The Effect of Glucagon on the Metabolic Rate of Rats

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DATA presented in a previous report showed that rats treated with small amounts of glucagon gained less weight and contained less protein, fat and water than pair-fed control rats.[1] Since the caloric intake of the two groups was identical, although the caloric value of the carcasses of the glucagon-treated animals was much lower than that of the control rats, the effect of glucagon on the metabolic rate was studied. The results of the investigation indicate that glucagon, under experimental conditions increases heat production thus altering caloric balance.

MATERIALS AND METHODS

Male Wistar rats weighing 200 to 250 gm. were fed Purina chow *ad libitum* and kept in a room maintained at a temperature of 28° ± 1°c. for a period of at least ten days before being subjected to experimental procedures. The thyroidectomized animals were used after a postoperative period of one month. Cessation of weight gain and subnormal oxygen consumption were accepted as evidence that the thyroid gland had been completely removed. The adrenalectomized animals were given 1 per cent saline to drink while those treated with cortisone were given a choice of saline or tap water. Upon completion of the investigation the adrenalectomized rats were sacrificed and then autopsied. The sites of the suprarenal glands were carefully examined through a dissecting microscope. No evidence of adrenal remnants were found.

Adrenal demedullation was performed by the enucleation technic of Evans.[2] The animals were given a 1 per cent sodium chloride solution to drink for seven days postoperatively. Three weeks were allowed for regeneration of the adrenal cortices. At autopsy, histologic examination of the glands showed no evidence of chromaffin tissue.

Crystalline glucagon§ was administered subcutaneously either as a suspension in neutral saline or as a solution in saline at a pH of 9 to 10. Injections of crystalline serum albumin‖ in neutral saline or in solution at a pH of 9 to 10 were administered to control animals as required. Adrenalin (epinephrine)¶ and cortisone** were administered intramuscularly.

All rats were fasted four hours before the experiments. Their metabolic rate was measured in terms of the oxygen consumption of each animal. For each experiment four treated and four control animals were used. The oxygen consumption was determined according to the method of Ferguson and Sellers[3] utilizing a closed circuit apparatus with eight chambers, each containing one rat. The apparatus was immersed in a water bath at 28.0° ± 0.5°c. The temperature in the chambers was 30.0° ± 0.5°c. Measurements were made at frequent

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§ Eli Lilly & Co., Indianapolis, Lot No. 258-234 B-54-2.

‖ Armour & Co. Ltd., Chicago, Illinois.

¶ Parke-Davis & Co., Brockville, Ontario, 1 Canada, cc. ampoules of adrenalin-in-oil 1:500.

** Merck & Co. Ltd., Montreal, Canada.

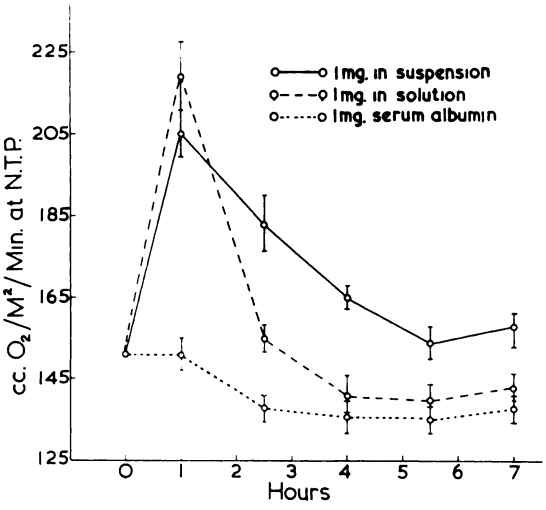

FIG. 1. Change in the oxygen consumption of normal rats following the subcutaneous injection of glucagon. Control rats were given an injection of albumin.

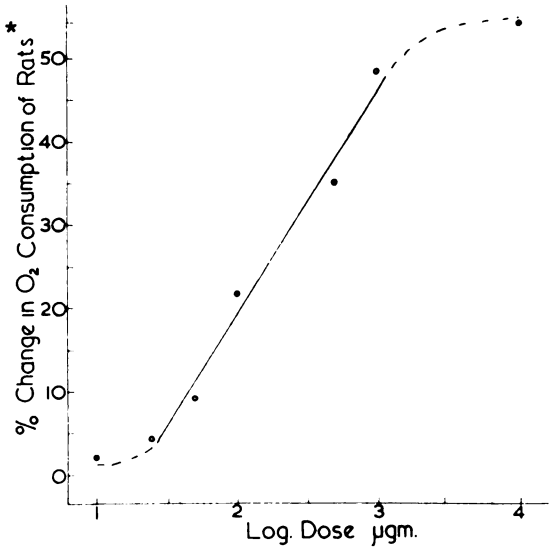

FIG. 2. Effect of glucagon on the metabolic rate: dose versus response.

