The Pathologic Effects of Large Amounts of Glucagon

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The work of Ingle[1] and Cavallero[2] has shown that glucagon will produce a temporary or mild glucosuria in partially depancreatized animals and in animals pretreated with cortisone, but a significant diabetogenic action of glucagon administered alone to intact rats has not previously been shown.

The results of early attempts made in our laboratory to show a diabetogenic action of the pancreatic hyperglycemic factor were disappointing. We reinvestigated the problem using much larger doses of glucagon attempting, at the same time, to prolong its activity by suspending it in corn oil and administering it subcutaneously at eight-hour intervals. Glucagon administered under these conditions appeared to exert a profound effect.

Figure 1 shows the average changes in weight and food intake of intact male controls injected with corn oil, and of normal male rats injected three times daily with 300 *u*g. of glucagon (Lilly, lot no. 258-234B-33) suspended in corn oil. Weight changes are shown for a second set of controls limited to the amount of food consumed by the glucagon-treated animals.

It is apparent that the glucagon-treated rats consumed much less food than the controls and lost weight rapidly. The weight loss cannot be completely attributed to the reduction in food intake, as the pair-fed controls lost much less weight.

The glucagon-treated animals were not glucosuric but animals similarly treated and encouraged to eat bread, frequently showed a transient but intense glucosuria. This stimulated us to investigate the effect of glucagon in force-fed rats.

Male Wistar rats weighing 150 to 160 gm. were fed by stomach tube at 8:00 a.m., 4:00 p.m., and 12:00 m., the high carbohydrate diet described by Reinecke, Ball and Samuels.[3] The volume administered was slowly increased until the tenth day; thereafter each rat received 10 ml. of the fluid diet, containing 6 gm. of solids at each feeding. Five animals served as controls and were injected with corn oil. The remaining five rats received subcutaneously at six-hour intervals a total of 1.2 mg. of glucagon daily. The glucagon was suspended in corn oil* in a concentration of 3 mg. per ml.

Figure 2 shows the average daily (a) glucose excretion, (b) urinary nitrogen excretion, and (c) body weight changes in the force-fed controls and in the glucagon-treated animals.

It is evident that the control rats excreted no glucose and gained weight during the experimental period. The glucagon-treated animals excreted approximately 4 gm. of glucose daily. Their urinary nitrogen excretion was nearly twice that of the controls and they lost weight rapidly. The blood sugar levels of the glucagon-treated animals remained between 350 to 450 mg. per cent throughout the day, while the controls showed only a slight increase in blood sugar concentration after each feeding.

During the experimental period the control rats remained in excellent health while the treated animals became emaciated and ill, with only two of the five rats surviving a seven-day period.

A second experiment was then performed in an attempt to produce permanent diabetes with glucagon. In this experiment twenty force-fed rats were used—ten control rats and ten glucagon-treated. The results substantiated those obtained in the first experiment. Intense glucosuria and hyperglycemia developed rapidly with some animals excreting as much as 10 gm. of glucose daily. However, this investigation was difficult to carry out. The force-fed control rats thrived but the glucagon-treated animals became increasingly ill until after a week they required constant attention. The treated rats suffered gastrointestinal disturbances and their stomachs frequent-

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* We have recently found that it is unnecessary to suspend the glucagon in oil. A neutral saline suspension is equally effective when administered subcutaneously.

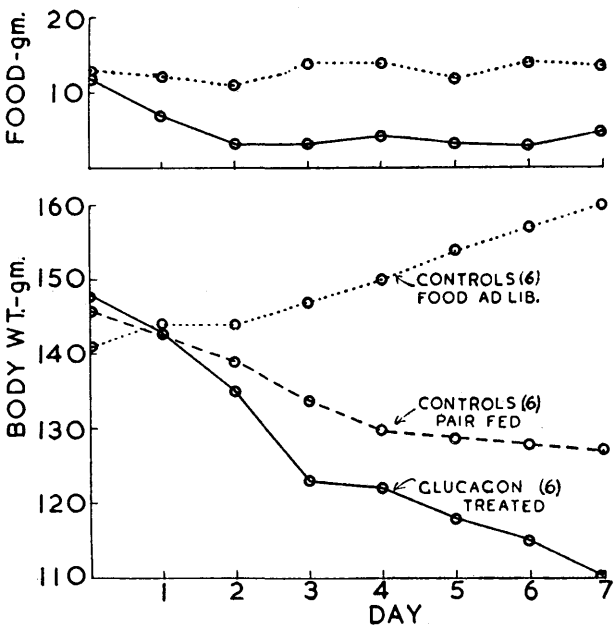

FIG. 1. Changes in the weight and food intake of male control rats injected with corn oil and of rats injected subcutaneously every eight hours with 300 gamma glucagon in oil.

