STUDIES ON BIOSYNTHESIS OF INSULIN BY PANCREAS SLICES*

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The study of the synthesis of insulin per se is of great importance from the point of view of the homeostatic mechanisms involved in the control of blood sugar and the eteology of diabetes mellitus. In the past, this study was made difficult because of the small amounts of insulin producing beta cells relative to acinar cells in pancreas. The presence of proteoletic enzymes in acinar tissue as well as the lack of an accurate assay method to measure newly synthesized insulin contributed to the difficulty of this problem. The development of anti-insulin serum (Moloney and Coval 1955 and Armin et al. 1960) has made possible the assay and isolation of small quantities of labeled insulin by incubating pancreas extracts with anti-insulin serum. The insulin antibody complex may be precipitated with sodium sulfite (Grodsky and Forsham 1960). The presence of proteolytic enzymes in pancreas may be greatly reduced by feeding an ethionine diet which results in atrophy of the ascinar tissue (Farber and Popper 1950). In this communication, studies on insulin synthesis by pancreatic tissues from rats fed ethionine and normal diet

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are reported. Male albino rats weighing 250 gms. were fed a normal chow diet and normal diet supplemented with ethionine (1 g/kg diet) for two to three weeks. The rats were killed by decapitation and pancreas removed from a number of rats and pooled together. Two to three gms. of slices were incubated with a mixture of C^{14} labeled L-amino acids as present in the insulin molecule[1]. The incubation was terminated with the addition of acidified ethanol by the procedure of Pettinga (1960) and the extract dialized for twenty-four hours against running distilled water. The undializable fraction was freeze-dried and the residue dissolved in 2.0 ml. of 0.15 M KCl (pH 2.0), and an aliquot assayed for radioactivity. Another set of aliquots was then incubated in glycine buffer (pH 9.4) with and without normal guinea pig serum and anti-insulin serum. The insulin antibody complex was then precipitated with sodium sulfite (Grodsky and Forsham 1960) and assayed for radioactivity. The results are given in Table I. The extract was also assayed for biological activity (Table II) by the rat epididymal fat pad method for insulin-like activity as reported by Renold et al.(1960). These results indicate that rat pancreas slices incubated in vitro incorporate C^{14} from labeled amino acids into an acid alcohol extract of pancreas and this activity is precipitated with anti-insulin serum. The acid alcohol extract also stimulates the oxidation of glucose-1-C^{14} to CO_2 by rat epididymal

1 (0.25) umoles of each of the following amino acids were added per flask: Alanine, Arginine, Aspartic Acid, Cystine, Glutamic Acid, Glutamine, Glycine, Histiding, Isoleucine, Leucine, Lysine, Phenylalanine, Proline, Serine, Tryosine, and Valine)

Table I

INCORPORATION OF C^{14} AMINO ACIDS INTO ACID ALCOHOL EXTRACT AND BINDING OF THIS RADIOACTIVITY BY ANTI-INSULIN SERUM

Condition	Incorporation of C^{14} A.A.* cpm/g**	Total Activity Precipitated without serum present cpm/g	Total Activity Precipitated with N.S. cpm/g	Total Activity Precipitated with A.I.S. cpm/g
Normal	5540 ± 530***	240 ± 20	1430 ± 120	4120 ± 210
Ethionine Fed	6890 ± 480	260 ± 40	1360 ± 130	4200 ± 190

* Following abbreviations are used: A.A., amino acids; N.S., normal serum; A.I.S., anti-insulin serum.

** 2-3 gm of pancreas slices were incubated in 6 ml. of Ringer-bicarbonate medium for ninety minutes with 2 - 2.5 x 10^6 cpm of a mixture of C^{14} amino acids. All activities are expressed per gm of original pancreas.

*** Each figure is an average of six values with S.E. as indicated.

Table II

OXIDATION OF GLUCOSE-1-C^{14} TO CO_2 BY RAT EPIDIDYMAL FAT PADS*

Extract Added	No. of Obs.	Activity in $C^{14}O_2$ cpm/gm Wet Tissue
None	4	2930 ± 150*
Normal Serum	4	2600 ± 220
Acid ethanol extract +Normal Serum	7	8700 ± 330
Acid ethanol extract +Anti-Insulin Serum	7	3150 ± 500
Insulin 300 microunits	3	10,500 ± 950
Insulin 900 microunits	3	20,200 ± 1860

*Approximately 500 mg of epididymal fat pad tissue was incubated in 6 ml. of Ringer-bicarbonate medium with glucose-1-C^{14} (2 - 2, 5 x 10^5 C.P.M.). Normal or anti-insulin serum was added with or without an aliquot of dialyzed acid alcohol extract equivalent to 25 mg of incubated pancreas slices. $C^{14}O_2$ was isolated and counted as $BaCO_3$.

fat pads, indicating the presence of insulin-like activity. Furthermore, the insulin-like activity of the acid alcohol extract was lost upon incubation with anti-insulin serum. This provides additional evidence that insulin activity was being measured. Ethionine feeding did not significantly increase C^{14} incorporation into the protein fraction precipitated by anti-insulin serum.

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