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Studies on the Material Responsible for Activity Attributed to the Glucose Tolerance Factor

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Chemistry

at Massey University

New Zealand

Peter Robin SHEPHERD

1989
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Abstract

An extract that was known to have activity attributed to the glucose tolerance factor (GTF) was isolated from brewers yeast and this was used as the starting point in attempts to isolate GTF.

The initial extract from brewers yeast was shown to be far from pure as it was separated into 15 fractions using high voltage paper electrophoresis. GTF activity was initially monitored using a simple yeast fermentation assay and the more active of these fractions were further purified using reverse phase high performance liquid chromatography. The most active fraction was anionic and contained little chromium or amino acid material although mass spectroscopy revealed the presence of adenine. However the exact nature of the material remained elusive.

Due to doubts about the specificity of the simple yeast assay a modified version of the yeast assay was investigated which measured the ability of the sample to stimulate the metabolism of yeast cells above the level that was accounted for by cell proliferation. This assay were shown to be very reproducible although the most active fraction from the simple yeast assay was not active in this assay.

The low chromium rat epididymal adipocyte assay was investigated as a possible means of verifying the results of the modified yeast assay. The importance of diet as a determinant of whether adipocytes would respond to GTF was investigated using 4 different diets. The adipocytes from rats fed on a torula yeast diet produced the maximum potentiations and it was found that a unique feature of these cells was a reduced ability to convert glucose to fatty acids via the glycolytic pathway. The potentiations seen in these cells were most obvious in the conversion of 1-14C-glucose to CO2 and fatty acids and it was concluded that this was due to either a potentiation of glucose transport or of acetyl-CoA carboxylase.

An extract of torula yeast was prepared in a similar method to that used to isolate the initial extract from brewers yeast. This extract showed high levels of activity in the both assay systems which indicated that these assay was not measuring GTF as originally defined. This was further indicated by the finding that no one compound was responsible for the activity in any of the assays investigated.

The chromium contained in the original yeast extract was also spread amongst many fractions and the chromium content of these fractions bore no correlation to the activity in the assay systems indicating that the active fractions were not chromium complexes.
Overall these results show that there is no unique factor responsible for the activity in the simple yeast assay and the low chromium rat adipocyte assay. Further it was concluded that none of the active material represented chromium complexes. As the activity in these assays was thought to be due to the presence of a GTF this firstly strongly argues against GTF being a chromium complex and secondly it questions the existence of GTF at all.
Acknowledgements

I have many people to thank for making this thesis possible and only a very small space to do it in. However I could start by saying;

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