* One hour after administration of glucagon solution

intervals over a seven hour period. The volume of oxygen consumed by each rat per minute per square meter of body surface at normal temperature and pressure was calculated using Lee's formula.[4]

PROCEDURES AND RESULTS

The Effect of Glucagon and Adrenalin on Intact Rats

The metabolic rates of rats treated with a single injection of a glucagon solution or a glucagon suspension were compared with those of control animals treated with an injection of crystalline plasma albumin (Fig. 1). In each instance, glucagon produced a marked elevation of the metabolic rate. The maximum increase occurred one hour after the injection, but diminished rapidly thereafter. The mode of administration of the hormone affected the magnitude and duration of the response. The injection of glucagon dissolved in alkaline saline caused a greater increase (47 per cent) in the metabolic rate than glucagon given as a suspension in neutral saline (35 per cent). However, the suspension produced a more prolonged effect (Fig. 1); the rate was still elevated by 15 per cent at the end of seven hours. Additional experiments showed that over a range of 25 to 1,000 µg. of glucagon, a linear

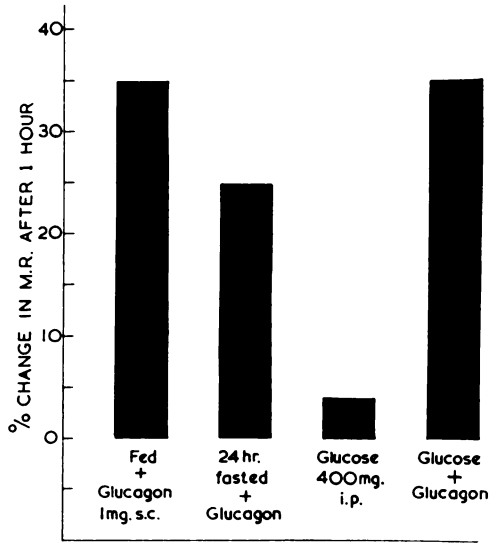

FIG. 3. Effect of glucagon and glucose on the metabolic rate of rats. No correlation between the hyperglycemic action of glucagon and its effect on oxygen consumption is evident.

relationship existed between the logarithm of the amount of glucagon administered and the percentage change in oxygen consumption (Fig. 2).

No correlation could be established between the hyperglycemic action of glucagon and its effect on oxygen consumption. When the rats were fasted for twenty-four hours the

TABLE I

Metabolic Rates of Rats One Hour After Adrenalin Administration*

Groups	No. of Rats	Metabolic Rate‡	Increase	per cent
Intact				
Control	10	143 ± 5		
Treated	10	213 ± 10	70	49.0
Adrenalectomized				
Control	15	146 ± 4		
Treated	16	171 ± 5	25	17.1
Adrenalectomized plus cortisone†				
Control	12	167 ± 9		
Treated	11	247 ± 14	86	47.9

* 0.1 mg. adrenalin-in-oil per rat.

† 2.5 mg. cortisone daily per rat.

‡ In this and other table, cc. O_2/M^2/minute at normal temperature and pressure ± standard error of the mean.

administration of glucagon did not produce a significant change in the blood sugar level but it did cause a 25 per cent increase in the metabolic rate (Fig. 3). This increase was 10 per cent less than that observed in the rats fasted four hours. Conversely, the intraperitoneal injection of 400 mg. of glucose (sufficient to produce a hyperglycemia of slightly greater

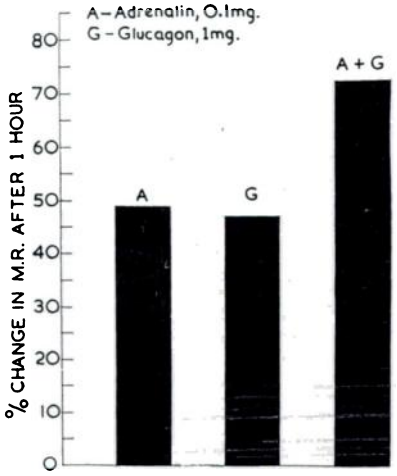

FIG. 4. Synergistic effects of adrenalin and glucagon in stimulating oxygen consumption of rats. The changes represented by bars A and G were the greatest that could be produced by injection of either of these hormones.

