Diabetologia

Insulin and the Glucose-Glucagon Feedback Mechanism in the Duck

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Summary. The relationship between insulin and the glucose-glucagon feedback mechanism was studied by testing the effectiveness of various routes, doses and timing of insulin administration prior to and during a glucose tolerance test in Peking ducks made transiently diabetic by subtotal pancreatectomy. Insulin injections or infusions given either before, or only during the glucose load, did not restore the A-cell response to glucose. Yet, if given both before and during the glucose test, in conditions which mimic the physiological basal insulin level and its variations (with, initially, intramuscular injections of 0.2 IU/kg and 8 µg/kg glucagon, every six hours, and then an intravenous injection of 3.6 mU/kg plus an infusion of 0.9 mU/kg/minute for one hour), the normal glucagon response to glucose was re-established. Insulin must therefore be present, both before and during glucose stimulation, for glucose to be effective as an A-cell suppressor.

Key words: Duck, glucose-glucagon feedback mechanism, glucagon, insulin, glucose.

The existence of a glucose-glucagon negative feedback mechanism has been demonstrated in normal man [34], dogs [5, 6, 26], ducks [13] and geese [13, 14, 32]. It applies under physiological conditions: plasma glucose variations of the order of 5% or less are sufficient to evoke a rapid response in geese [32]; similar data have been obtained in hypophysectomized ducks [18, 7].

This feedback mechanism would be expected to reduce the elevated plasma glucagon levels of diabetic animals, but such a response has never been observed in any of the species studied: man [1, 34], dogs [24] or ducks [13].

The present investigation[1] was undertaken to see if insulin is a prerequisite for the normal functioning of the feedback system. We chose to use ducks made transiently diabetic by subtotal pancreatectomy: the duck is a species in which glucagon assumes a particular importance [21, 22] and where the operation provokes a reversible diabetic state [21].

Material and Methods

1. Animals

90 white Peking male ducks, 3 to 6 months old, were used. They were installed indoors, at room temperature, one day before, and after the operation, and fed "ad libitum" fowl pellets ("Fortis Duquesne") plus tap water.

2. Subtotal Pancreatectomy

The operation was performed under local anaesthesia according to the technique described by Mialhe [21]; only the round end of the splenic lobe was left.
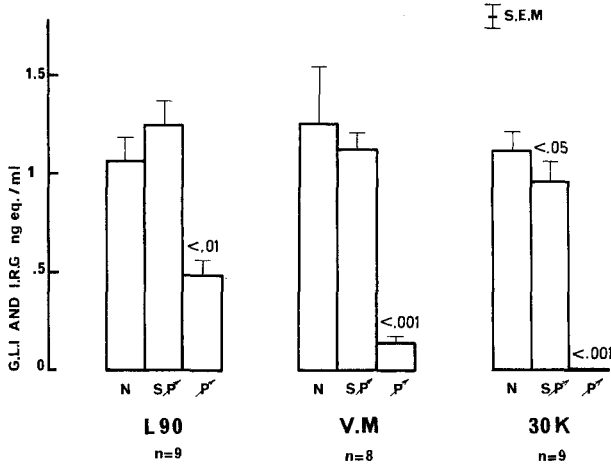
3. Plasma Samples

Ducks were fasted for 14 to 18 hrs and the blood, drawn from a wing vein in the morning, kept on ice until centrifugation. Plasma samples were kept frozen until use. Trasylol has been shown to be unnecessary in our method of glucagon determination [17].
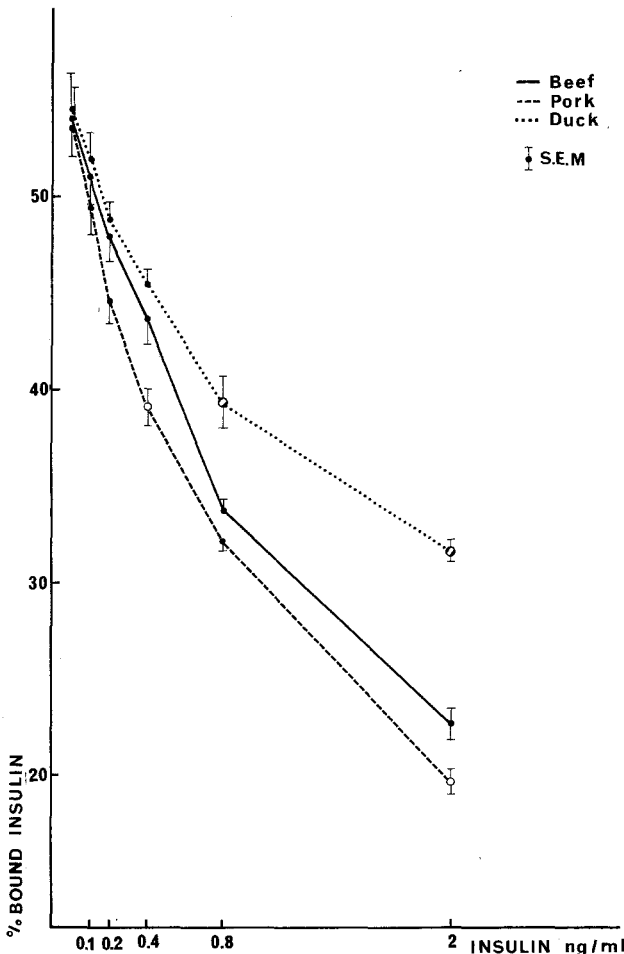

