Phosphoglucomutase and phosphatase, necessary for the two additional steps, have been shown to be available in excess or at least in optimal amounts.

Sutherland also has shown that, in contradistinction to epinephrine, intravenous administration of glucagon to the intact animal, although causing as rapid an increase in blood glucose, is not accompanied by a rise in blood lactate, indicating that such a single injection did not influence muscle glycogen.

Myers[10] has shown, by using hepatic vein catheterization, both in animals and in human beings, that hepatic venous glucose concentrations rose more abruptly and more intensely than did peripheral blood glucose values,

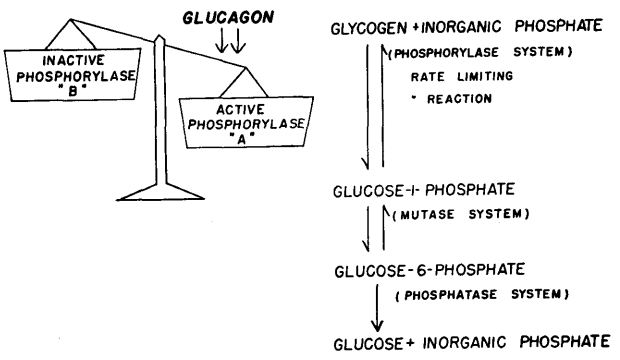

FIGURE 2—Site of action of glucagon. Stimulation of phosphorylase system.

again confirming earlier work of Collens and Murlin[11] that the site of the hyperglycemic effect was in the liver.

PRESENT STUDY

In an effort to delineate more exactly the effects of glucagon in man, the purified preparation previously described was given intravenously to normal persons and to diabetics of various degrees of severity. All the subjects were eating well, had no complaints and were hospitalized only for the tests. A purely arbitrary dose of 20 units per kilogram weight was selected and utilized throughout the study.

On account of the transient effect of glucagon, an intravenous infusion over a half-hour period was employed to bring out measurable physiological effects which might be too fleeting to be significant using a single injection.

The procedure used was as follows: All subjects were given the substance in the fasting state. The diabetics did not receive insulin on the morning of the test. Glucagon in dosage based upon 20 units per kilogram of body weight was administered over a half-hour period, blood samples were drawn before and at periodic intervals after the infusion, and determinations were made for blood glucose, pyruvate, lactate, inorganic phosphate and potassium. Fractional steroid excretion of urinary 17-hydroxycorticoids and 17-ketosteroids was determined as well as creatinine excretion.

The hepatic glycogenolytic effects of both glucagon and epinephrine have been shown to occur in the same site, that is on the activation of liver phosphorylase and, therefore, it was important to determine differences between the two if such existed. Consequently, several subjects who received glucagon were also given an epinephrine tolerance test. The latter consisted of the intramuscular injection of a 1:1000 solution of epinephrine, dosage being 0.01 cc. per kilogram body weight. Comparative blood determinations were made.

In the normal persons (Figure 3), blood glucose values were at their peak at the termination of the infusion of the glucagon and fell below initial levels between 60 and 90 minutes. The serum inorganic phosphate showed a significant fall with a return towards control levels within 120 minutes. The response of blood glucose and phosphate to epinephrine was consistent with the known effects. The interesting difference was the pyruvate response which rose after epinephrine but failed to rise and, in fact, fell after glucagon.

The typical response to glucagon and epinephrine

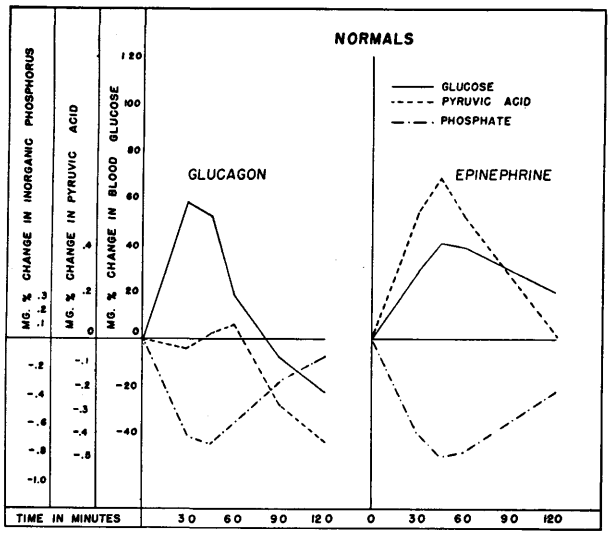

FIGURE 3—Typical glucose and phosphate response in nondiabetic after glucagon and epinephrine.