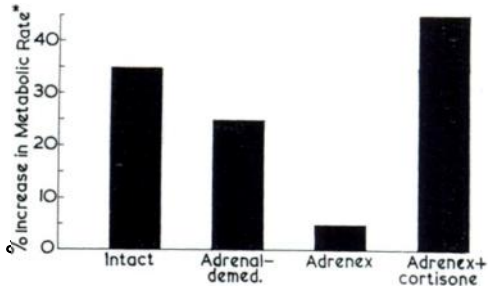

FIG. 5. Effect of adrenalectomy and adrenal-demedullation on the metabolic response to glucagon. The small increase shown for the adrenalectomized rats is not significant statitiscally.

* One hour after 1 mg. of glucagon was administered subcutaneously.

magnitude and duration than that produced by glucagon, increased the metabolic rate by only 4 per cent. It was also observed that the concomitant administration of glucose potentiated the hyperglycemia induced by glucagon but did not enhance its effect on metabolic rate (Fig. 3).

The effect of adrenalin on the metabolic rate of rats was studied under the same conditions used for the experiments with glucagon. It was found that adrenalin, like glucagon, was most effective one hour after administration (Table I).

An increase in oxygen consumption of 47 to 49 per cent appeared to be the limit of the change that could be elicited in intact animals with either glucagon or adrenalin, but when these hormones were administered together increases of 72 to 75 per cent were observed (Fig. 4).

Adrenalectomy and Adrenal-demedullation

The administration of glucagon to adrenalectomized rats sixteen to twenty-one days after surgery did not elicit a significant change in the metabolic rate (Fig. 5). Treatment with 2.5 mg. cortisone daily for eight to ten days not only restored but also potentiated the response to glucagon (Fig. 5). The magnitude of the response was closely related to the duration of cortisone therapy. Three to four days of treatment with this steroid were required before the metabolic rate of the adrenalectomized rats could be altered by glucagon. Full metabolic effect was not restored

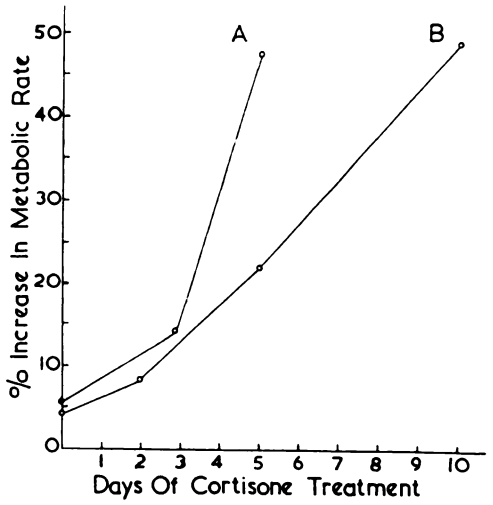

FIG. 6. Time required for cortisone therapy (2.5 mg. daily) to restore the metabolic response of adrenalectomized rats to glucagon. The per cent increase in metabolic rate refers to the change one hour after the injection of 1 mg. of glucagon in alkaline solution. Curve A represents animals that received injections of cortisone one day after adrenalectomy. Curve B represents rats that received injections of cortisone sixteen days after adrenalectomy.

until nine to ten days of cortisone treatment (Fig. 6, curve B).

The metabolic response to glucagon was immediately abolished by adrenalectomy and could not be maintained by starting cortisone injections the same day the suprarenal glands were removed. However, the immediate application of cortisone therapy restored the glucagon effect in a shorter time than when similar treatment was started sixteen days after the rats had been adrenalectomized (Fig. 6, curve A).

The administration of a single dose of glucagon increased the metabolic rate of adrenaldemedullated rats by 25 per cent in one hour. This response was 10 per cent less than that obtained in the intact animal (Fig. 5). At autopsy, the adrenal glands of these animals were found to be very small in comparison to the glands of intact animals of similar weight. A histologic examination of the glands revealed that the regenerated cortices were smaller than normal.