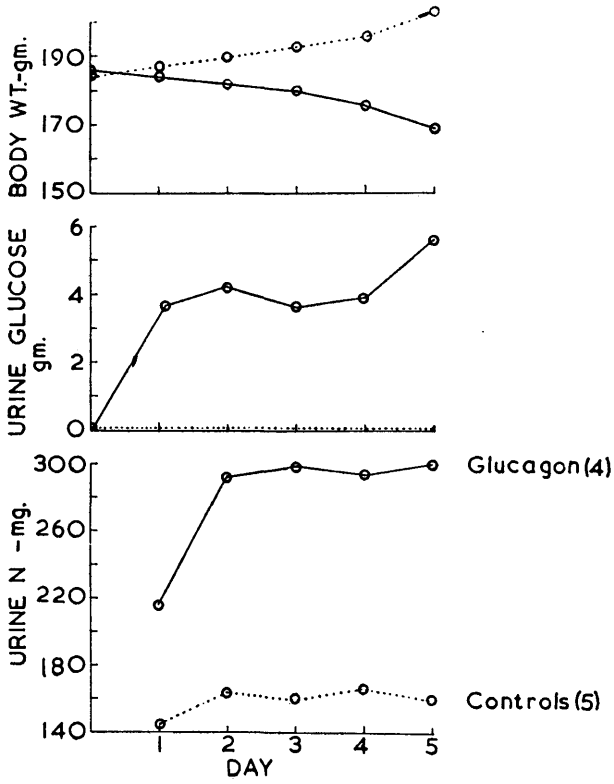

FIG. 2. Urinary nitrogen and glucose and body weight of force-fed controls and of force-fed rats injected every six hours with 300 gamma of glucagon.

ly became so distended that it was necessary to aspirate the contents. In other instances the animals would suddenly lapse into a state resembling diabetic coma with blood sugar levels between 800 to 900 mg. per cent; this latter condition responded dramatically to insulin therapy. We succeeded in maintaining only two force-fed rats on glucagon for a period of ten days. The animals remained glucosuric and hyperglycemic for six days following the cessation of all treatment. We feel that if treatment can be maintained for a longer time permanent diabetes may be produced.

In two normal dogs studied with Dr. James Campbell, glucagon in oil produced transient hyperglycemia and mild glucosuria. Since the supply of glucagon was limited the treatment of these animals was neither intensive nor prolonged. The dogs were sacrificed after one week.

Histological examination of pancreas revealed degranulation of the β-cells. The extractable insulin content of the pancreas was found to be only 15 per cent of the normal.

The histological appearance of the pancreatic β-cells in the glucagon-treated rats has been variable. In some instances these cells show degranulation and hydropic degeneration, in other cases the β-cells appear to be more intensely granulated than normal. In both dogs and rats, glucagon consistently produces marked degranulation and atrophy of the acinar cells. The significance of this observation is not evident.

Although the glucagon used in these investigations was highly purified, it contained considerable amounts of other proteins. The possibility that the diabetogenic effect was due to an unknown contaminant had to be considered. However, we have since found that pure crystalline zinc glucagon has the same diabetogenic action; thus we attribute our initial results to the action of glucagon alone. The results of these investigations clearly show that under our experimental conditions glucagon possesses marked diabetogenic properties when administered to force-fed rats and to dogs fed ad libitum. It is not known, at this time, that a permanent diabetes can be produced with the pancreatic hyperglycemic factor.

The mechanism(s) through which glucagon acts to produce diabetes is unknown. The effect of this substance on extrahepatic carbohydrate utilization remains a controversial subject. Studies carried out in vitro by Candela[4] and Snedecor, De Meio and Pincus[5] indicate that glucagon inhibits the stimulating effect of insulin on glucose uptake and glycogen synthesis by the isolated rat diaphragm. However, Smith working with Dr. F. G.

