

A CLINICAL ROLE FOR INSULIN BIOSYNTHESIS

by ARNOLD J. HILL

Halifax, N. S.

Considerable emphasis is placed upon the study of the structure and action of insulin in the pre-clinical years. This is probably so because more is known about the activities of insulin than is known about most other peptides. The clinical importance of insulin has been attributed chiefly to its inherent hormonal properties.

Recently, it has been suggested that the fundamental key to diabetes mellitus may be directly related to insulin biosynthesis - an area to which little attention has been paid by students or clinicians.

Theories of Insulin Biosynthesis

The synthesis of insulin takes place in the microsomes of the beta cells of the islets of Langerhans in the pancreas. Insulin is then transferred to the secretin granules either by direct transfer or through transformation of the endoplasmic reticulum into the secretin granules (1). The current wide-spread view of insulin biosynthesis has been that two peptide chains were synthesized separately and then later joined by the formation of disulphide bonds in the presence of an enzyme. The amino acid sequence containing the intrachain disulphide bonds is referred to as the A chain; the longer amino acid sequence linked to chain A by interchain disulphide bonds is known as the B chain (see Fig. 1).

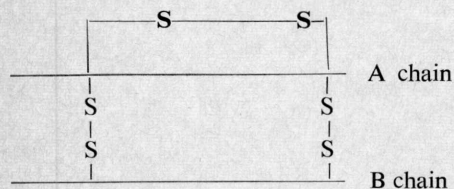


Fig. 1 - Structure of Insulin (Schematic)

Mol. Wt. approx. 6500

Most of the evidence supporting this theory has been obtained from the studies in vitro employing non-human or synthetic in-

sulin chains (2,3,4,5) which were observed to recombine under certain conditions to give high yields of insulin.

A different interpretation of insulin biosynthesis is that a structure is first formed which consists of one long peptide chain with intrachain disulphide bridges. One or more of the peptide bonds of this long chain is subsequently cleaved to yield two or more peptide chains connected by interchain disulphide bonds (6). Steiner and Oyer (7) in an interesting study which lent support to this theory, used material obtained from an islet cell adenoma removed from a patient with severe hypoglycemic episodes. This type of tumor of the islets of Langerhans contains mostly beta cells which are well separated from surrounding acinar tissue. The islet cells were freed of acinar cells and were incubated with tritiated leucine or phenylalanine. The crude insulin fraction was purified by chromatography and was shown to contain three peaks arbitrarily named A, B and C. The elution pattern was essentially the same for the phenylalanine labelled material as for the leucine labelled material. Peak A proved to be a mixture of ultra-violet absorbing materials appearing in the void volume of the column. Peak C was shown to be insulin. The high specific activity (radioactivity per unit mass) in peak B prompted further investigation.

The Relationship of Component B to Insulin

Labelled material from Peak B or C was next incubated with an excess of guinea pig anti-serum or normal serum, and then subjected to zone centrifugation on a sucrose gradient. By this means, the antigen - antibody complex was separated completely from the unreactive material.

The labelled insulin became bound to globulin when incubated with guinea pig antisera. Addition of small amounts of non-

*fourth year medicine.

*Abstracted from an article submitted to CAMSI Journal, October, 1967.

— Compliments of —

J. F. HARTZ CO. LTD.

5561 MORRIS STREET

HALIFAX, N. S.

PHONE 423-7172

Student Discounts

Suppliers of:

Welch Allyn Diagnostic Instruments

Stille Surgical Instruments (made in Sweden)

other high quality surgical equipment

**Various makes Stethoscopes, Laboratory Coats and
other Student Requirements**

CANADA PERMANENT TRUST COMPANY

- Estate Planning
- Executor and Trustee
- Investment Fund
- Investor Service

Established 1855

Dial 422-1531

Barrington at Sackville

Halifax, N.S.

labelled porcine insulin before incubation, displaced the labelled material from the globulin complex as was anticipated for a specific antigen - antibody reaction. Labelled component B behaved essentially the same way as labelled component C. Because the separated chains were noted for not reacting with anti-insulin sera it was concluded that component B must be closely related immunologically to native insulin.

