

compared on the basis of gram wet tissue. "Mitochondria-free" fractions of rat liver had 67% of the insulinase activity of the "nucleus + debris-free" fractions; whereas, "mitochondria-free" fractions of rat kidney had 28%.

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Effect of Growth Hormone and Glucose on ^{14}C Incorporation by the Perfused Dog Pancreas* (33045)

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The feasibility of using the isolated, recirculated perfusion of the dog pancreas to study the biosynthesis of insulin *in vitro* has been demonstrated (1). Recent reports have indicated that pituitary growth hormone (GH) is necessary for or augments the release of immunoreactive insulin (IRI) (2-6), and stimulates the incorporation of tritiated leucine into insulin in rat pancreas slices (7). These observations suggested that an effect of GH on the incorporation of ^{14}C labeled amino acids into insulin might be demonstrated in the perfused dog pancreas.

Methods. Dog pancreata were perfused with a medium containing ^{14}C labeled amino acids as described previously (1). One series of perfusions was carried out at low glucose levels (approximately 50 mg/100 ml) with the *in vitro* addition of one or three units of porcine GH.² Another series was carried out at glucose levels of 250-350 mg/100 ml with or without the *in vitro* addition of GH. In addition, two dogs were pretreated with GH at a dosage of 3 mg/day given in equal subcutaneous injections morning and evening for 5 days prior to taking the pancreas. The

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² Porcine GH was purchased from General Biochemicals, Chagrin Falls, Ohio 44022.

last GH injection was given approximately 18 hours before the pancreas was removed. These pancreata were perfused at the high glucose level with the same amount of amino acids- ^{14}C as those described above (1).

All perfusions were terminated at the end of 2 hours. Each pancreas was extracted for insulin as previously described (1) and the immunoreactive insulin (IRI) determined by method C of Hales and Randle (8)³ and the ^{14}C content of the insulin containing fraction was determined. As described in the earlier report (1), the major portion of the IRI eluted in peak 2 from the Sephadex G-50 column, and since this peak contained the major portion of the radioactivity, it was assumed that the incorporation of ^{14}C into this fraction represented the biosynthesis of insulin.

Results. The results of the extractions of the perfused pancreata are shown in Fig. 1. As previously reported, the pancreas extract typically eluted from the Sephadex columns in 3 peaks. All three peaks from each fractionation were assayed for IRI and the ^{14}C content was determined. It may be seen in Fig. 1 that virtually all of the IRI and with one exception the major portion of the ^{14}C

³ The guinea pig anti-insulin serum and the crystalline porcine insulin used as standards were generously supplied by Dr. Mary Root of the Eli Lilly Research Laboratories.

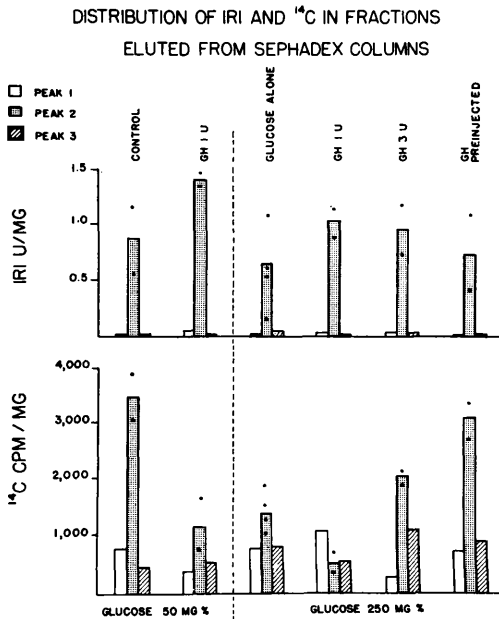


FIG. 1. The IRI and ¹⁴C recovered in the three peaks of pancreas extract eluted from Sephadex G-50 columns with 1 M acetic acid. The dots on peak 2 represent the actual values obtained and indicate the number of perfusions performed.

was found in peak 2. The data of Fig. 1 indicate that major changes of ¹⁴C incorporation occurred only in peak 2 in response to the experimental conditions employed. The small changes in ¹⁴C incorporation into peaks 1 and 3 were considered to be within limits of the experimental error. Therefore, the changes in ¹⁴C incorporation in peak 2 have been interpreted as representing changes in insulin biosynthesis.