Fig. 1. GLI and IRG decrease in subtotally depancreatized ducks subjected to a total pancreatectomy two days later. These value are determined with three different antibodies: L90, VM, and 30K. N = normal fasted ducks, S. P = two days subtotally depancreatized ducks, P = four hours totally depancreatized ducks, n = number of animals

Fig. 2. Comparison of IRI standard curves obtained with beef (——), pork (---) and duck (......) insulins. ○ slightly significant difference ($p < 0.05$) from beef standard curve; ● significant difference ($p < 0.01$) from beef standard curve. n = 8 determinations

4. Plasma Determinations

a. Plasma Glucose was measured with a Technicon autonalyser by the method of Hoffman [11] after dialysis of the plasma.

b. Plasma Total Glucagon (GLI) was determined by the radioimmunoassay of Leclercq-Meyer et al. [17] using Novo pork glucagon as a standard. The results are expressed in ng porcine glucagon equivalents/ml and, in Figure 6, as a % of the control value (this was necessary because the data, obtained with two different antibodies, were scattered, even if the variations observed were of the same amplitude and direction).

Neither of the two antibodies, VM and L90, used to determine plasma GLI, are specific for pancreatic glucagon. We are, however, able to assert that the plasma GLI variations correspond to real changes in pancreatic glucagon (IRG) from the following series of experimental observations:

– After total pancreatectomy, a mean GLI value of 0.20 ng Eq/ml was determined using the VM antibody [10, 16] and of 0.56 ng Eq/ml with the L90 antibody. These basal GLI values correspond to gut GLI.

– When subtotally depancreatized ducks are subsequently *totally* depancreatized after the time interval normally observed before the initiation of a glucose tolerance test, i.e. two days later, plasma GLI levels fall to the basal level characteristic of one-stage depancreatized animals, i.e. 0.14 ± 0.03 ng Eq/ml with VM antibody and 0.48 ± 0.08 ng Eq/ml with L90 antibody (Fig. 1). For comparative purposes, Figure 1 also includes the GLI and IRG values of normal fasted, two days partially depancreatized and four-hour totally depancreatized birds, obtained with three different antibodies: VM and L90, raised in our laboratory, and 30K purchased from Unger. The last, which is known to be pancreatic-specific, confirms the high glucagon values observed with our antibodies: it gives levels of 1.1 ng Eq/ml and 0.96 ng Eq/ml in the normal fasted and in the partially depancreatized duck, respectively, and shows moreover that the main glucagon species in the duck is indeed of pancreatic origin: four hours after total pancreatectomy, the glucagon level, as measured with the 30K antiserum, is zero.

– Furthermore, the only variation of plasma gut GLI values so far observed in pancreatectomized ducks occurred after an amino-acid infusion [31]; it was small and delayed; free fatty acids [10] and glucose [29] have no effect on basal gut GLI values.

We can therefore confidently conclude that the GLI variations we measure are of pancreatic origin.
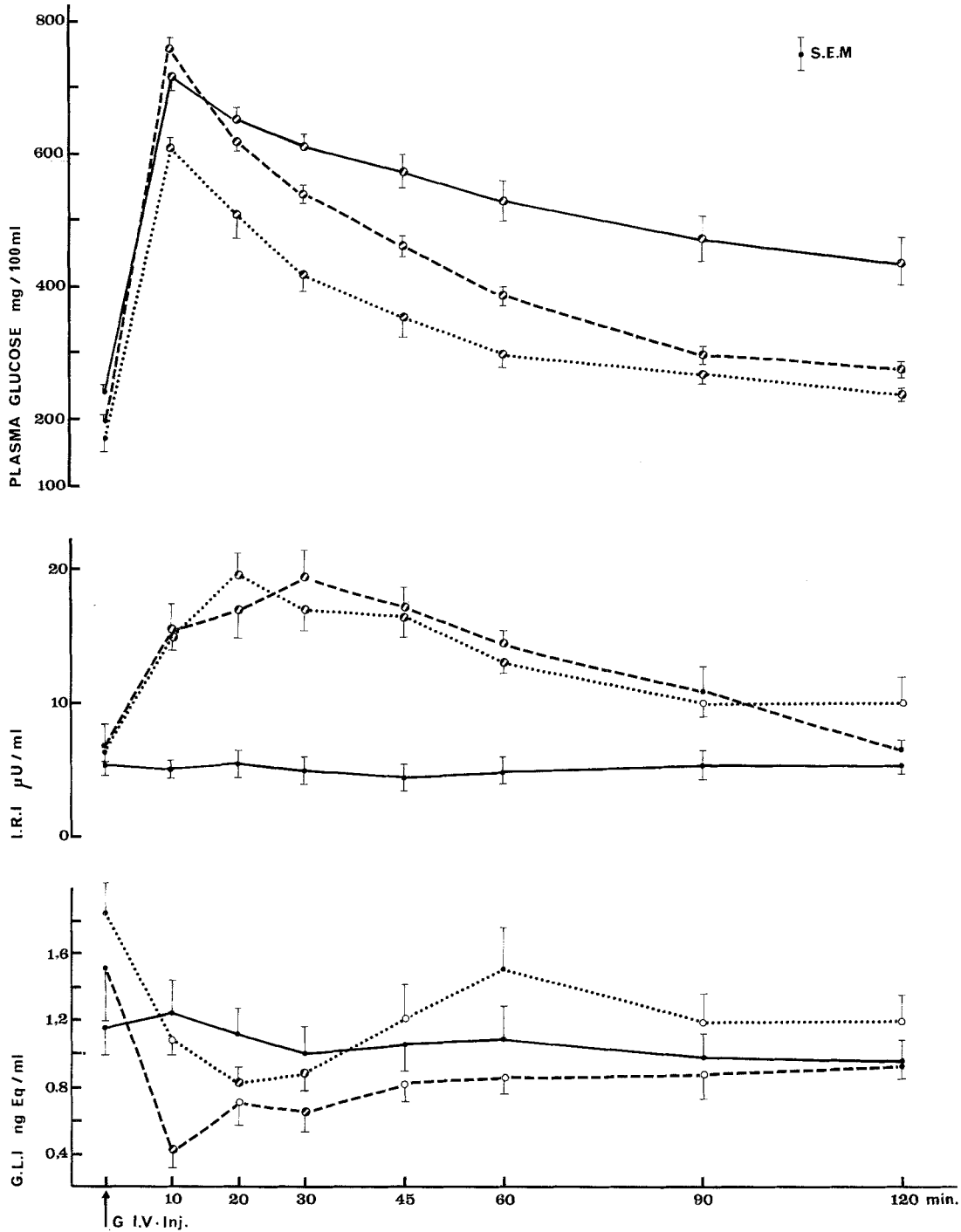
Fig. 3. *Glucose tolerance test in normal ducks, in transiently diabetic ducks and after transient diabetes.* Effect of an intravenous glucose injection (1.75 g/kg) on plasma glucose, IRI and GLI: – in 12 normal fasted ducks ------, – in 8 to 16 transiently diabetic ducks, i.e. two days after subtotal pancreatectomy ——, – in 8 to 16 ducks after transient diabetes, i.e. twelve days after subtotal pancreatectomy .... In this figure, as in the following ones, ● and ○ respectively correspond to highly significant ($p < 0.01$) and significant ($p < 0.05$) differences from the zero time value, i.e. the glucose injection (G. IV inj.)

