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## Precipitin reaction between bovine insulin, porcine insulin, monocomponent insulin and guinea pig antibovine insulin sera

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— BRIEF NOTE —

**PRECIPITIN REACTIONS BETWEEN BOVINE INSULIN,  
PORCINE INSULIN, MONOCOMPONENT INSULIN  
AND GUINEA PIG ANTIBOVINE INSULIN SERA**

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It is unquestionably recognized that insulin has antigenicity. Although the formation of visible precipitin lines between insulin and insulin antibody has been demonstrated by several workers (1, 2, 3), some recent reports have brought up some doubt on this point (4, 5). BERSON and YALLOW (6) stated that insulin was univalent in its reaction with antibody. While, the study of ARQUILA and FINN (7) presented evidence that the insulin molecule had more than one antigenic determinant site.

Our results, in this preliminary report, showed the presence of the precipitating antibody to insulin.

The experiment was performed as follows. Bovine crystalline insulin (Lot. 121C-13, 26.4 I. U. /mg) was obtained from Sigma, porcine recrystalline insulin (Lot. #V-1416, 24.4 I. U. /mg) from Schwarz/Mann and Actrapid monocomponent pork insulin (F-20011-00-101-1, 40 I. U. /ml) from Novo.

Five guinea pigs were immunized with bovine crystalline insulin mixed with Freund's complete adjuvant (Iatron). The immunization was repeated at 5 weeks' intervals for 11 months and the animals received a total injection of 14.4 mg of the insulin. Blood samples were obtained by cardiac puncture six weeks after the last injection. The experiment was carried out by using the multiple-well double diffusion technique. The plate was prepared by 1.0% agar-NOBLE gel of DIFCO and adjusted by neutral buffer (0.05 M, pH 7.2, PBS).

The results of the precipitin reactions are shown in Figs. 1 and 2. Antisera from guinea pigs 1, 2, and 3 produced one clear precipitin line with bovine (Fig. 1), porcine and monocomponent pork insulin, respectively. Antisera from the guinea pigs produced one precipitin line with monocomponent pork insulin as well as porcine and bovine insulin, respectively. The bovine and porcine precipitin lines were fused completely with the monocomponent precipitin line (Fig. 2).

Although proinsulin also reacts with insulin antisera, it is thought that

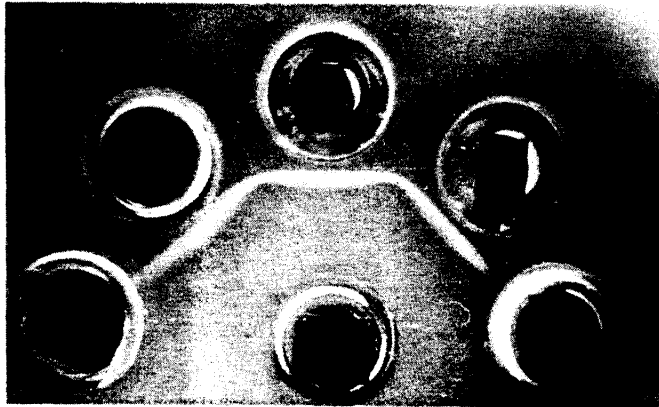


Fig. 1. Precipitin lines between bovine insulin (AG) and antisera from the guinea pigs 1 (0), 2 (1), 3 (2), 4 (3) and 5 (4).

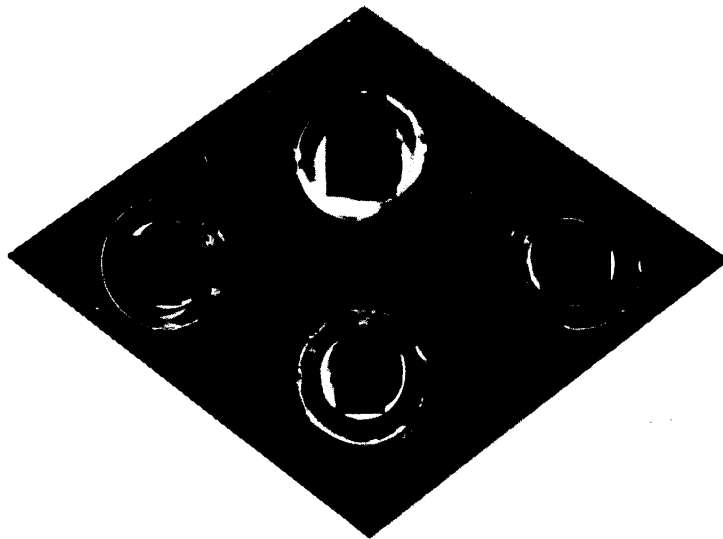


Fig. 2. Precipitin lines between antiserum from the guinea pig (0) and porcine insulin (1), monocomponent pork insulin (2) and bovine insulin (3).

the precipitin line observed in this experiment is responsible for insulin itself, because monocomponent insulin does not contain proinsulin. Furthermore, from the fact that guinea pig antiproinsulin serum reacted only weakly with monocomponent insulin (8), it is presumed that precipitin lines observed by us in this experiment were produced by the anti-insulin antibody, but not by the anti-proinsulin antibody.

Our results confirmed that bovine insulin, when used as an immunizing antigen in guinea pigs, produces precipitating antibody to insulin. The fact that pork insulin also produces precipitin lines with these antisera seems to demonstrate that there is an antigen common to pork and bovine insulin.

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