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Association of Thyrotropin with γ-Globulin in Myxoedema.* (23348)

J. M. MCKENZIE (Introduced by E. B. Astwood)
Ziskind Research Laboratories, New England Center Hospital, and Department of Medicine, Tufts University School of Medicine, Boston.

In human serum, thyrotropin has been stated to be associated with β-globulin(1,2). However, Postel(3) demonstrated that when bovine pituitary thyrotropin was added to human serum, and this solution fractionated by electrophoresis in a starch block, thyrotropic activity was present only in the gamma globulin fraction. Using a highly sensitive bioassay for thyrotropin(4), endogenous thyrotropic activity has now been found only in association with gamma globulin.

Methods. The bioassay procedure was

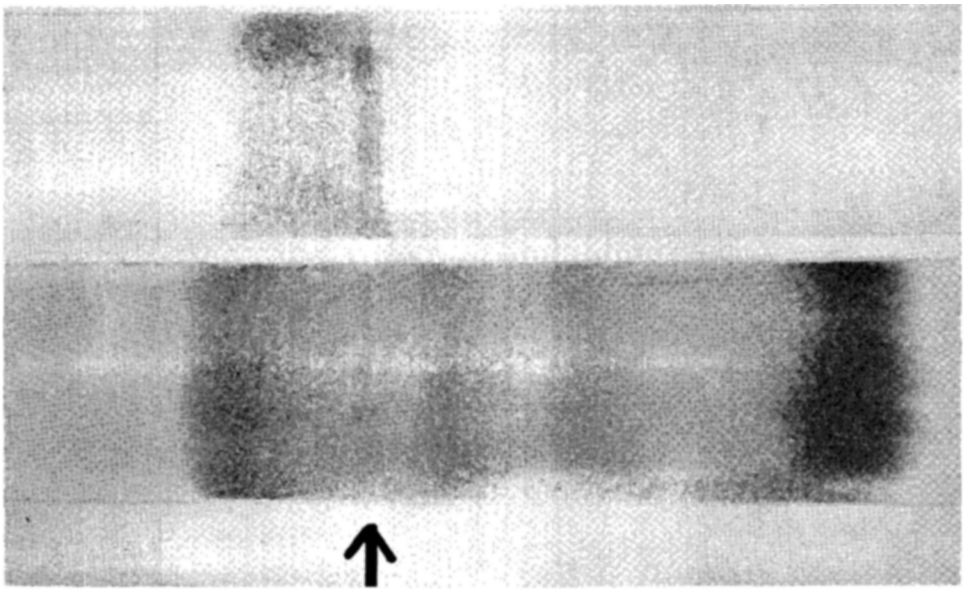

FIG. 1. Identification of a fraction of serum obtained by electrophoresis on a starch block. The gamma globulin fraction (top) and whole serum (bottom) simultaneously electrophoresed on paper at pH 8.6.

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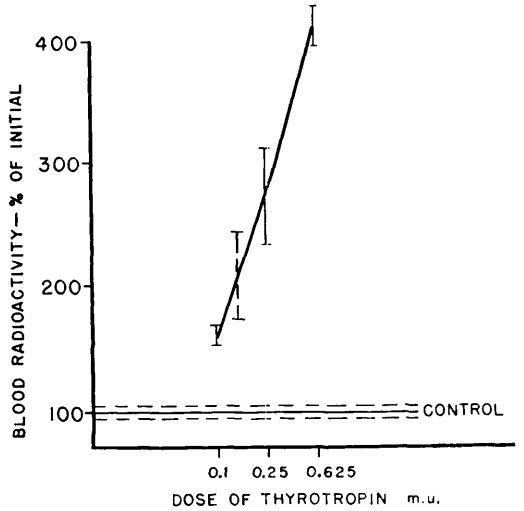

FIG. 2. Assay of gamma globulin from the serum of a patient with myxoedema. Mean response ± stand. error of the mean shown for 6 observations of each dose level of U.S.P. Reference Standard Thyrotropin (continuous vertical lines) and of the unknown (interrupted line). For calculations of the result see text.

adapted from the method of Adams and Purves(5). Twenty g female mice, on a low-iodine diet, were given an intraperitoneal injection of I^{131} followed immediately by a subcutaneous injection of 10 μg thyroxine. 0.066% Thyroid (U.S.P.) was then added to their diet and the animals used for thyrotropin assays 4, 5, and 6 days later. The radioactivity was measured in 0.1 ml of venous blood, obtained 2 hours after intravenous injection of U.S.P. Reference Standard Thyrotropin or the solution being assayed. This radioactivity was compared with that present before the injection. With groups of 6 mice used in this manner it was possible to detect 0.025 international milliunits (i.m.u.) of thyrotropin and the precision of the assay (λ) averaged 0.25.

Three to 5 ml samples of serum from patients with myxoedema were fractionated by the method described by Kunkel and Slater (6), on a block of washed starch suspended in borate buffer of pH 8.6. After elution of 1-cm segments of the block, the protein distribution was determined by spectrophotometry (λ 278). The appropriate eluates were combined, dialyzed, and lyophilized. They were subsequently redissolved and assayed for thyrotropin content. The identity of the protein fraction was confirmed by paper electrophoresis at pH 8.6, comparison being made with simultaneously electrophoresed whole serum. Separation of gamma globulin was particularly satisfactory by this method (Fig. 1).

Results. Serum fractions from 4 patients with myxoedema were assayed. Responses typical of thyrotropin were produced by the gamma globulin fraction in each case. Negative responses were obtained by assay of the other protein fractions. In a typical experiment (Fig. 2) the mean response of the gamma globulin was equivalent to 0.141 i.m.u. thyrotropin. This was obtained with injections of 0.2 ml of a 2.4% solution, obtained from the electrophoresis of 3 ml serum. The whole fraction was dissolved in 1.5 ml saline and this serum was thus calculated to contain 0.302 i.m.u. thyrotropin/milliliter.

Summary. A bioassay technic able to detect 0.025 international milliunits of thyrotropin was developed. Using this technic, fractions obtained from the electrophoresis of serum of patients with myxoedema were assayed. Thyrotropic activity was found only in association with γ-globulin.

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