Young[6] has been unable to confirm this observation and Clarke of our department finds that the stimulating effect of insulin on glucose uptake by the rat diaphragm in vitro is enhanced if the animals are pretreated with glucagon.

Drury, Wick and Sherrill[7] have reported that the hyperglycemic factor slightly inhibits the disposal of blood glucose in eviscerated rabbits while Ingle, Nezamis and Humphrey[8] claim that it has no effect on glucose utilization in eviscerated rats. Studies of a-v differences lead Elrick et al[9, 10, 11, 12] to conclude that glucagon enhances peripheral utilization in normal human beings and in normal or depancreatized dogs. The observations made by Elrick in normal humans have been confirmed and extended by Van Itallie, Morgan and Dotti.[31] Bondy and Cardillo[14] could find no evidence of inhibition of glucose utilization in glucagon-treated humans. In our own department Dr. Margaret Henderson working with Dr. G. Wrenshall, has found in one experiment using C^{14}-labeled glucose an increase in the utilization of glucose following the administration of glucagon to depancreatized dogs.

Although a definite conclusion cannot be reached, the experimental data presently available do not favor the view that the genesis of glucagon diabetes in the intact animal is due to a reduction in peripheral glucose utilization.

Our own data indicate that increased gluconeogenesis contributes to the glucagon-induced diabetes. Figure 3 shows the average urinary nitrogen excretion of intact fasting control rats and of comparable animals treated with glucagon. It is apparent that the fasting glucagon-treated animal excretes about 40 per cent more nitrogen than the controls. Under these conditions no glucosuria or hyperglycemia occurs. The glucagon-induced increase in nitrogen excretion can be almost completely attributed to the increase in urea excretion.

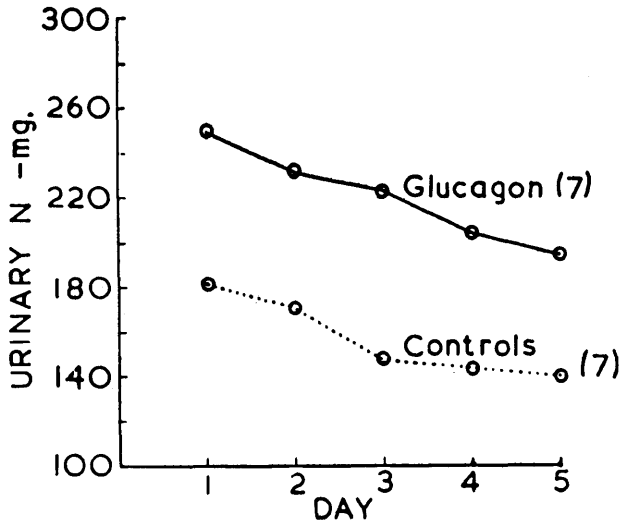

FIG. 3. Urinary nitrogen of fasting male controls and of fasting rats injected every eight hours with 400 gamma of glucagon.

Figure 4 shows the changes in the amino acid and sugar levels in the blood of controls and in rats injected with 1 mg. of glucagon suspended in saline. The blood amino acids fell much more rapidly in the glucagon-treated animals and remained lower throughout the five-hour observation period. The blood sugar rose immediately after glucagon administration but fell within three hours to normal levels. It does not appear that the glucagon-induced fall in blood amino acids can be attributed to an increase in the rate of peripheral utilizations since urinary nitrogen excretion also increased during this period.

In some respects the changes induced by glucagon administration are similar to those induced by adrenal glucocorticoids. The possibility that the marked increase in gluconeogenesis following glucagon administration was due to stimulation of the adrenal cortex was investigated.

Figure 5 shows the total nitrogen and urea nitrogen excreted by adrenalectomized glucagon-treated rats and adrenalectomized controls. The food intake of the controls was limited to the amount consumed by the treated animals. It is apparent that the glucagon-treated ad-