Component B sedimented more rapidly on the sucrose gradient than C. Its molecular size was estimated to be 10,800 by gel filtration, therefore, component B had a higher molecular weight than insulin (component C).

Further experiments showed that insulin can be released by tryptic digestion of the B component, but did not help decide whether this heavier protein was a complex of insulin with another protein, or was a discrete protein which contained insulin sequences bound covalently to other peptide material. Disulphite bond cleavage (sulfitolysis) of labelled component B did not give rise to labelled chains A or B as did labelled insulin. When sulfitolysis was carried out after treatment with trypsin, leucine-labelled material B gave labelled material corresponding to A and B chains of insulin on electro-phoresis. End group analysis was able to show that the N-terminal amino acid in component B was phenylalanine as is the case for the mammalian B chain of normal insulin.

As the next approach Steiner *et al* (8), using rat islet cells, undertook a study to examine the time course of incorporation of radioactive amino acids into components B and insulin. It was reasoned that if component C was a precursor on insulin, radioactivity would first appear in it, and upon subsequent incubation radioactivity would be transferred into the fraction containing insulin. This was found to be the case.

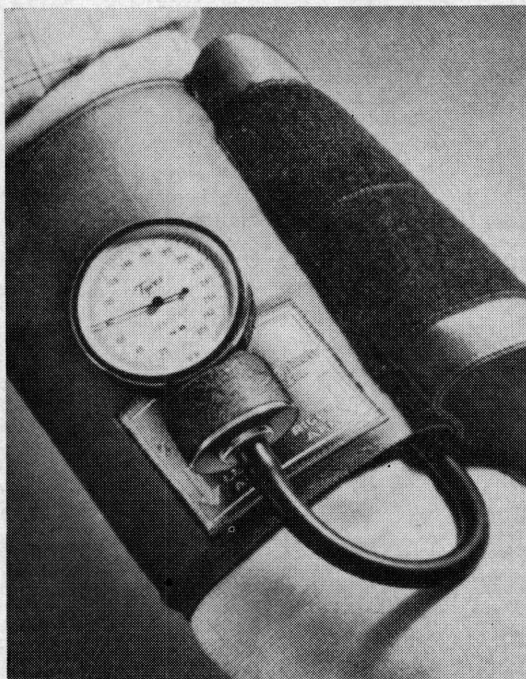
These experiments demonstrated that the B component becomes labelled earlier than insulin, in insulin synthesizing tissues from man and from rat, and that the radioactivity is transferred to insulin as incubation proceeds in the absence of new peptide bond synthesis. Component B is a large protein and it contains the amino acid sequences of

insulin in a form which can be released by limited tryptic proteolysis.

The fact that component B reacted with antibodies to insulin suggests that some antigenic properties of native insulin were already present in the structure. Steiner and his group have designated component B as a precursor of insulin or "proinsulin".

Several investigators had predicted the existence of "proinsulin" but it had not been demonstrated prior to this time. Ultrastructural and fractional studies by Caro and Palade (9) have indicated many similarities in the secretory and synthetic mechanisms of the acinar and the beta cells of the pancreas. It is of particular interest that in the exocrine cells approximately 40 to 60 minutes elapse before newly synthesized zymogen proteins first begin to appear. Recent work (5) has shown that there may be a similar delay in the transfer of newly synthesized insulin into the granule fraction of the beta cells. Others (10) have found a delay of one hour before newly synthesized insulin is secreted into the medium from slices of rabbit pancreas incubated *in vitro*. The findings of Steiner *et al* (8) that there was a delay of at least 30 minutes before significant amounts of labelled insulin appeared during the incubation *in vitro*, suggested that during intracellular transport of proinsulin from the region in the rough endoplasmic reticulum where it is probably formed, to the secretory granules, a proteolytic process takes place which results in the liberation of insulin from proinsulin.