The ¹⁴C incorporation into the insulin containing fraction was greatest when the perfusion medium glucose was 50 mg/100 ml. The addition of 1 unit of GH at the low glucose level reduced the average ¹⁴C incorporation to a third of that seen with low glucose alone. When the pancreas was perfused with a high glucose level alone, the ¹⁴C incorporation was similarly reduced to only a third that of the low glucose perfusions. The addition of 1 unit of GH under conditions of high medium glucose levels resulted in a still further reduction of ¹⁴C incorporation into the insulin containing fraction. The ¹⁴C recovery in peak 2 after the

addition of 3 units of GH at a high glucose level was above that with high glucose alone but was below that with low glucose alone.

Pancreata removed from dogs pretreated with GH for 5 days prior to perfusion and perfused with the high glucose load had approximately the same ¹⁴C incorporation that was found in the low glucose alone perfusions and was substantially higher than with high glucose alone or when GH was added *in vitro*.

The IRI recovered in peak 2 ranged from 0.6 units/mg to 1.4 units/mg of extract. These figures reflect only the IRI activity of the insulin containing fraction on a unit/mg extract basis and cannot be related to the experimental condition employed. The method of extraction did not lend itself to a determination of the total amount of insulin present in the pancreas.

Discussion. The data indicate that the incorporation of ¹⁴C labeled amino acids into the IRI containing fraction of the isolated, perfused dog pancreas is reduced in conditions which cause increased IRI secretion. Preliminary experiments have indicated that elevation of the glucose level of the perfusion medium results in the release of IRI in this preparation (unpublished observations), and this is what might be expected from the results of others (2-7, 9). Furthermore, GH added to the perfusion medium augments the insulin response induced by glucose load. Thus, the greatest incorporation of ¹⁴C into pancreas IRI occurred at glucose levels of about 50 mg/100 ml, a level that does not stimulate IRI release. The addition of 1 unit of GH at low glucose levels or the increase of medium glucose alone to 250 mg/100 ml resulted in a marked reduction of the ¹⁴C incorporation. The simultaneous elevation of the glucose level and the addition of 1 unit of GH resulted in a further reduction of the ¹⁴C content of the IRI containing fraction. With the information presently available there is no way to account for the observation that 3 units of GH added to the perfusion medium or injected into the pancreas donors did not reduce the ¹⁴C content of the IRI containing fraction as effectively as 1 unit of GH at a high glucose level.

Taylor and Parry (10,11) earlier suggested that insulin secretion and synthesis were interdependent, based on the observation that rabbit and ox pancreas slices incorporate more ^3H labeled leucine into insulin and secrete more insulin in a high glucose medium than in a low glucose medium. More recently Martin and Gagliardino have made the same suggestion (7). However, Taylor and Parry (12) using a potent stimulator of insulin secretion, tolbutamide, have concluded that increased synthesis of insulin is not an obligatory accompaniment of increased insulin secretion. The data presented here suggest that conditions favoring IRI release are independent of insulin synthesis. This is in accord with the report of Lazarow *et al.* (13) that islet tissue of the marine teleost fish incorporate only half the ^3H labeled leucine into insulin in a high glucose medium as compared to a low glucose medium. Our observation of reduced ^{14}C incorporation into the insulin fraction in the presence of high glucose levels is in accord with the recent report of Wells *et al.* (14, 15). Using histological evidence for insulin synthesis and/or storage in rat pancreata grown in tissue culture these workers have concluded that high glucose levels inhibit insulin synthesis and/or storage. It is possible that conditions that favor insulin release such as high glucose levels (9) and which is augmented by the presence of the GH (2-7) cause a diversion of energy and/or substrate away from the synthetic process. Alternatively, factors leading to increased IRI secretion could conceivably stimulate the incorporation of sufficient amounts of unlabeled precursors into insulin that the ^{14}C incorporation would appear to be relatively reduced. Further work is required for the resolution of this problem.

As previously indicated (1), the maximum activity of the pancreas fractions was about 1.5 units/mg. It is generally accepted that pure insulin has an activity of 20-25 units/mg which indicates that fractions produced here while having the greatest IRI and ^{14}C activity, still contained much inert material. The resolution of the factors governing the biosynthesis of insulin in this *in vitro*

preparation await the development of a method which will permit the preparation of pure insulin from less than a whole, single dog pancreas.

Summary. The isolated, recirculated, perfused dog pancreas has been used to study the effect of elevated glucose concentrations and GH on the incorporation of ^{14}C labeled amino acids into the insulin containing fraction. The greatest incorporation occurred at the low glucose level. High glucose levels alone and 1 or 3 units of GH at low or high glucose levels caused a reduction of ^{14}C amino acid incorporation into the IRI containing fraction below that seen with low glucose levels alone. Pretreatment of the pancreas donors with GH restored the ^{14}C incorporation to that seen with low glucose alone. The results are discussed.

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