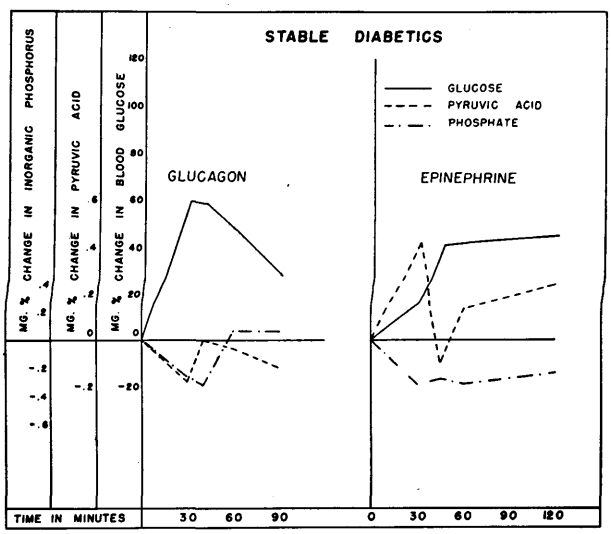

FIGURE 4—Prolonged glucose response and diminished phosphate fall in the stable diabetic.

of the *stable* diabetic is seen in Figure 4. In our classification, these are overweight, middle-aged patients who can tolerate insulin withdrawal and do not develop acidosis readily and whose blood sugar values tend to remain relatively stable throughout the day. Blood glucose values were abnormally elevated after both glucagon and epinephrine. There was a relatively minor fall in blood inorganic phosphorus after both glucagon and epinephrine; and no change in pyruvate occurred after glucagon but elevation did occur after epinephrine.

The most striking differences in response were exhibited in the *unstable* diabetics (Figure 5). These patients are usually, but not always, young, thin diabetics who show a remarkably wide variation in blood sugar when measured four times a day and who display a high degree of insulin sensitivity and are prone to develop acidosis easily.

After glucagon, blood glucose values did not rise as high as in the stable diabetic or normal subject. However, inorganic phosphate fell to levels comparable with the nondiabetic. In the case depicted, there was a rather profound fall in pyruvate. Low pyruvate levels were seen in others of this group, but were not as striking as in this instance. After epinephrine, blood glucose and pyruvate rose to unusually high levels, and inorganic phosphate fell to low levels indicating active phosphorus utilization. Lactate values paralleled pyruvate changes. Potassium changes were inconsistent and probably without definite significance, although there was a tendency for potassium levels to fall in a manner similar to phosphate.

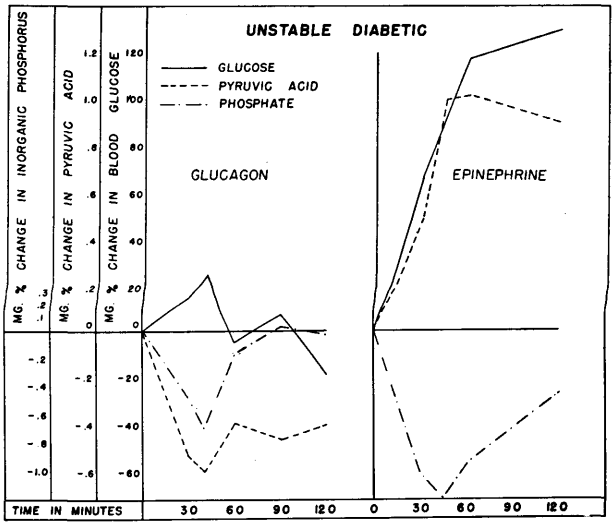

FIGURE 5—Marked response to epinephrine. A "normal" fall in phosphate after glucagon with diminished glucose response.

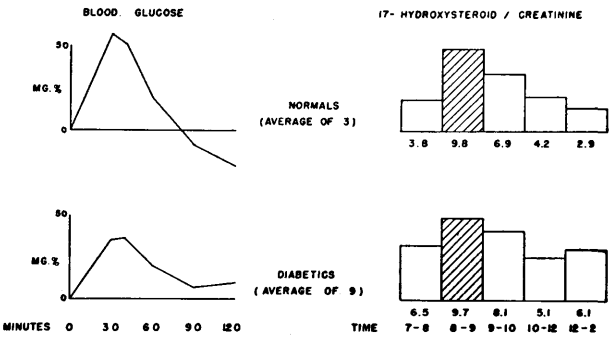

FIGURE 6—Shaded block indicates hour during which glucagon was administered.