c. Plasma Immunoreactive Insulin (IRI) was determined by radioimmunoassay using a dextran coated charcoal method of separation [8] and Novo beef insulin as a standard. As the ducks were treated with pig insulin, a comparison between pig, duck and beef insulins seemed necessary. Figure 2 shows the standard curves obtained with the different insulins: duck insulin determinations read on beef standard curves are under-estimated ($p < 0.01$), from 0.8 ng/ml ($\simeq 20$ µU/ml) onwards, while pig determinations read on the same standard curve are slightly over-estimated ($p < 0.05$), from 0.4 ng/ml ($\simeq 10$ µU/ml) onwards. The errors in the results are therefore small under our experimental conditions[1].

5. Insulin Injections and Infusions

Novo Actrapid insulin was used; the timing and doses are detailed for each experiment.

6. Glucose Tolerance Tests

The ducks were tied to a board, lying on their back, they remained calm and immobile under these conditions and did not need to be anaesthetized.

The large dose of glucose used (1.75 g/kg IV) corresponded to that known to provoke maximal suppression of glucagon secretion in normal birds [15].

7. Statistical Methods

Student's "t" test was used. All the mean values were compared to zero time and are given with the standard error of the mean.

Results

1. Effect of Intravenous Glucose on Glucagon Secretion in Normal and Diabetic Ducks

In normal ducks (Fig. 3), intravenous glucose induces a significant decrease of plasma GLI ($p < 0.01$ from 10 to 30 min; $p < 0.05$ from 45 to 90 min) and a significant increase of plasma IRI ($p < 0.01$ from 10 to 60 min).

During a transient diabetic state [21, 22], i.e. two days after subtotal pancreatectomy (Fig. 3), the ducks are hyperglycemic and there is no significant decrease

of plasma GLI and no increase of IRI despite the huge rise in plasma glucose concentration.

When, however, the same animals regain a normal, or near-normal glucose tolerance, i.e. 12 days to 3 months later (Fig. 3), a dramatic decrease of GLI level is observed ($p < 0.01$ at 20, 30 minutes, $p < 0.05$ at 10, 45, 90 and 120 minutes) following the glucose injection, and an increase of IRI level ($p < 0.01$ until 60 minutes; $p < 0.05$ from 90 to 120 minutes). They nonetheless differ from normal ducks in having a high fasting glucagon level; this high level may be related to the glycaemia which is significantly lower (176 ± 9 mg/100 ml) than in the normal (199 ± 7 mg/100 ml).

In three ducks (Fig. 4) a permanent severe diabetes occurred after subtotal pancreatectomy. The

