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Note

Improved radioimmunoelectrophoretic assay of human serum thyroxine-binding globulin

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Direct determination of human serum thyroxine-binding globulin (TBG) concentration was first described by Freeman and Pearson [1], using two-dimensional immunoelectrophoresis and autoradiography. The method was time consuming, and costly in its use of antiserum and [^{125}I]thyroxine (T_4). A more rapid assay resulted from the adaptation of the Laurell monorocket technique for use in TBG measurement [2, 3]. Barbital, an inhibitor of T_4—TBG binding, was used as a buffering medium, as originally described by Laurell [4]. Improved results were reported by Drysdale et al. [5], who used a phosphate buffer to increase the binding of [^{125}I]T_4 to TBG and an antiserum-free starter gel to stabilize the [^{125}I]T_4—TBG complex. In this report we describe a radioimmunoelectrophoretic assay of TBG, in which labelling of serum samples with high specific activity [^{125}I]T_4 occurs during electrophoresis, thereby simplifying sample preparation and speeding the assay.

MATERIALS AND METHODS

Materials

High-specific-activity [^{125}I]T_4 (approximately 600 mCi/μmole) was synthesized by the method of Weeke and Örskow [6]. Antiwhole human serum was obtained from Wellcome (Beckenham, Great Britain). Antiserum to a human α-globulin fraction, rich in TBG, was produced in sheep. The fraction for immunization was obtained by electrophoresis of whole human serum in agarose gel. The globulin fraction was cut from the undried gel, emulsified with complete Freund's adjuvant, and injected intramuscularly. RP-XOmat X-ray film was obtained from Kodak UK (London, Great Britain). Agarose and other reagents were obtained from BDH (Poole, Great Britain).

Experimental procedure

Laurell monorocket electrophoresis was carried out using 0.05 *M* phosphate buffer at pH 7.4 according to the method of Drysdale et al. [5]. Electrophoresis continued for 12 h, at a voltage of 2 V/cm across the plate.

In an important modification, high specific activity [^{125}I]T_4 was added to the starter gel (Fig. 1) in a concentration of 500 nCi/ml. Antigen wells, 2 mm diameter, were punched in the starter gel 5 mm from the starter gel—antibody gel interface, and serum samples of 2 μl were added to each well.

The antibody gel consisted of either a 25% dilution of Wellcome antiwhole human serum or a 2% dilution of anti-α-globulin antiserum.

The dried electrophoretic plates were subjected to autoradiography for 12 h followed by rapid processing in an RP-XOmat Processor, Model M 6-N [2].

RESULTS

The results of a typical assay using anti-α-globulin antiserum are shown in Fig. 1. Use of 25 × 8 cm glass plates allowed measurement of twenty samples and six standards in one assay.

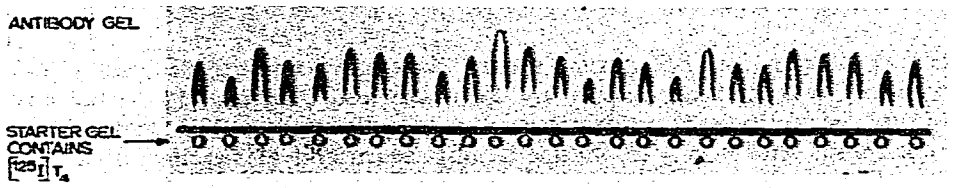

Fig. 1. Autoradiograph showing TBG peaks. Antibody gel contains sheep anti-α-globulin.

TBG results were expressed as a percentage of a working standard serum [5]. In thirteen consecutive assays, measurement of a single standard yielded a mean of 48 (±2.7 S.D.), giving a relative standard deviation of ±5.6%. The sensitivity of the method was 5% of the working standard serum, although smaller amounts could be measured by increasing the sample size.

DISCUSSION

The use of monorocket electrophoresis for TBG measurement has been described using both barbital [2, 3] and phosphate buffers [5]. In both systems, serum samples were individually labelled and incubated with [^{125}I]T_4 before electrophoresis. In the modified method described here, labelling of TBG with [^{125}I]T_4 occurs during electrophoresis, thereby simplifying the assay and reducing the time required for completion. The antigen wells were situated near the starter gel—antibody gel interface, so that [^{125}I]T_4, which has a greater electrophoretic mobility than TBG in the buffer system used, could flow past the serum samples during electrophoresis. The use of a high-specific-activity preparation of [^{125}I]T_4 enables a reduction in the time needed for autoradiography, and reduced the total time for TBG measurement to 24 h.

The assay could be performed using a commercial antiwhole human serum,

but clearer peaks were obtained, using less antiserum, when an antibody to α-globulin was used. The method is sufficiently rapid, precise and inexpensive to make it an acceptable alternative to the indirect and expensive T_3 uptake test.

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