The average excretion of urinary steroids of the three normals and the nine diabetics are shown in Figure 6. It would appear that all had some stimulation of steroid excretion during the hour in which glucagon was given. This occurred simultaneously with the hyperglycemic response. These data are based upon hourly 17-hydroxycorticoid/creatinine ratios.

Eosinophile determinations, made before and three hours after the glucagon administration, failed to disclose any consistent pattern of change.

COMMENTS

Different clinical types of diabetes respond differently to glucagon. The height of the glucose response can probably be related to the amount of liver glycogen immediately available. The unstable diabetic, who may be said to have no circulating insulin[12], presumably would have less liver glycogen than the normal as insulin will maintain adequate liver glycogen stores. Therefore, the rise in blood sugar after glucagon would be small. In contrast, the stable diabetic with some circulating insulin may have larger glycogen stores and a greater glucagon response.

If the decline in inorganic phosphate is indicative of carbohydrate utilization, then the unstable diabetic after glucagon can dispose of the hexose derived from liver glycogen to the same degree as the nondiabetic. This is difficult to explain in the light of Bornstein's[12] findings that these patients are deficient in circulating insulin and the known fact that phosphorylation is dependent upon the presence of insulin.

The stable diabetic shows a higher and more prolonged blood glucose elevation and a lesser fall in inorganic phosphate. This may indicate that glucose metabolism is retarded and glycogen mobilized from the liver by glucagon is retained in the blood for a longer period as glucose.

The lack of pyruvate response after glucagon could be accounted for if the hexose phosphate derived from liver glycogen did not follow the complete course of carbohydrate breakdown but was reconverted to glycogen, perhaps in the muscle. These data indicate that glucagon acts primarily upon liver phosphorylase. Elevation of pyruvate after epinephrine can be explained by its glycogenolytic action on muscle.

The enhanced urinary excretion of 17-hydroxycorticoids during the hour of infusion of glucagon may be due to stimulation of the adrenal as a result of a nonspecific stressful situation resulting from the infusion. The possibility of an increased rate of excretion of steroid by the kidney must also be considered.

SUMMARY

The administration of purified glucagon to normal and to diabetic subjects caused an elevation in blood glucose in all individuals.

Stable and unstable diabetics showed differences in their response to glucagon. The stable diabetic had a greater and more prolonged rise in blood sugar and a lesser fall in serum inorganic phosphate than the unstable diabetic.

In contrast to the effects of epinephrine, the infusion of glucagon did not result in an increase in blood pyruvate.

During the hour of glucagon infusion, there was an increase in steroid excretion as shown by determination of the 17-hydroxycorticoid/creatinine ratio.

REFERENCES

[1] Kimball, C. P., and Murlin, John R.: Aqueous extracts of pancreas. III. Some precipitation reactions of insulin. J. Biol. Chem. *58*:337-46, 1923.

[2] Sutherland, E. W., and de Duve, C.: Origin and distribution of the hyperglycemic-glycogenolytic factor of the pancreas. J. Biol. Chem. *175*:663-74, 1948.

[3] Sutherland, E. W.: The effect of the hyperglycemic factor of the pancreas and of epinephrine on glycogenolysis. Recent Progess in Hormone Research. *5*:441-63, 1950.

[4] Sutherland, E. W., Cori, C. F., Haynes, R., and Olsen, N. S.: Purification of the hyperglycemic-glycogenolytic factor from insulin and from gastric mucosa. J. Biol. Chem. *180*:825-37, 1949.

[5] Ferner, H.: Studien über die Histiophysiologie des Inselsystem der Bauchspeicheldrüse und den Diabetes mellitus. Virchows Arch. f. path. Anat. 390-432, 1951.

[6] Zimmermann, B., and Donovan, T. J.: Hyperglycemic effect of insulin. Am. J. Physiol. *153*:197-204, 1948.

[7] Staub, A., Sinn, L., and Behrens, O. K.: Purification and crystallization of glucagon (hyperglycemic-glycogenolytic factor). Science. *117*:628-29, June 5, 1953.

[8] Sutherland, E. W., and Cori, C. F.: Influence of insulin preparations on glycogenolysis in liver slices. J. Biol. Chem. *172*:737-50, 1948.