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[1] In diabetic ducks treated with pig insulin, we measure a mixture of duck insulin at low concentration (when the duck and beef curves almost coincide) and of pig insulin – at low concentration (for example I. M. "physiological" injections): pig und beef curves are close together, so that the values read are near to the real ones. – at higher concentration (I. M. and I. V. injections, infusions): pig and beef curves are slightly different, so that the values obtained are slightly overestimated. Thus, if we find IRI levels close to the normal range, we can be sure that the treatment restored physiological conditions, as errors only lead to over-estimation.
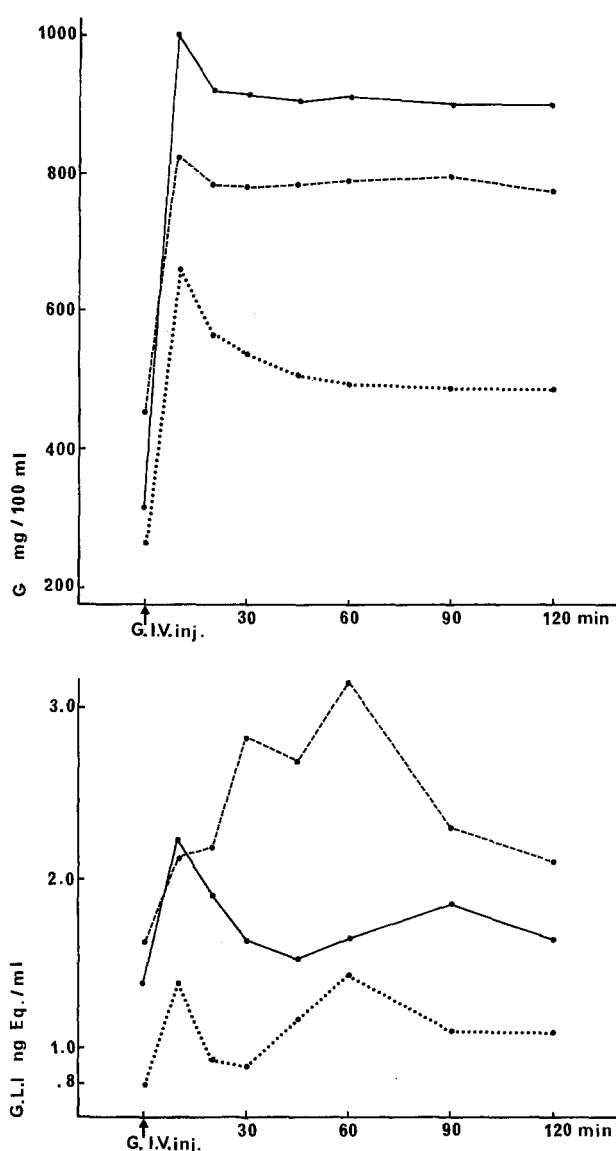

Fig. 4. *Permanent diabetes following subtotal pancreatectomy.* Evolution of plasma glucose and GLI during a glucose tolerance test in 3 subtotally depancreatized ducks
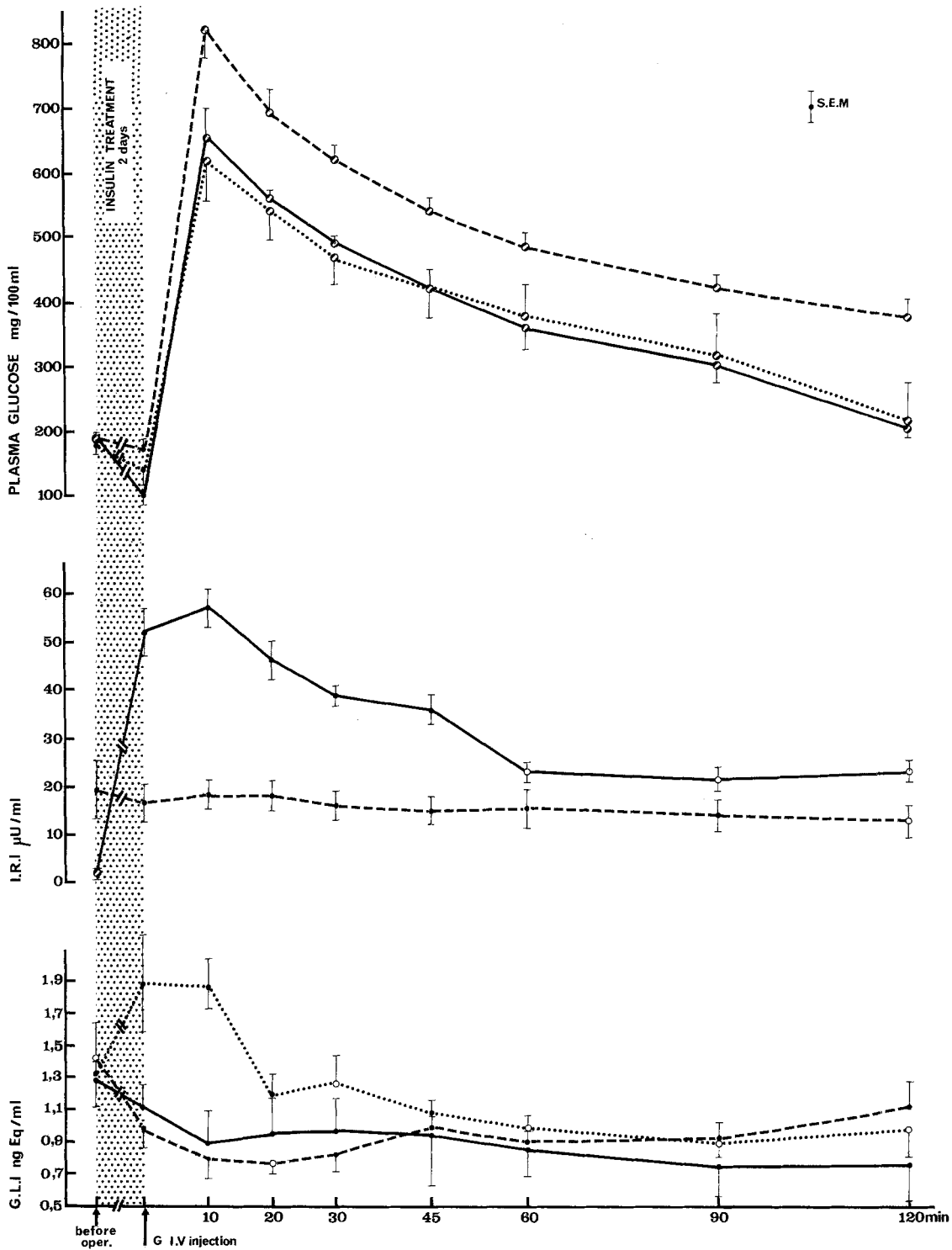
Fig. 5. *Glucose tolerance test in transiently diabetic ducks treated with different doses of insulin before the test.* – small doses of insulin. (10 ducks) -----: 0.2 U/kg I. M. insulin plus 8 μg/kg I. M. glucagon were injected every six hours between the operation and the test. – high doses of insulin. (5 ducks) ——: 1 U/kg I.M. insulin plus 40 μg/kg I.M. glucagon were injected every 12 hrs between the operation and the test. – high doses of insulin (6 ducks) ......: 2 U/kg I. M. insulin and 80 μg/kg I. M. glucagon were injected every 12 hrs between the operation and the test. The curves show the evolution of plasma glucose, IRI and GLI, before subtotal pancreatectomy, after two days of treatment (time zero of the test) and during the glucose test. In this figure, as in the following ones, the dotted area corresponds to the two post-operative days. ● and ○: same symbols as in Fig. 3