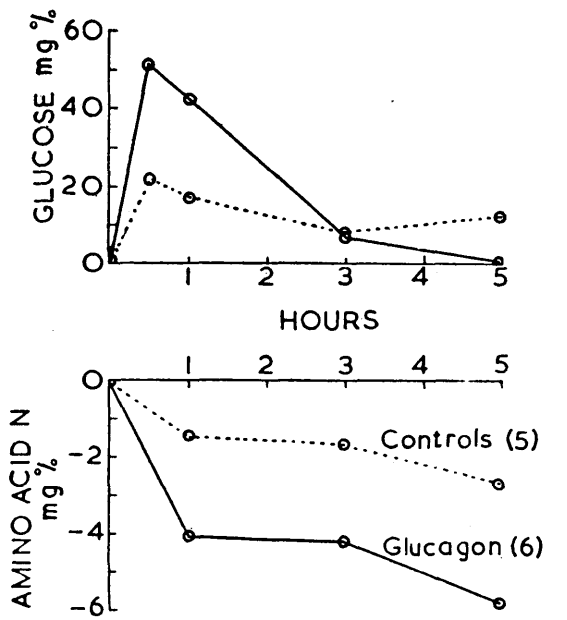

FIG. 4. Changes in the blood amino acid and sugar levels of male control rats and of comparable animals given one subcutaneous injection of 1 mg. glucagon suspended in neutral saline.

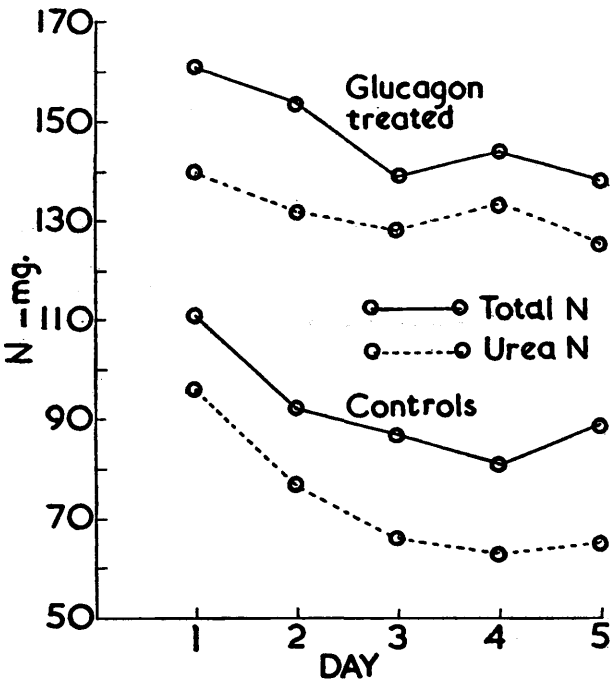

FIG. 5. Urinary nitrogen and urea nitrogen excretion of seven male adrenalectomized rats (maintained on saline) injected with 400 gamma of glucagon every eight hours and of seven adrenalectomized pair-fed controls.

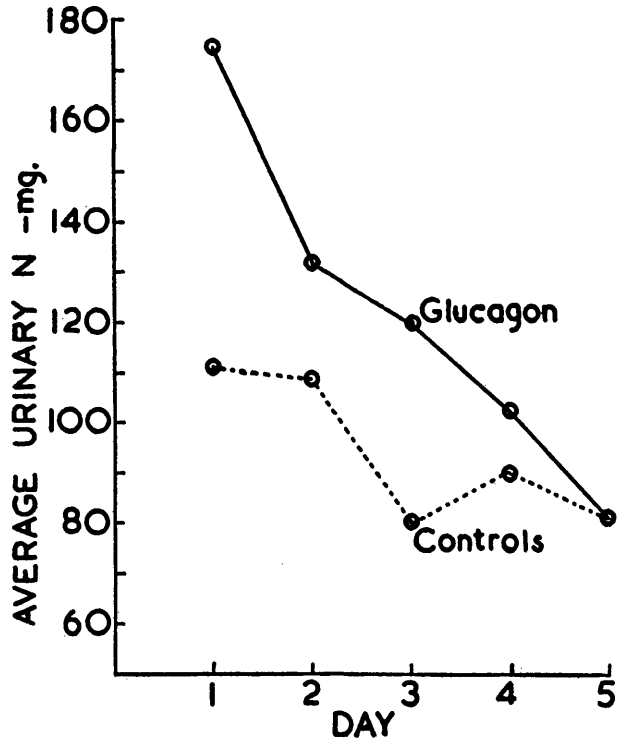

FIG. 6. Urinary nitrogen excretion of five fasting adrenalectomized rats injected with 400 gamma glucagon every eight hours and of five fasting adrenalectomized controls.

renalectomized rats excreted about 50 per cent more nitrogen and urea than the controls.