Adrenalectomy also reduced the response of the animal to adrenalin (Table I). The administration of a single dose of the hormone fourteen days after operation increased the metabolic rate of the rats by only 17 per cent. This response was a third of that obtained in intact animals. The daily administration of cortisone from the time of adrenalectomy (Table I) restored the effect of adrenalin.

Thyroidectomy

The metabolic rate of the thyroidectomized rats fell and then stabilized at a value 28 per cent below the metabolic rate of the intact control animals during a three week postoperative period. The administration of glucagon to these animals one month postoperatively did not alter the oxygen consumption significantly (Table II).

TABLE II

Effect of Glucagon on the Metabolic Rate of Thyroidectomized Rats

Group	No. of Rats	Metabolic Rate*			
		Hours after Injection			
		1.0	2.5	4.0	5.5
Intact†	24	151 ± 4	138 ± 3	136 ± 4	135 ± 3
Thyroidectomized					
Control†	15	109 ± 5‡	100 ± 3	108 ± 6	100 ± 3
Treated§	15	121 ± 5	108 ± 3	105 ± 2	107 ± 5

* See footnote (‡) in Table I.
† Injected with 1 mg. albumin dissolved in saline.
§ 1.0 mg. glucagon suspended in saline.
‡ The differences are not significant at the 5 per cent level of confidence.

COMMENTS

It appears that the effect of glucagon and of adrenalin on the metabolic rate is similar in some respects. The greatest increase in oxygen consumption obtained after a single injection of glucagon was about 47 per cent. It has been shown[5,6] that the maximum response to adrenalin is of a similar order of magnitude. The effect of both substances is rapid and transient. The metabolic rate was increased to the greatest extent one hour after a single injection of either of these hormones and returned to control levels during the next few hours.

The similarity in the patterns of response produced by the two substances suggested that the effect of glucagon on the metabolic rate might be due to the liberation of adrenalin. This does not appear to be true since the glucagon was obtained in adrenalectomized animals maintained on cortisone and in adrenal-demedullated animals. The observation that the response to both adrenalin and glucagon was greatly reduced by adrenalectomy, but was gradually recovered and even potentiated (Fig. 5; Table 1) by treating these animals with cortisone, suggests that the adrenal cortex plays an important role in the regulation of respiratory metabolism. The small reduction in the response obtained in the adrenal-demedullated animal compared to that observed in the intact (36 per cent) or the cortisone-treated adrenalectomized animal (47 per cent) may be explained by the decrease in total secretory capacity noted in glands which have regenerated after adrenal enucleation.[7]

The influence of adrenalin on respiratory metabolism, i.e., its "calorigenic action," has been ascribed to an increase in the production and utilization of glucose and lactic acid, an increased activity of skeletal muscle, a peripheral vasoconstriction leading to a rise in body temperature and subsequent increase in metabolic rate, and to the hormone acting as a respiratory catalyst at the cellular level.[8-10] Griffith[10] believes the calorigenic action is the resultant of the sum of these effects. Glucagon does not produce hyperlacticacidemia or cardiovascular effects[11-15] and there is no evidence of a stimulating effect on skeletal muscle. The only feature that glucagon and adrenalin have in common is the ability to produce hyperglycemia by stimulating hepatic glycogenolysis. Both Griffith[10] and Ellis,[9] after reviewing the literature, concluded that the hyperglycemia induced by adrenalin plays no significant role in its calorigenic action and, in the present investigation, no evidence has been obtained that glucagon-induced hyperglycemia is directly related to its effect on oxygen consumption.

Both adrenalin and glucagon are dependent on the presence of the thyroid for their calorigenic action. Swanson[5] found that adrenalin had no effect on the oxygen consumption of rats in the absence of thyroxin. Extending the findings of earlier workers,[6,16] she observed that an increase in the metabolic rate induced by adrenalin was directly related to the circulating level of thyroxin. This finding suggested that adrenalin increases the rate of production of substrate (lactic acid) while thyroxin increases its utilization. Thus the calorigenic effect would be limited not only by the level of circulating thyroid hormone but also by the availability of substrate.

This concept might be used to explain the relationship between glucagon, thyroxin and the metabolic rate. In this case the substrate could be the deaminated residues of amino acids. It has been shown that the administration of glucagon is followed immediately by an increase in the rate of urea synthesis and amino acid catabolism.[17,18]

It is clear that glucagon does increase the oxygen consumption of rats. The assumption that this respiratory change reflects an increase in the oxidation of body nutrients with a consequent increase in heat production is strongly supported by a previous investigation which showed that rats treated with glucagon grow more slowly than untreated animals consuming identical amounts of food.