glucose tolerance was greatly impaired; plasma GLI levels were high and rose paradoxically during the test.

2. Effect of Insulin on the Glucose-Glucagon Feedback Mechanism in Diabetic Ducks

The normal functioning of this feedback mechanism may require not only the presence of insulin but, in addition, its intervention at specific stages, i.e.: A = before, B = during C = before and during a glucose load = A and B combined. In order to determine at which moment an insulin presence becomes crucial, we injected insulin according to three different schedules.

a. Insulin Treatment before the Glucose Load

Small Doses. Ten partially depancreatized ducks were treated intramuscularly with insulin and glucagon in the molar ratio of glucagon to insulin of 1.66 to 1 (1 to 1, weight by weight); this proportion is not far from that found in normal conditions (Mialhe [21] quotes a figure generally falling in the range of 2 to 4, determined by the quantities of intramuscular glucagon and insulin necessary to restore the blood glucose of totally depancreatized birds to normal). Our animals were treated immediately after the operation, and every six hours, for two days, the last injection being given three hours before the glucose test.

After several attempts, we found that 0.2 U/kg insulin and 8 µg/kg glucagon every six hours maintained a blood glucose level and an IRI level next to normal.

After this treatment (Fig. 5), basal plasma glucose and IRI were not significantly different from preoperative levels. Two hours after the glucose load, the plasma glucose level was still twice as high as before the injection. During the glucose test, plasma IRI does not increase significantly (because of the removal of most of the B-cells), and the decrease of plasma GLI compared to time zero is only significant ($p < 0.05$) at 20 minutes. This plasma GLI variation is shorter and smaller than in normal or twelve days subtotally depancreatized ducks.

We then tried treatments with higher amounts of insulin and glucagon.

High Doses. Eleven partially depancreatized ducks received higher amounts of intramuscular insulin and glucagon, still respecting the same ratio, immediately after the operation and thence for two days; the last injection was given three hours before the glucose test.

Five ducks received intramuscular injections of 1 U/kg insulin and 40 µg/kg glucagon twice a day (Fig. 5). In this case, plasma glucose decreases from 190 ± 4

mg/100 ml before treatment, to 95 ± 17 mg/100 ml after the treatment; this represents a highly significant decrease of glycaemia. Plasma IRI level significantly increases from 2.1 ± 0.4 µU/ml to 51.5 ± 5.7 µU/ml after treatment. In spite of the high plasma glucose and IRI during the glucose test, there is no significant decrease of plasma GLI.

Six ducks were injected with still higher doses, that is 2 U/kg insulin and 80 µg/kg glucagon twice a day, under the same conditions. After this treatment (Fig. 5), glycaemia decreases significantly (141 ± 28 mg/100 ml), and the glucose curve looks more like a normal one; there is a significant GLI decrease ($p < 0.05$) at 30, 60, 90 and 120 minutes. In normal ducks, the GLI decrease is more important and occurs earlier.

Thus, when insulin is given to diabetic ducks in physiological amounts *before* the glucose test, glucagon secretion is not suppressed by glucose; a small and late glucagon suppression is observed only with pharmacological quantities of insulin.

b. Insulin Treatment at the Time of Glucose Injection

Insulin Injection. Six operated ducks received simultaneous intravenous injections of insulin and glucose (Fig. 6). The amount of insulin used (0.2 U/kg) is one which normalizes the plasma glucose curve in totally depancreatized ducks given the same glucose load [20].

No decrease of plasma GLI is observed at the beginning of the glucose test; it falls slightly at 90 minutes and significantly ($p < 0.05$) at 120 minutes.

"Physiological" Insulin Variation Curve. In order to get closer to physiological conditions, we thought it might be preferable to give a small priming intravenous dose of insulin at the same time as the glucose load, and then an infusion of insulin for one hour.

After several attempts, we found that a priming dose of 3.6 mU/kg insulin, followed by an infusion of 0.9 mU/kg/minute insulin, closely mimics the normal insulin response to glucose. Ten ducks were treated in this way.

Under these conditions, (Fig. 7) the plasma IRI variation curve obtained after the glucose load is similar to the normal one. Plasma IRI increases significantly ($p < 0.01$) from 10 to 60 minutes; the values are only slightly higher than in normal ducks given the same glucose load. Plasma GLI decreases significantly at 10 minutes ($p < 0.05$), but from the 20th min. onwards, it is no longer significantly different from the basal value.

