The Effect of Blood Glucose Concentration on Insulin Output

Robert Metz, M.B., Chicago

The available evidence concerning the effect of the blood sugar level on the output of insulin from the pancreas permits only the limited conclusion that extra insulin is liberated in the presence of hyperglycemia.[1,2] There is no evidence to support a more precise delineation of this relationship.

The purpose of the present study was to explore the quantitative aspects of the effect of blood glucose concentrations, both high and low, on the rate of insulin secretion in the dog. The insulin-like activity of blood drawn from the pancreatic vein was determined by bioassay, using the isolated rat-diaphragm technic. Hypoglycemia was produced by pretreatment with phloridzin, and hyperglycemia was produced by intravenous glucose infusion.

The results of this experiment indicate that the rate of insulin secretion is a continuous function of the blood sugar concentration. Information has been obtained about the form of this relationship, as well as the actual quantities of insulin secreted in the presence of normoglycemia, hypoglycemia and hyperglycemia.

MATERIALS AND METHODS

Seven male dogs were used. Four of them were given 10 ml. of a 20 per cent solution of phloridzin in propylene glycol, administered subcutaneously twice daily on the two days preceding the experiment. All animals were fasted for eighteen hours before the experiment. They were anesthetized with intravenous sodium pentobarbital. The abdomen was entered and the pancreaticoduodenal vein exposed. A plastic cannula, having about the same diameter as the vein, was introduced in a retrograde direction and secured with its tip lying just within the margin of the pancreas. The dog was heparinized, and the pancreatic venous effluent was allowed to drain continuously into graduated tubes; by timing the inflow into these tubes a measure of the flow rate was obtained. Aliquots of the effluent were collected before, and at intervals after, the start of an infusion of dextrose in water into a femoral vein. The infusion rate was varied from time to time to produce differing rates of increment of the blood glucose levels. The aliquots were collected under oil into the graduated tubes, and the plasma was separated by centrifugation and stored at 4° C. pending the performance of the assays.

The technic used for the assays was essentially that first successfully used by Groen and his associates,[3] and described more recently by Wright.[4] Male Sprague-Dawley rats weighing between 100 and 200 gm. were used. In any one run the weights of the rats did not vary by more than 10 gm. The rats were fasted for eighteen to twenty-four hours and killed by decapitation. The diaphragms were carefully removed and bisected, and each pair of hemidiaphragms was soaked in a Glucose-Krebs-Ringer-Bicarbonate (G.K.R.B.) solution at 4° C. for fifteen minutes. After soaking, hemidiaphragms were transferred to separate 10 ml. Erlenmeyer flasks for incubation. This was done on a Dubnoff incubator for one hour at 37° C. under an atmosphere of 95 per cent O_2 and 5 per cent CO_2. For incubation 1 ml. of either plasma (unknowns), G.K.R.B. (controls) or G.K.R.B. containing known concentrations of insulin (standards), adjusted to equal glucose concentrations, was used. At the end of the incubation period the hemidiaphragms were removed and placed in an oven at 110° C. overnight and weighed the next day. The glucose concentration of the medium, which had been measured before incubation, was remeasured, and the quantity of glucose consumed by the hemidiaphragm during incubation was calculated. The glucose uptake was expressed as milligrams of glucose per gram dry weight of diaphragm per sixty minutes. Each sample was assayed in duplicate. In each run the mean uptake of the diaphragms incubated in the control (G.K.R.B.) was subtracted from the mean uptakes of the diaphragms incubated in the respective unknowns or standards, to give the increment in glucose uptake produced by the insulin in the medium. The insulin concentration of

From the Departments of Medicine and Physiology, Northwestern University, Chicago, Illinois. Present address: Charles H. Best Institute, 112 College Street, Toronto, Ontario, Canada.

each plasma sample was read from a calibration curve prepared by plotting increments in glucose uptake as a function of known insulin concentration, and expressed as milli-units (mU.) of insulin per 100 ml. of plasma.

