

Acta Medica Okayama

Volume 28, Issue 5

1974

Article 8

OCTOBER 1974

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— BRIEF NOTE —

**FORMATION OF ANTIBODY TO MONOCOMPONENT
INSULIN IN GUINEA PIGS**

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Received for publication, July 22, 1974

It has been well known that conventional insulin preparations induce insulin antibodies in man and animals. Recently SCHLICHTKRULL *et al.* (1) demonstrated lack of antigenicity of monocomponent insulin (MC insulin) in rabbits after immunization with soluble MC insulin alone. The following study was designed to clarify the antigenicity of the MC insulin in guinea pigs.

Fifteen male guinea pigs were divided into three groups of five animals each. Group I received injections of 0.5 ml of Freund's complete adjuvant every two weeks for four weeks in six subcutaneous areas. Group II was injected with 20.0 IU of the MC insulin mixed with 0.5 ml of Freund's complete adjuvant. And Group III was given conventional pork insulin with the adjuvant in the same way. Serum samples were collected by cardiac puncture. Insulin antibody was assayed by using ^{125}I -insulin and ethanol precipitation (2) in an 1:26 dilution of the serum. The antibody titer was indicated by the percentage of bound/total count.

Four of five guinea pigs immunized with the MC insulin induced antibody formation (Fig. 1). Following the first injection of the MC insulin, binding antibody to the insulin appeared already to some degree in two of five animals. Upon the second exposure to the MC insulin, the level of the antibody titers rose rapidly and it was 25.0% in guinea pigs. While that to the conventional insulin was 31.2% and Group I showed no elevation of antibody titers.

Insulin easily produces antibody and it has been also speculated that proteins of high molecules or polypeptides contained in the conventional insulin potentiate the formation of antibody to insulin. The present study showed that the formation of binding antibody to insulin was observed in the MC as well as in the conventional insulin, both of which had been given together with Freund's complete adjuvant. From this finding, it appears that the absence of contaminants in the MC insulin is not sufficient to make the insulin non-immunogenic. The observation of HIRATA (3) that three of the five rabbits

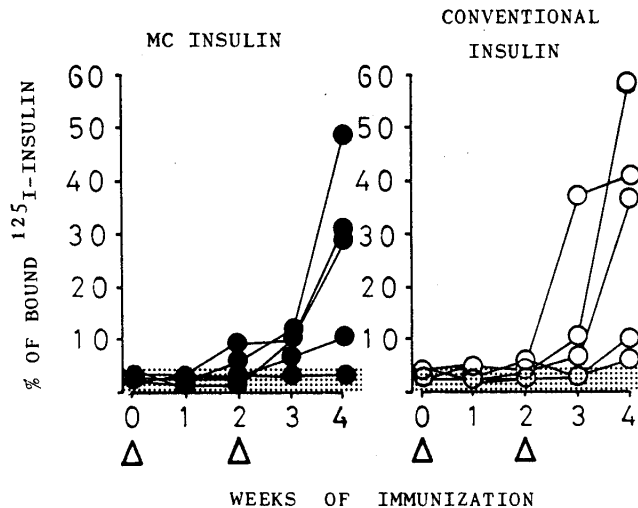


Fig. 1. Formation of binding antibody to MC insulin in guinea pigs.

immunized with MC insulin together with Freund's complete adjuvant developed insulin antibody also coincides with our results.

Insulin preparations in a modified form or in acid solution readily induce antibody. And it is well known that the use of an adjuvant potentiates antibody formation (4). However, insulin preparations in the soluble form or in the neutral solution result in weak antigenicity (5). SCHLICHTKRULL *et al.* (1) immunized rabbits with the MC insulin dissolved in neutral solution and did not use the adjuvant, so that under their conditions it may be that they could not detect any antibody binding to insulin.

It seems reasonable to conclude from the present study that antigenicity of insulin is not induced by the contaminants in the insulin preparations, but by the insulin itself.

Acknowledgement: Thanks are tendered to Prof. Y. HIRATA of Tottori University for providing the MC pork insulin.

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