Therefore, when insulin is given to diabetic ducks *during* the glucose load, glucagon secretion is not suppressed by glucose, or only for a shorter time than in the normal.
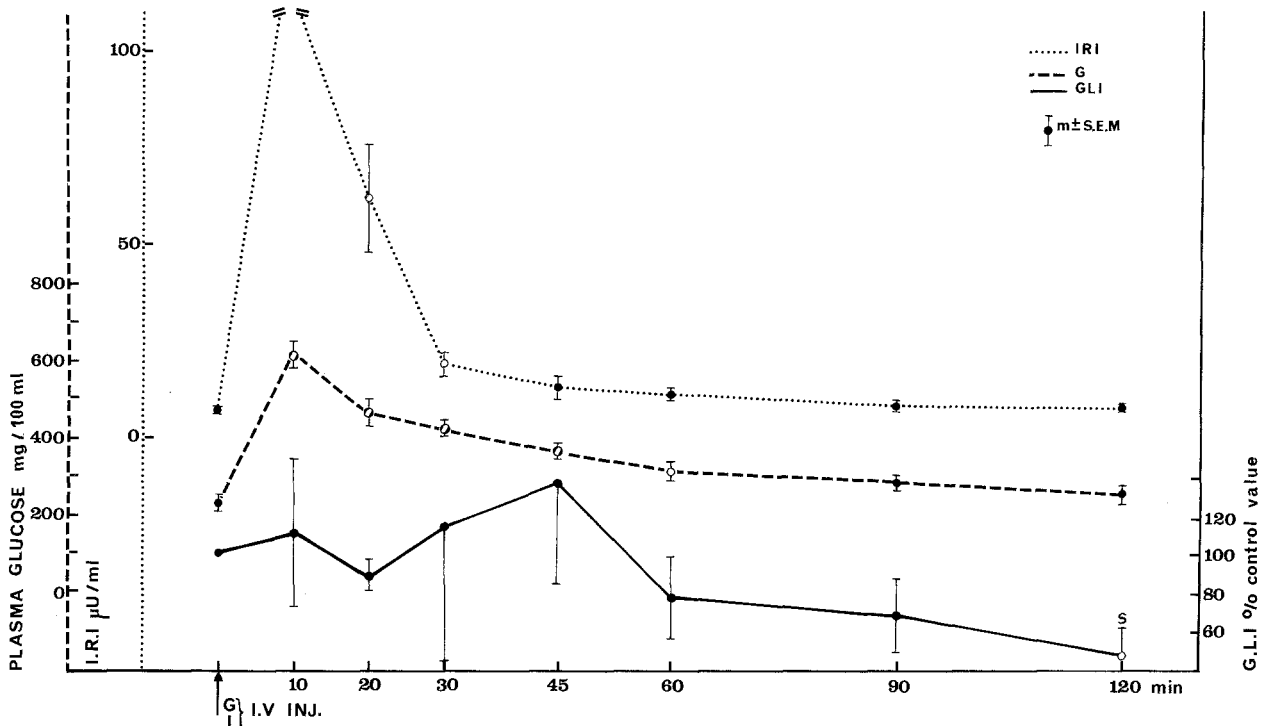
Fig. 6. *Glucose tolerance test in transiently diabetic ducks given an insulin injection at the beginning of the glucose test.* 0.2 U/kg insulin was injected I. V. at zero time. Plasma glucose, IRI and GLI during the test (6 ducks). ⊙ and ○: same symbols as in Fig. 3. In this figure, as in the two following ones {$_I^G$. I. V. inj. = injection of glucose + insulin at time zero