RESULTS

Standardization and estimation of the reliability of the assay: From time to time during the course of this investigation, assay runs were performed on standard solutions of insulin in G.K.R.B. The results from all these determinations were pooled (table 1) to construct the calibration curve. The results indicated an apparently linear log-dose-response curve, as reported by other investigators. The slope of the curve, 0.6, was in close agreement with the range of slopes reported by Randle.[5]

Several samples of arterial blood, obtained from anesthetized dogs, were assayed. The mean insulin concentration of thirteen samples was 16 mU. per 100 ml., a value similar to those reported for both human and dog venous blood by others.[3,4] As a further check of the assay, insulin was added to samples of pooled peripheral blood plasma to produce insulin concentrations in the range encountered in pancreatic venous plasma, and the insulin enriched plasma was then assayed. Duplicate assays were run on ten samples to which 0, 100, 500 and 1,000 mU. per 100 ml. were added, respectively. That the method was not subject to gross systematic error is indicated by the small systematic deviation of the points from the line (figure 1), which theoretically would represent perfect recovery.

TABLE 1

Increment in glucose uptake produced by various concentrations of insulin in G.K.R.B. (mean values)

Insulin concentration (mU./100 ml.)	No. of determinations	Increment in glucose uptake	S.E.
10	9	2.5	.69
100	14	10.5	.62
500	5	15.3	.84
1,000	13	19.4	.50

To assess whether the random error of the assay method could have accounted for the observed differences in insulin concentration in the pancreatic venous blood samples, the deviation of paired results from their respective means was examined. An analysis of variance performed on the data indicated that the differences between paired observations were significantly different ($p<.01$) from the differences within pairs.

Measurement of insulin output: Twenty-five samples of pancreatic venous blood were obtained from seven

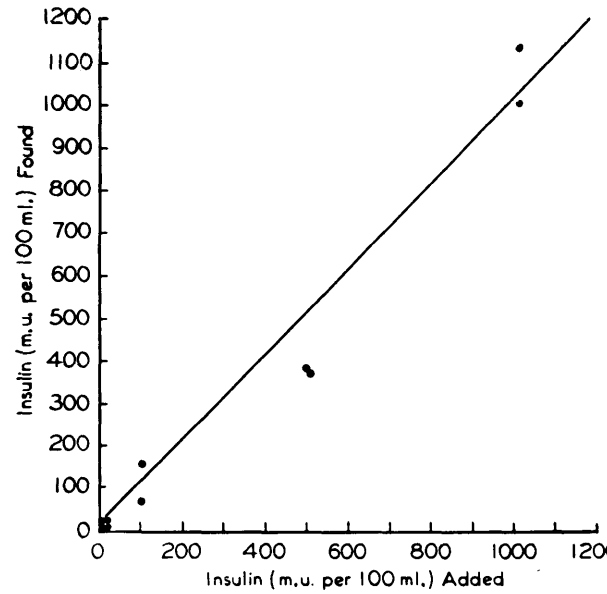

FIGURE 1

dogs. The blood glucose concentrations at the time of collection of the various samples ranged from 37 to 655 mg./100 ml. In the four phloridzinized dogs the blood sugars at the time of collection of the first pancreatic venous samples, that is, before the start of the glucose infusions, were between 37 and 72 mg./100 ml. The corresponding values for the other dogs were between 86 and 151 mg./100 ml. The flow rates from the cannula varied from 3.5 to 8 ml./min. In some of the animals there was an increase in flow rate as the experiment progressed; in others there was no change. Whereas plasma, and not whole blood insulin was measured, the figures for blood flow were multiplied by 6/10 in order to convert blood flow values to plasma flow. The insulin content of each sample, as determined by the bio-assay, was multiplied by the calculated plasma flow, the resultant being insulin output in mU./min. (table 2).

The results revealed a very wide range of insulin outputs, from 0.2 to 40 mU./min. It is apparent from table 2 that the insulin outputs increased as the blood sugar rose during the experiments. The data do not allow of a reliable estimation of the form of the relationship in each animal individually. But when the data from all the animals are simply combined, and plotted as log insulin output against log blood sugar, the result is apparently linear. Figure 2 presents this plot of the experimental points and the fitted regression equation, $\log Y = -3.14 + 1.64 \log X$. Figure 3 presents the original data and the corresponding exponential equation, $Y = 0.0007\ (X^{1.64})$. Obviously

TABLE 2

Blood glucose, pancreatic venous insulin concentration and blood flow, and calculated insulin output