[9] Sutherland, E. W.: The effect of the hyperglycemic factor and epinephrine on enzyme systems of liver and muscle. Ann. New York Acad. Sc. *54*:693-706, 1951.

[10] Myers, J. D., Kibler, Robert F., and Taylor, W. Jape: Effects of pancreatic hyperglycemic factor on hepatic carbohydrate metabolism of man. Federation Proc. *11*:111, March, 1952.

[11] Collens, W. S., and Murlin, John R.: Hyperglycemia following the portal injection of insulin. Proc. Soc. Exper. Biol. & Med. *26*:485-90, 1929.

[12] Bornstein, J., and Lawrence, R. D.: Plasma insulin in human diabetes mellitus. Brit. M. J. *2*:1541-44, 1951.

DISCUSSION

I. J. PINCUS, M.D. (*Philadelphia*): I think Dr. Kirtley and his collaborators are to be congratulated for this very interesting study which set out to do three things: First, to confirm in the human, findings that had previously been made in experimental animal; this they have succeeded in doing. Secondly, to learn new facts about the physiological effect of this hyperglycemic factor; this, too, they have done. Thirdly, to try to determine what differences there might be between so-called stable and labile diabetics, and here a start has been made.

I should like to comment on many phases of their findings, but shall limit my comment to two. My associates and I are particularly interested in the results that Dr. Kirtley presented on the effect of HGF in regard to the hyperglycemia produced in the labile diabetic. Some years ago we found that these patients showed a large rise in sugar as compared to the normal and the stable diabetic. Our experiments, however, were done under different circumstances, using different preparations, and I think, more importantly, different dietary conditions.

Our patients had been fed and had received their normal morning's insulin before the study was done. It is possible, I believe, that these labile diabetics have a more marked rise, because they rapidly store, then rapidly lose their glycogen stores in the liver. It seems to me possible that more important than the level of liver glycogen is the responsiveness of liver glycogen to HGF, or other factors. It may be that the difference in responsiveness which Dr. Kirtley has discussed is extremely important in explaining some of the phenomena that occur in these patients.

I should like to speak for a moment about the results of the pyruvate determinations. The fact that epinephrine causes a rise and HGF no rise is of considerable interest and shows that the two operate differently on at least some enzyme systems. I should like to suggest the possibility that HGF actually does cause a fall in pyruvate, does act on a muscle enzyme in inhibitory fashion. Some of Dr. Kirtley's results seem too striking to be coincidence, and it is possible he has pointed to another physiological effect of this very interesting substance, which I think can explain some of the discrepancies that occur in clinical diabetes.

The Undernutrition Treatment of Diabetes

In 1916 the whole aspect of diabetes was changed by the revolutionary methods proposed by Frederick M. Allen. It is true that Naunyn and von Noorden had used fasting and vegetable days on occasion, that Guelpa had crudely employed the same and that in this country Hodgson had pointed out the advantages even as early as 1911 of restricted diet in the treatment of diabetes, but the fact remains that it was F. M. Allen who demonstrated on animals and then upon human beings that fasting and undernutrition would give new life to a diabetic, mild, moderate or severe.

I wrote in the preface of my first edition in 1916 "I would not have wished to write a book on diabetes three years ago; today it is a pleasure and an inspiration, because the improvement in treatment is beyond question. The introduction of fasting and emphasis on physical exercise in the treatment of diabetes by Dr. F. M. Allen, of the Rockefeller Institute of Medical Research, has decidedly changed the outlook for this class of patients. "Fasting" is in itself a distinct advance, but the practical simplification of treatment which it entails is an almost greater advantage. Now doctor and patient may know at once whether or not the treatment is successful."

The Allen method brought great alterations. The duration of life went up to 6.1 years, a gain of 25 per cent, and at that time this seemed especially notable. Age at death advanced to 46.7 years and the per cent of those living 20 or more years was changed to 3.1. Still more striking was the fall in deaths due to diabetic coma, from 63.8 per cent to 41.5 per cent, due to the low diet, the administration of small quantities of carbohydrate, liberal amounts of liquids and the prevention of nausea and vomiting largely by the avoidance of alkalies which in their turn we felt formerly blocked the kidneys through excretion of sodium oxybutyrate. Practically no cases of diabetic coma at this period originated in the hospital, a marked contrast to what occurred at the end of the last and the first few days of this century.

From *Diabetes Yesterday, Today, and Tomorrow,* by Elliott P. Joslin, M.D., in Proceedings of the American Diabetes Association, 1:122-123, 1941.