Fig. 7. *Glucose tolerance test in transiently diabetic ducks given a priming injection of 3.6 mU/kg insulin followed by an one hour insulin infusion of 0.9 mU/kg/min., during the test.* Plasma glucose, IRI and GLI, before subtotal pancreatectomy, two days thereafter, and during the test (10 animals). ⊙ and ○: same symbols as in Fig. 3
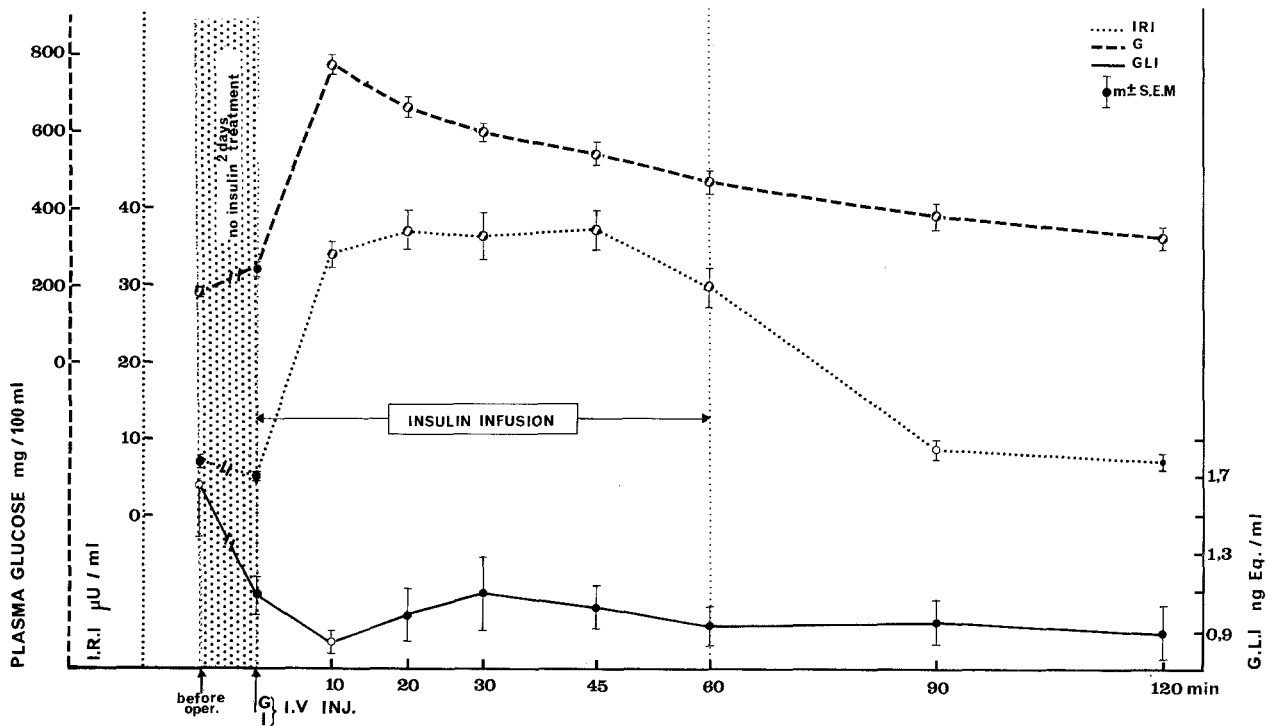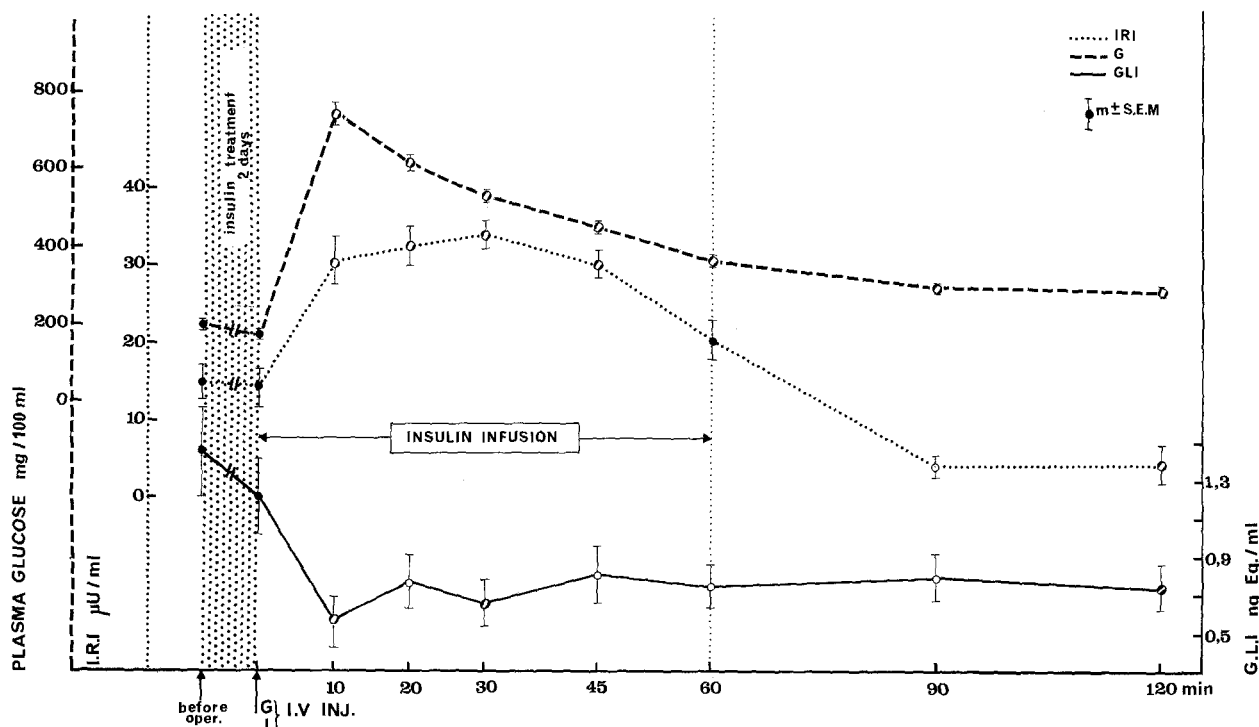
Fig. 8. *Glucose tolerance test in transiently diabetic ducks treated with insulin before and during the test.* 0.2 U/kg I. M. insulin and 8 µg/kg I. M. glucagon were injected every 6 hrs between the operation and the test; a priming intravenous injection of 3.6 mU/kg insulin followed by an one hour infusion of 0.9 mU/kg/min insulin, was given during the glucose test. Plasma glucose, IRI and GLI, before the operation, two days thereafter, and during the test (13 animals). ● and ○: same symbols as in Fig. 3

c. Insulin Treatment before and during the Glucose Tolerance Test

In a further attempt to approach physiological conditions, we combined the two treatments A and B: we injected intramuscularly small doses of insulin (0.2 U/kg) and glucagon (8 µg/kg) every six hours after the operation (the last injection being given three hours before the glucose test as before), and then a priming intravenous dose of insulin (3.6 mU/kg) at the time of the glucose injection, followed by an insulin infusion (0.9 mU/kg/min.) for an hour.

Under these conditions (Fig. 8), the plasma IRI level measured at zero time is not significantly different from the preoperative one. The plasma IRI curve during the glucose test looks like the normal one: there is a very significant rise ($p < 0.01$) of IRI from 10 to 45 minutes; in normal ducks, this rise is significant ($p < 0.01$) from 10 to 60 minutes, the IRI values being slightly lower.