Dog number	Pancreatic venous blood sample number	Peripheral venous blood glucose conc. (mg./100 ml.)	Insulin concentration (mU./100 ml.)	Flow rate (ml./min.)	Insulin output* (mU./min.)
1	1	37	10	3.5	0.2
	2	40	25	3.5	0.5
	3	389	425	4.5	11.5
	4	539	830	4.5	22.5
	5	528	500	4.5	13.5
2	6	151	52	3.5	1.1
	7	340	780	5.0	23.4
	8	422	505	7.0	21.2
	9	574	560	7.0	23.5
3	10	86	31	3.5	0.6
	11	306	570	3.5	12.0
	12	514	720	5.0	21.6
4	13	65	16	7.0	0.7
	14	426	135	7.0	5.7
	15	655	970	7.0	40.7
	16	577	380	7.0	16.0
5	17	42	28	3.5	0.6
	18	391	730	8.0	35.0
	19	658	790	8.0	37.9
6	20	127	90	5.0	2.7
	21	116	58	5.0	1.7
	22	236	130	5.0	3.9
	23	296	330	5.0	9.9
	24	246	205	5.0	6.2
7	25	72	26	4.5	0.7

*Insulin output = insulin concentration x 6/10 (blood flow rate).

this strictly empirical equation will not bear extrapolation, for at still higher glucose concentrations the insulin output would presumably level off at some maximum.

In view of the fact that the blood sugar levels were generally changing throughout these experiments, the results do not establish that the response of the pancreas is exclusively an instantaneous one to the concentration of glucose in the blood. There may be a lag in the response, or the response may be modified by the rate of change of glucose concentration. Although the data are not adequate to rule out these possibilities, it seems likely that if the level of insulin liberation is in fact modified by the rate of blood sugar change, the deviation of each of the individual points from the regression line might have been due in part, at least, to the rate of change at the time of collection of each of the samples.

Investigation of this point showed no significant correlation ($r = 0.17$) between deviation and the rate of change, suggesting that this factor had not influenced the pancreatic response.

DISCUSSION

The problem of the relationship of insulin output to the blood sugar level has been under investigation for more than thirty years (see Houssay[1] and Pozza[2] for a review of the literature on this subject). Previous studies have dealt almost exclusively with the effect of hyperglycemia, and the methods that have been used have not provided quantitative information. Ricketts[6] pointed out twenty years ago that the elucidation of the phenomena of insulin secretion would have to await a method for the measurement of the amount of insulin in the blood stream. The results reported in this study have been obtained by means of such a method. The reliability of these results depends, of course, on the reliability of the insulin assay method used.

The question of the specificity of this type of insulin assay procedure has not been completely settled, and doubt has been expressed concerning the nature of the "insulin-like activity" in blood.[7,8] In the present study, the finding of very much higher levels of "insulin-like activity" in the pancreatic venous blood as compared

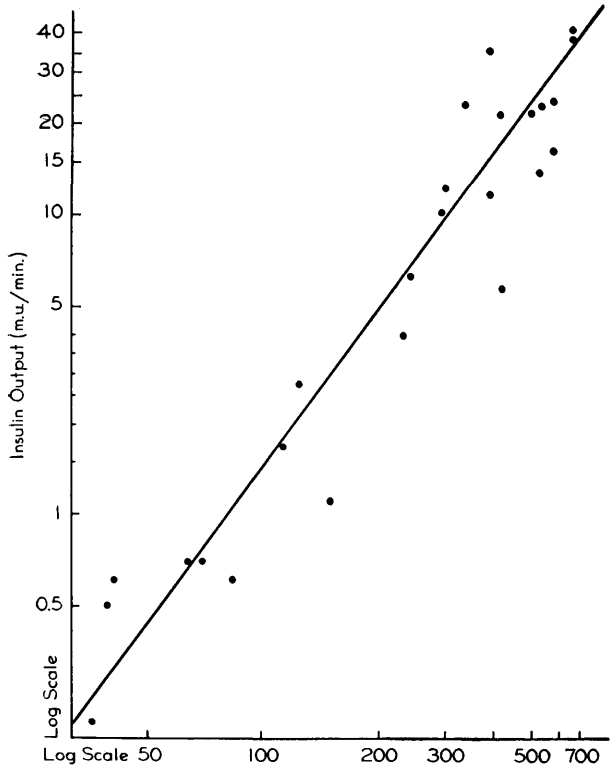

FIGURE 2

to peripheral blood, strongly supports the conclusions of those workers who have claimed that such activity is due to insulin itself.[3,5] Although a precise estimate of the systematic error of the assay was not made, there is reason to believe that the units used in this report do, in fact, correspond closely with the standard units used in the quantitation of insulin. The few assays that were performed on insulin enriched plasma showed almost quantitative recovery of the added insulin. Moreover, a rough calculation* of the daily insulin secretion in the dog, based on the figures obtained in this study, lends further support to the reliability of the figures.