Figure 6 shows the average urinary nitrogen excretion of fasting adrenalectomized rats treated with glucagon and of the fasting controls. The glucagon induced a marked increase in nitrogen excretion the first day but the effect became progressively less until on the fourth day there was no significant difference between the amounts of nitrogen excreted by each group. It will be recalled that in the fasting intact rat the glucagon-induced increase in nitrogen excretion was undiminished at the end of five days (see figure 3).

The results of these last two experiments indicate that the catabolic action of glucagon is not mediated by way of the adrenal gland. However, the effect of glucagon on the catabolism of amino acids during the fasting state is partly dependent upon the presence of adrenal cortical secretions. Recent experiments have shown that the diabetogenic actions of glucagon and cortisone are markedly synergistic. Rats treated with both substances develop severe diabetic symptoms despite a 75 per cent reduction in their food intake. Weight loss is precipitous and death occurs in from five to seven days.

SUMMARY

The data presented show that glucagon when administered in large amounts has a marked diabetogenic action in force-fed rats. It produces intense glucosuria, hyperglycemia and weight loss. Preliminary experiments indicate that glucagon has a diabetogenic action in dogs fed ad libitum. The specific mechanisms responsible for glucagon diabetes are not known, but the data strongly indicate that glucagon induces a marked increase in gluconeogenesis and suggest that overproduction of glucose contributes to this phenomenon. The protein catabolic effect of glucagon is not mediated by the adrenal glands although a synergy between the actions of adrenal cortical secretions and glucagon appears to exist.

SUMMARIO IN INTERLINGUA

Effectos Pathologic de Grande Quantitates de Glucagon

Le datos presentate monstra que glucagon administrate in grande quantitates ha un effecto marcatemente diabetogene in rattos alimentate per compulsion. Illo produce intense grados de glucosuria, hyperglycemia, e perdita de peso. Experimentos preliminar indica que glucagon ha un effecto diabetogene in canes alimentate ad libitum. Le mechanismos specific que es responsabile pro diabete a glucagon non es cognoscite, sed le datos indica fortemente que glucagon induce un marcate augmento de

gluconeogenese, e illos pare suggerer que un hyperproduction de glucosa contribue a iste phenomeno. Le effecto de glucagon in le catabolismo proteinic non es mediate per le glandulas adrenal, ben que il pare que il existe un forma de synergismo inter le actiones del secretiones adrenocortical e de glucagon.

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GROUP DISCUSSION

CHAIRMAN BRANDALEONE: Thank you, Dr. Salter. The three previous papers on glucagon will be discussed by Dr. Joseph L. Izzo of Rochester, New York.

JOSEPH L. IZZO, M.D.: Three years ago crystalline glucagon was first prepared; now, its chemical structure has been determined. This is a singular accomplishment by Dr. Bromer and his colleagues at the Lilly Research Laboratories. As Dr. Bromer points out, it should now be possible to study the relation of the structure of glucagon to biological activity. I am not qualified to discuss critically the actual data presented. However, the methods which have been employed are standard, and the results seem straightforward. One thing which has impressed me is the plethora of OH groups in the single straight chain glucagon molecule. Does this have any biological significance? Perhaps Dr. Bromer can tell us if they are essential for activity of the hormone, as they are in insulin.

The brilliant advances in the chemistry of glucagon serve to point up the poignant and still unsettled question of the physiologic or pathologic importance of glucagon. Since a convincing example of a deficiency picture has not yet been established, either clinically or experimentally, its hormonal status has not been proved to the satisfaction of all. Dr. Anderson considers glucagon a hormone whose physiologic role is that of an adjuvant and orderly synergist to insulin function. This is said to be accomplished by glucagon triggering hepatic glucose output through its stimulation of phosphorylase activity and thereby providing the stimulus for insulin production in the postabsorptive state. This represents an extension of an hypothesis which, I believe, was first proposed by Bürger, one of the most distinguished students of this substance, several years ago. Unfortunately, as Dr. Anderson himself admits, the hypothesis can be neither proved nor disproved by studies the type of which were just presented. Whether or not the irregular undulations in hepatic glucose output demonstrated by Dr. Anderson as well as others are attributable to glucagon is a moot point. It would be of interest in this respect to know what happens to these "hepatic glucose spurts" in the animal deprived of intrinsic glucagon either by pancreatectomy or by selective destruction of the *a*-cells of the pancreas,