The plasma GLI decrease is significant ($p < 0.05$) or highly significant ($p < 0.01$) for all values (10 to 120 minutes). This GLI decrease is similar to that seen in normal or twelve days depancreatized ducks.

Thus, if physiological insulinemia and physiological insulin variations are restored in diabetic ducks, glucagon secretion is suppressed by glucose: insulin seems essential for the normal functioning of the glucose-glucagon interplay.

Discussion

1. Glucagon Secretion during Diabetes

The plasma glucagon levels measured in our studies (GLI) correspond to glucagon (IRG) of almost exclusively pancreatic origin (see Methods section).

In the three cases of severe diabetes observed in this series of experiments, high GLI levels were noted. High GLI plasma levels have already been reported during spontaneous or experimental diabetes in several species: in man [1, 34], dogs [24] and geese [12, 13, 14]. In addition a GLI rise is observed after i. v. glucose in these three ducks. A similar IRG rise has already been reported in diabetic man after a carbohydrate meal [23]. At the moment however, an interpretation of this phenomenon could only be speculative, since only three ducks responded in this manner.

However, in ducks, during transient surgical diabetes for two days [22], GLI levels are low, but sufficient for a true diabetic state to exist. In the complete absence of pancreatic glucagon after total pancreatectomy, hypoglycemia accompanies fasting, and hyperglycemia the fed state. This means that, in the duck, the presence of a small level of circulating pancreatic glucagon is necessary for a true diabetes to appear; IRG levels are not high during the diabetes following

Fig. 9. *Postulated double action of insulin on the A-cell:* ① corresponds to a long term action of insulin, for example on glucose metabolism within the A-cell and ② to a short term action of insulin: this might be the insulin-glucagon feedback mechanism, which might come into play when insulin release is provoked by a factor like hyperglycemia

subtotal pancreatectomy, because of the reduced pancreatic mass. It is remarkable that in twelve days or less, the pancreatic remnant is able to secrete enough glucagon and insulin to restore the metabolic state to one approaching normal.

2. Glucose-Glucagon Feedback Mechanism during and after Diabetes

a. During Diabetes. During transient diabetes, an induced hyperglycemia does not decrease GLI plasma level: this suggests that a diabetic state of two days or less, suppresses the sensitivity of the A-cell to glucose. Similar findings have been reported for chronic diabetic states in man [23, 35], dogs [2, 3] and geese [13, 14].

When the diabetic episode is over, glucagon secretion is reduced by a hyperglycemic load, and insulin secretion (and glucose tolerance) also become normalized: this observation favours an action of insulin in the glucose-glucagon feedback mechanism. Curiously enough, the glucagon basal level is high in these animals. This could be due to the slight hypoglycemia; alternatively some metabolite other than glucose may not have regained its normal plasma level twelve days after the operation.

b. Insulin Treatment. Insulin treatment of diabetic ducks gives different results according to the injection schedule. Insulin injections made only before, or only during, the glucose tolerance test do not normalize the glucose-glucagon feedback mechanism, except if pharmacological amounts of insulin, provoking hypoglycemia, are given before the test. In contrast, when insulin is given both before and during the glucose load, so as to mimic physiological basal insulin

levels and variations, a normal glucagon response to glucose is observed.

It is interesting to compare these results with those obtained in other species. In the normal human newborn [19], glucose alone does not suppress glucagon secretion; this is probably due to an unresponsiveness of the B-cell to glucose, since glucose + exogenous insulin decrease the plasma glucagon level. In alloxan-diabetic dogs, hyperglucagonemia [24] and A-cell unresponsiveness to glucose [35] are suppressed by a huge insulin infusion, even after long-standing diabetes [2, 3]. In man, the hyperglucagonemia of severe diabetic ketoacidosis is also suppressed by insulin therapy [1, 25], but the administration of exogenous insulin during a carbohydrate meal, or a glucose infusion [36], fails to restore the A-cell suppressibility of diabetic subjects, even when plasma insulin rises above normal concentrations. This discrepancy may be explicable in the light of our results: diabetic patients have passed through a prolonged phase of insulin deprivation, or abnormal secretion, before the diabetic state is detected.

It is of interest that the presence of insulin is necessary both before, and during, a glucose load for a normal adjustment of glucagon secretion.

Lack, or insufficient secretion of insulin for even two days suppresses the glucagon response to glucose. Glucagon release was, however, found to be abnormally increased, as in diabetes, when glucose metabolism within the A-cell was impaired by inhibition of substrate oxidation in vitro [4], and by 2-deoxyglucose or mannoheptulose in vivo in normal dogs [24, 37]. These facts suggest that insulin could be necessary for glucose metabolism within the A-cell; this would be a long term action. Any other explanation, such as the induction or suppression of some enzymes within the A-cell, might be valid, since such mechanisms exist in the hepatocyte [38]. The inhibitory effect of glucose on A-cell secretion may also be related to its interaction with membrane receptors; indeed, it has been suggested that glucoreceptors exist at the A-cell level [27, 9].

The absence of normal insulin secretion during a glucose load in the duck also suppresses the glucagon response; this is a short term action of insulin and could mean that the direct insulin-glucagon feedback mechanism [28, 30] may play an important role in the suppression of glucagon by glucose. This postulated double action of insulin on the A-cell could then be as described in Figure 9.

Conclusion

Insulin is able to restore the A-cell suppressibility by glucose in ducks made transiently diabetic by subtotal

pancreatectomy. It must be given both between the operation and the glucose load (regularly spaced I. M. injections), and during the glucose load (i. v. injection + infusion).

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