The results obtained in this experiment clearly confirm the belief that hyperglycemia provokes the liberation of extra insulin from the pancreas. They show further that insulin output is a continuous positive function of the concentration of glucose in the blood, and that the response of the pancreas to changes in the blood glucose concentration probably is prompt, as even with a rapidly rising blood sugar, there was no evidence of a modification of the relationship between insulin output and the level of glycemia. It is apparent that this effect of blood sugar on insulin output may function as a negative feedback loop in the homeostatic regulation of blood sugar.

The lowest blood sugar recorded in the experiments was 37 mg./100 ml. and the corresponding insulin output was 0.2 mU./min. However, the 0.2 mU./min. flowing from the pancreatic vein may have represented merely circulating insulin, and there may have been no actual output at the time. At a blood sugar of 660 mg./100 ml. the output was about 40 mU./min. Thus the part of the pancreas drained by the pancreaticoduodenal vein was apparently able to respond to hyperglycemia by increasing insulin secretion from approximately zero to a level of about 2.5 units per hour.

The substantial decrease, or even cessation, of insulin output in the presence of low blood sugar values could have important consequences because of the differences in insulin responsiveness among the various tissues of the body. The shutting-off of the insulin supply in hypoglycemia would have the effect of decreasing glucose transport into insulin-sensitive tissues, such as muscle and adipose tissue, but would not, presumably, affect transport into brain cells with their insulin-independent glucose transport system. The brain would, therefore, be expected to receive a greater proportion of the available circulating glucose in time of glucose deficiency and less vital tissue a lesser proportion.

Some of the findings in this study may have a bearing on certain aspects of diabetes in the human being. The normal dog pancreas showed an enormous capacity to increase rapidly the supply of insulin to the body. If the human pancreas is similarly constituted, it seems not unlikely that the prediabetic and "mild" diabetic states could be an expression of an impairment of this capacity. This speculation receives support from the fact that appreciable and even, in some cases, normal quantities of insulin may be extracted from the pancreases of maturity onset diabetics.[9] Yet the distinguishing feature of this condition is an impaired ability to handle a glucose load, possibly due to an inadequate response on the part of the secretory mechanism of the pancreas.

It is seen that the effect of the blood sugar on the level of insulin liberation may be expressed graphically by a curved line with the concavity upward (figure 3). This suggests that insulin output might be a power

*Insulin output from the pancreatic vein in the presence of a blood sugar of 80 mg./100 ml. was about 1 mU/min. (figures 2 and 3). The pancreatic vein drains about one third of the pancreas, and that part of the organ where there is a lesser concentration of islets than in the tail. Therefore, insulin output from the whole organ would be, say, 4 mU/min., or approximately 6 units/24 hours. This figure must be close to the twenty-four-hour insulin requirement of a fasted dog.

