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THE VALUE OF SHORT-TERM INCUBATION PERIODS IN ASSESSING THE IMMUNO-REACTIVITY OF 125 I-LABELLED LUTEINIZING HORMONE AND PROLACTIN

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Where preparation of samples is unnecessary the technique of radioimmunoassay is particularly convenient to processing large numbers of samples and several hundred determinations can be set-up in one day.

The antiserum dilution used in the assay is usually chosen so that approximately 50% of the labelled antigen is bound to the specific antibody. The percentage observed depends on the immunoreactivity of the radioactive antigen and since the assay usually incorporates incubation of reactants for several days, failure to achieve adequate binding may become apparent only after a large number of samples have been committed for assay. It is therefore important to examine the labelled tracer prior to addition to the assay medium and since such substances commonly deteriorate on ageing it is an added benefit where an evaluation can be obtained within a few hours. In our hands the rapid charcoal-dextran method of Herbert, Lau, Gottlief & Bleicher (1965) did not produce the favourable results reported by Abraham (1968). The pre-precipitation technique suggested by Midgley (1969) was also not satisfactory and we therefore examined the possibility of short incubation periods, as suggested by Midgley (1969), for the normal post-precipitation procedure.

The immunoreactivity of three samples each of ovine LH and prolactin was examined by observing the percent 125I-labelled hormone bound by various dilutions of the appropriate antiserum. The incubation periods were varied as detailed in Fig. 1 and included the standard incubation with first antiserum for 24 hr. and exposure to immunoprecipitating antiserum for 72 hr (Midgley 1969).

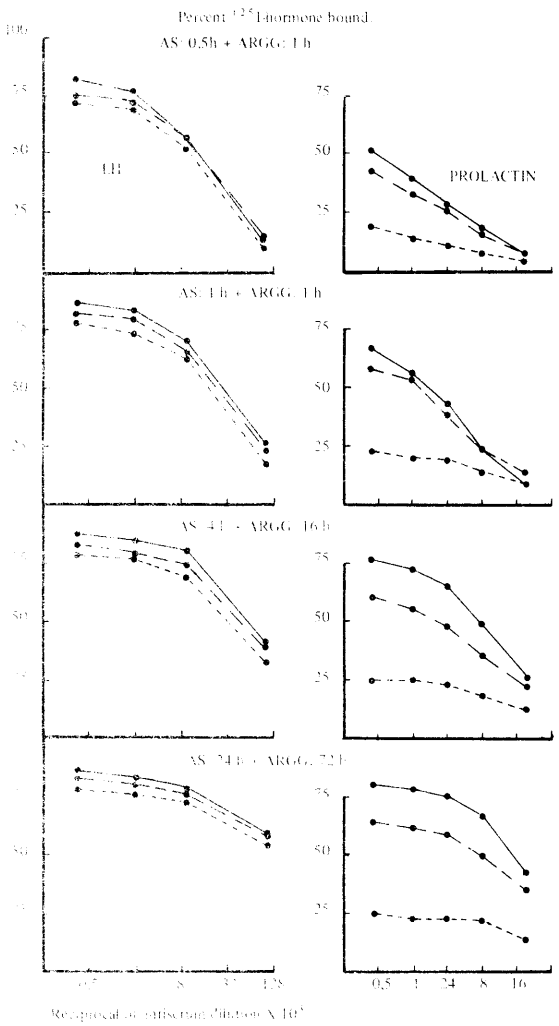

Fig. 1. *The influence of the duration of incubation on the antiserum dilution curves for three samples (═══) of LH and prolactin incubated with antisera (AS) to LH or prolactin and antiserum to rabbit gamma globulin (ARGG).*

The assay procedure was that described for ovine LH (Niswender, Reichert, Midgley and Nalbandov, 1969) and ovine prolactin (Davis, Reichert and Niswender, 1971). Antisera to the hormones studied were raised in rabbits and used at the dilutions given in Fig. 1.

From the appearance of the antiserum titration curves presented in Fig. 1 it is evident that an accurant ranking of the relative immunoreactivity of the various samples of LH and prolactin was obtained with an antiserum dilution of 1 : 400 and a total incubation time of 1,5 hr. A lengthening of the incubation period did not markedly increase the binding of 125 I-LH, except when the antibody dilution exceeded 1 : 400. However, in the case of samples 1 and 2 of prolactin each increase in the duration of the incubation period resulted in a greater proportion of the 125 I-prolactin bound at all dilutions of the anti-prolactin serum.

The results of this study demonstrate that the maximum immunoreactivity of a labelled peptide hormone can be measured when the hormone and appropriate antisera are associated for intervals which are considerably shorter than those normally recommended. Furthermore, the "50 percent level" can be determined after incubation of reactants for as little as 1,5 hr.

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