The demonstration of the existence of proinsulin may have considerable clinical significance. It is known, for instance, that in many adult diabetics, and in the early stages of juvenile diabetes that plasma insulin levels appear to be normal or elevated as measured by immunological techniques. These findings would be explained by the presence in the circulation of an abnormal form of insulin having the immunological properties of insulin but having altered or reduced biological activity. It is possible that proinsulin could be responsible for this immunological activity, and that diabetes could possibly be due to the loss of the enzymatic mechanism(s) necessary for the conversion of the precursor to the normal hormone.



The Cuff-Mounted TYCOS Aneroid

No Doubt About it - When it's a TYCOS

For more than fifty years the TYCOS Aneroid has been a favorite with doctors everywhere. There are four principal reasons for this popularity. Accuracy: the accuracy of a TYCOS Aneroid can be visually checked each time it is used. If the pointer returns to "zero" when pressure is released, the aneroid is accurate. Quality Construction: We have received letters about TYCOS Aneroids that have been used for more than 20 years; Warranty: The famous TYCOS Warranty states that any TYCOS Aneroid losing accuracy during the first 10 years will be repaired without charge. Convenience: The TYCOS Aneroid is available in either the Cuff-Mounted Model (No. 5090 - \$56.50) or the Hand Model (No. 5098 - \$56.50) with gage on inflating bulb. Each weighs only 18 ounces and is available with hook or Velero 80 cuff. See and try them at your Surgical Supply Dealer.

**TAYLOR INSTRUMENT
COMPANIES OF CANADA LTD.**
75 Tyecos Drive, Toronto 19, Ontario
Taylor Instruments Mean Accuracy First



House of Rodney

where Quality
is our great
concern

featuring
Shiffer-Hillman
Quality Clothing

Lord Nelson Arcade

CORTEL

ANSWERING SERVICE

WAKE - UP SERVICE
ALARMS

TELEX

422-6427

24 hours daily

"CORTEL

Will Serve You Well"

5212 Prince St.

Halifax, N. S.

Bibliography

1. Leibel B. S. and Wrenhall, G. A. (Ed.): "On the Nature and Treatment of Diabetes", Excerpta Medica Found., The Hague, 1965.
2. Katsoyannis, P. G. and Tometsko, A.: P.N.A.S., 55, 1554 (1966).
3. Humbel. R. E.: P.N.A.S., 53, 853 (1965).
4. Humbel. R. E.: Biochem. Biophys. Acta, 74, 96 (1963).
5. Bauer, G., Lindall, A. W., Dixit, P. K., Lester, G., and Lazarow, A.: J. Cell. Biol., 28, 413, (1966).
6. Wang, Su-Sum, Carpenter, F. H., J. Biol. Chem., 240, 1619 (1965).
7. Steiner, D. F. and Oyer, P. E.: Proc. Nat. Acad. Sci., U.S., 57, 473 (1967).
8. Steiner, D. F., Cunningham, D., Spigelman, L., and Aten, B.: Science, Vol. 157, No. 3789, 11 Aug. 1967.
9. Caro, L. G., Palade, G., J. Cell. Biol., 20, 473 (1964)
10. Howell, S. L. and Taylor, K. W.: Biochem. J. 102, 922 (1967).

Purchase a car in Europe

— go there and pick it up

— pay all tariffs, shipping, etc.

and

still have \$2 - 300* vacation money to spend



ATLAS TRAVEL SERVICE LTD.

Lord Nelson Arcade Halifax, Nova Scotia

422-8588

*depending on make of car.

Compliments

MARITIME TRAVEL SERVICE LTD.

A Complete Travel Service

AMERICAN EXPRESS

REPRESENTATIVE

1704 Granville St.

Phone 422-4441



**Talk to
Royal Trust**

About SAVINGS, INVESTMENTS,
MORTGAGES, REAL ESTATE,
EXECUTORSHIP SERVICES

Canada's Largest Trust Company

(Trustee C.M.A. Retirement Plan)

HALIFAX

DARTMOUTH

EDUICATION is the Brick
with which
you build your life
... firmly, wisely
and for lasting security!