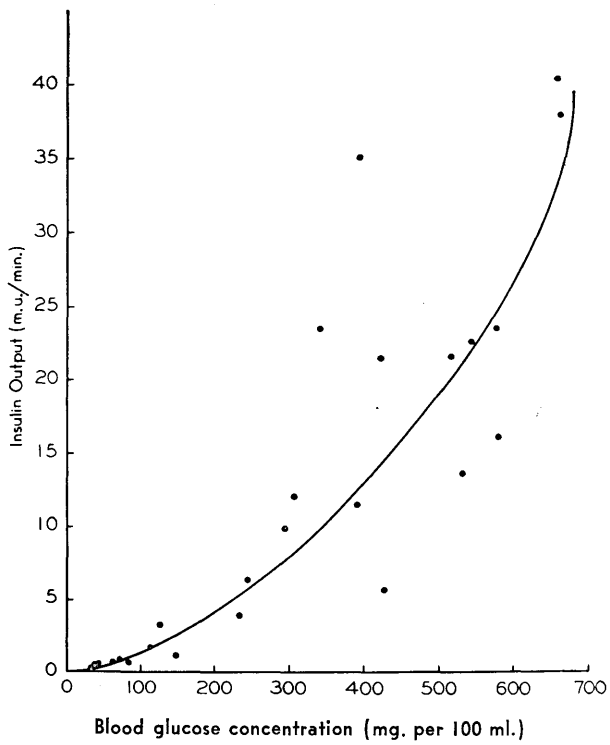

FIGURE 3

function of the glucose concentration, in which case a rise in blood sugar would result in a disproportionate increase in the demand on the pancreas, and the higher the blood sugar, the more potent would be the stimulatory effect. It may be seen (figures 2 and 3) that with a rise in blood sugar from 70 to 300 mg./100 ml., insulin output would be expected to increase from about 0.7 mU./min. to over 7 mU./min., that is, a fourfold increase in the glucose concentration at this level might result in a tenfold increase in the quantity of insulin liberated. If this "forced draught" effect of hyperglycemia exists in the human being, hyperglycemia, even if sporadic, could well hasten the exhaustion of an already compromised pancreas, such as probably exists in prediabetics and those diabetics with residual β-cell function.

SUMMARY

In acute experiments on anesthetized dogs, the venous effluent from a major part of the pancreas was continuously collected, its rate of flow recorded and its insulin concentration measured by the rat diaphragm technic. The rate of insulin secretion was thereby determined in response to lowering the blood sugar by pretreatment with phloridzin, and to elevating the blood sugar by continuous infusion of glucose intravenously. The rate of insulin secretion was found to be a continuous function of the blood sugar level, falling as low as 0.2 mU./min. in hypoglycemia (30-40 mg./100 ml.) and rising as high as 40 mU./min. in severe hyperglycemia (600-700 mg./100 ml.). This response of the pancreas to blood sugar concentration may well serve as a negative feedback loop in the regulation of the blood sugar level.

SUMMARIO IN INTERLINGUA

Le Effecto del Concentrationes de Glucosa Sanguinee Super le Rendimento de Insulina

In experimentos acute con canes anesthesiate, le effluxo venose ab un parte major del pancreas esseva colligite continuemente, le intensitate de su production esseva mesurate, e su concentration de insulina esseva estimate per le technica a diaphragma de ratto. Esseva determinate assi le responsa del secretion de insulina al reduction del sucro de sanguine effectuate per un pretractamento con phlorizina e al elevation del sucro de sanguine effectuate per un continue infusion intravenose de glucosa. Esseva constatate que le intensitate del secretion de insulina esseva un function continue del nivello del sucro de sanguine, descendente usque a 0,2 mU/min in hypoglycemia (30 a 40 mg per 100 ml) e montante usque a 40 mU/min in hyperglycemia sever (600 a 700 mg per 100 ml). Il es ben possibile que iste responsa del pancreas al concentration del sucro de sanguine age como un negative circuito de retro-effecto in le controlo del nivello de sucro in le sanguine.

REFERENCES

[1] Houssay, B. A.: Diabetes as a disturbance of endocrine regulation. Am. J. M. Sc. *193*:581, 1937.

[2] Pozza, G., Galansino, G., Hoffeld, H., and Foa, P. P.: Stimulation of insulin output by monosaccharides and monosaccharide derivatives. Am. J. Physiol. *192*:497, 1958.

[3] Groen, J., Kamminga, C. E., Willebrands, A. F., and Blickman, C. E.: Evidence for the presence of insulin in blood serum: A method for an approximate determination of the insulin content of blood. J. Clin. Invest. *31*:97, 1952.

[4] Wright, P. H.: Plasma-insulin estimation by the rat-diaphragm method. Lancet *2*:621, 1957.

[5] Randle, P. J.: Assay of plasma insulin activity by the rat-diaphragm method. Brit. M. J. *1*:1237, 1954.

[6] Ricketts, H. T.: Carbohydrate tolerance after protamine insulin: Its bearing on the physiology of insulin secretion. J. Clin. Invest. *17*:795, 1938.

[7] Edwards, J.: Estimation of plasma insulin. Lancet *1*:314, 1954.

[8] Leonards, J. R.: Insulin-like activity of blood: What is it? Fed. Proc. *18*:272, 1959.

[9] Wrenshall, G. A., Bogoch, A., and Ritchie, R. C.: Extractable insulin of pancreas. Diabetes *1*:87, 1952.