SUMMARY

The administration of glucagon to intact rats increased their oxygen consumption by as much as 47 per cent. The change was greatest one hour after the administration of glucagon and decreased slowly thereafter. A linear relation-

ship was observed between the percentage increase in the metabolic rate and the logarithm of the dose of glucagon. No correlation could be established between the hyperglycemic action of glucagon and its effect on metabolic rate. The increase in oxygen consumption produced by glucagon was similar in magnitude and duration to the change affected by adrenalin. The administration of glucagon and adrenalin together caused a greater increase than that produced by giving optimal amounts of only one of these substances. The effect of glucagon on respiratory metabolism was abolished after thyroidectomy and adrenalectomy had been performed. Its action appeared to be independent of the adrenal medulla since oxygen consumption was stimulated in adrenal-demedullated rats and in adrenalectomized rats treated with cortisone. Adrenalectomy also diminished the effect of adrenalin on the metabolic rate by 60 per cent. The administration of cortisone potentiated the effects of both glucagon and adrenalin.

The significance of the similarity between the calorigenic action of glucagon and adrenalin are briefly discussed.

REFERENCES

1 SALTER, J. M. *Am. J. Clin. Nutrition*, 8: 535, 1960.
2. EVANS, G. *Am. J. Physiol.*, 114: 297, 1936.
3. FERGUSON, J. K. W. and SELLERS, E. A. *J. Pharmacol. & Exper. Therapy.*, 97: 177, 1949.
4. LEE, M. O. *Am. J. Physiol.*, 89: 24, 1929.
5. SWANSON, H. E. *Endocrinology*. 59: 217, 1956.
6. SCHAEFFER, G. and THIABAULT, O. *Compt. rend. Soc. de biol.*, 139: 855 and 857, 1945.
7. HOLZBAUER, M. and VOGT, M. *J. Physiol.*, 138: 449, 1957
8. LUNDHOLM, L. *Acta physiol. scandinav.* (supp. 67), 19: 1, 1949.
9. ELLIS, S. *Pharmacol. Rev.*, 8: 485, 1956.
10. GRIFFITH, F. R. *Physiol. Rev.*, 31: 151, 1951.
11. SUTHERLAND, E. W. *Recent Prog. Hormone Res.*, 5: 441, 1950.
12. VUYLSTEKE, C. A. and DE DUVE, C. *Arch. Internat. physiol.*, 57: 445, 1950.
13. BOGOCH, A. and MCINTOSH, H. W. *Canad. M. A. J.*, 745: 875, 1956.
14. PINCUS, I. J. *J. Clin. Endocrinol.*, 10: 556, 1950.
15. CAHILL, J. F., ZOTTU, S. and EARLE, A. S. *Endocrinology*, 60: 265, 1957.
16. RING, G. C. *Am. J. Physiol.*, 137: 582, 1942.
17. TYBERGHEIN, J. M. *Arch. internat. physiol.*, 61: 104, 1953
18. SALTER, J. M., DAVIDSON, I. W. F. and BEST, C. H *Diabetes*, 6: 248, 1957.

DISCUSSION

DR. ESTELLE R. RAMEY (*Washington, D. C.*): Dr. Salter, does the chronic administration of glucagon maintain any of these phenomena? Past history with respect to the hyperglycemic effect, for example, indicates that continuous administration may show some kind of either adaptation or lack of effect with this hormone, and I wondered whether these other effects behave similarly.

DR. SALTER: We have administered glucagon to rats for periods up to six months, and we have seen no sign of any adaptation. The rats lose weight and stay thin, while their metabolic rate remains elevated.

We have administered glucagon for five to six months to rabbits, and in so doing have produced what is truly a "metaglucagon" diabetes. These animals have remained diabetic for as long as seventy days after the cessation of treatment with glucagon.

Occasionally a rabbit seems to develop an antibody to glucagon. These animals, of course, become completely resistant; however, this is rare.

DR. G. C. KENNEDY (*Cambridge, England*): I was struck by Dr. Salter's saying that there was a similarity between the effects of cortisone and of glucagon, because I have had exactly the same experience with high doses of cortisone on appetite and weight change. Has he tried the effect of glucagon treatment on rats with hypothalamic lesions?