HEAD OFFICE: HALIFAX, N.S.

PLANTS AT: HALIFAX • DARTMOUTH • LANTZ • NEW GLASGOW •
SYDNEY • WELLINGTON • SAINT JOHN • CHIPMAN • FREDERICTON •

COLIN

ROBERTSON LTD.

Your authorized V.W. Dealer

215 Wyse Road, Dartmouth

Phone 469-6411

Garnett Optical Co. Ltd.

A Maritime Enterprise

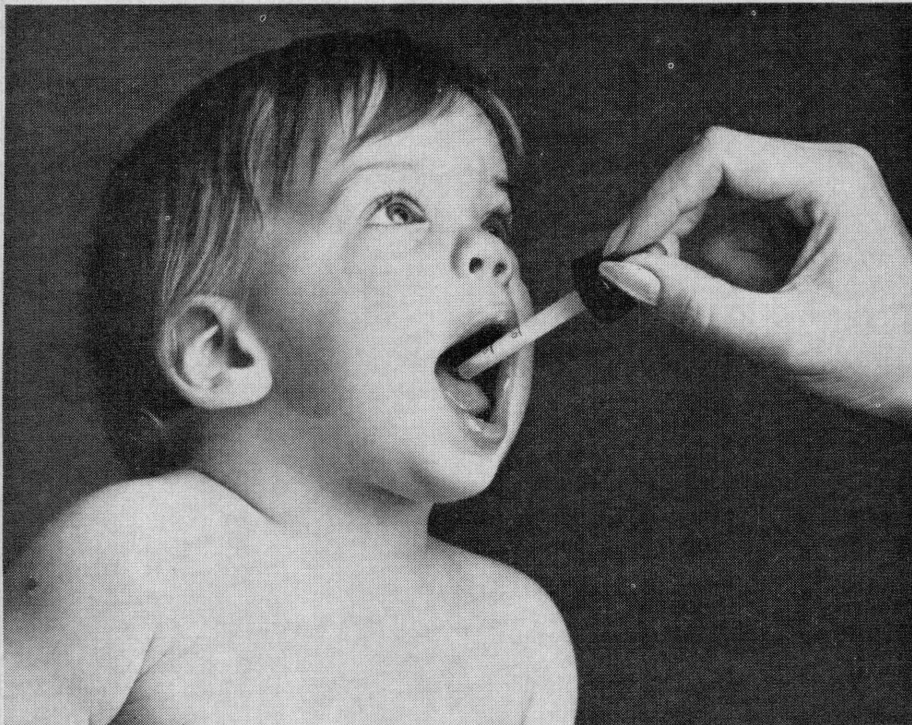
Ophthalmic Equipment

Leitz Microscopes

Olympus Microscopes

and

Allied Equipment



water-dispersible, hypoallergenic,
pleasant-tasting

OSTOCO[®] DROPS

Children relish the flavour of multivitamin OSTOCO Drops, and welcome their administration directly on the tongue, thus ensuring the most effective absorption. OSTOCO Drops provide the vitamins which are most important to infants and young children. Equally acceptable in formula, fruit juice or milk.

Each 5-drop dose (0.25 cc.) contains:

Vitamin A 2500 I.U.	Vitamin B ₁ 1 mg.
Vitamin D 400 I.U.	Riboflavin (vitamin B ₂) 0.6 mg.
Vitamin C 30 mg.	Niacinamide 4 mg.
	Vitamin B ₆ 0.6 mg.

Dosage: Infants and children under 6 years of age, 5 drops daily; children 6 years and older, 8 to 10 drops daily.

Bottles of 8, 15, 30 and 50 cc., each with calibrated dropper for waste-free dosage.

Full information on request.