The effect of cortisone on inhibiting the appetite is abolished if one punctures the hypothalamus and produces hyperphagia first, and it would be interesting, I think, to know whether there is any possibility that a similar hint of central action of glucagon could be elicited by using a hypothalamic obese preparation.

DR. SALTER: We have not done that, but Dr Stevenson and I have discussed it several times.

DR. THEODORE B. VAN ITALLIE (*New York, New York*): Dr. Salter has implied that glucagon may have a direct effect on amino acid catabolism. While this may be true, I think it is important not to underestimate the fact that glucagon depletes the liver of glycogen and that a liver depleted of glycogen may in itself be a powerful stimulus to gluconeogenesis from amino acids.

In some studies performed by Dr. Shoemaker and myself, studying the flux of various metabolites across the dog liver in response to glucagon, we found that shortly after the administration of glucagon, with the release of considerable amounts of glucose from the liver, the liver began to remove appreciable amounts of alpha amino nitrogen from the blood. This would tend to support the notion that to some degree the drop in amino acid nitrogen would be due to liver uptake of amino acids with their transformation into new glycogen.

DR. SALTER: The drop in amino acid nitrogen cannot be due to anything else, because the urea excretion

and the blood urea rise markedly. I would like to emphasize again that this effect is striking.

With regard to the glycogenolytic action, in liver slices we have not been able to demonstrate an effect of epinephrine on urea synthesis, and we have considered the same possibility. Our original contention was that epinephrine was acting peripherally as well as hepatically, whereas glucagon only acts on the hepatic tissue. We thought that perhaps the feedback of lactic acid from the muscle was helping maintain the energy equilibrium in the liver. If this were true some of the effects of glucagon might be done by giving epinephrine coincidentally. But this does not happen. If epinephrine is administered along with glucagon, it does not modify its effect on blood amino acids.

Dr. Kenneth Crispell (*New York, New York*): You seemed to show that cortisone is permissive for this reaction because you used very small doses. Do you know if small doses of thyroid are permissive in the thyroidectomized animal?

Dr. Salter: Yes. The administration of thyroid in small amounts (5 or 10 gamma a day) will restore the metabolic rate response completely.

The permissive effect of cortisone is just on the metabolic rate, not on the amino acid metabolism.

Dr. John R. Brobeck (*Philadelphia, Pennsylvania*): Therefore, you do not have to suppose that there is an increased secretion of thyroxin to cause the increase in metabolic rate.

Dr. Salter: No.

Dr. Brobeck: If there is enough thyroxin there, glucagon will then cause the increase?

Dr. Salter: On a constant does of thyroxin, you get the increase.

Dr. Rachmiel Levine (*Chicago, Illinois*): Does insulin counteract the effect of glucagon on raising the metabolic rate? The reason I am asking is that depancreatized dogs, within twenty-four hours after pancreatectomy, have a high metabolic rate, which is diminished by insulin. I wonder whether the metabolic rate is due to some peripheral interference with carbohydrate metabolism and, therefore, the appearance of intermediates, raising the rate. Does insulin counteract this reaction?

Dr. Salter: I don't know. There was another point that I should have mentioned. We think that the increase in metabolic rate may be completely due to a change in oxygen consumption by the liver, even though it would mean a tremendous increase. We have tried at various times to detect a change in the temperature of the liver, but without success. Dr. Van Itallie and his group showed that the blood flow through the liver was greatly increased following glucagon administration, which could easily account for this failure.

If you give glucagon, take the liver out and incubate it *in vitro*, the oxygen consumption of the organ rises by as much as 150 per cent.

Dr. Jean Mayer (*Boston, Massachusetts*): The evidence for possibly increased glucagon secretion in obese hyperglycemic mice is almost entirely circumstantial: they have hypertrophy of alpha cells, an increased turnover of liver glycogen, increased phosphorylase activity of the liver, plus a reaction to alpha cell-destroying agents. On the other hand, the evidence that they have increased insulin secretion is now well documented. This may bear on Dr. Levine's comment, in that the increased pancreatic content of insulin has been shown, in particular at the Banting and Best Institute. Dr. Renold and his group have shown that there is also considerably increased circulating insulin-like activity.

It may well be that the action of glucagon is quite different in the presence of an excess of circulating insulin, and certainly some of the evidence presented by Dr. Elrich on the possible synergism of glucagon and insulin on carbohydrate metabolism may bear on that point.

I think the action of insulin is established while the action of glucagon is hypothetical.