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RADIOIMMUNOLOGICAL EVALUATION OF RABBIT ANTI-SERA TO OVINE LUTEINIZING HORMONE

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OPSOMMING: RADIO-IMMUNOLOGIESE EVALUASIE VAN KONYN ANTISERUMS TEEN LUTEINISERENDE HORMOON

Antiserums verkry van vyf konyne, geimmuniseer teen skaap luteiniserende hormoon (LH) was getoets vir hul vermoë om met ^{125}I-gemerkte skaap LH te verbind. Vyf en dertig dae na die aanvang van immunisasie het antiserum verkry van slegs een konyn na verdunning (1:20 000) ongeveer 50% van die bygevoegde ^{125}I-LH gepresipiteer. Daaropvolgende immunisasie het tot gevolg gehad dat slegs een konyn antiserum geproduseer het wat nie ten minste tot 1:50 000 verdun kon word nie, terwyl dit in die geval van drie konyn moontlik was om serum tot 1:100 000 te verdun. Die kompeterende reaksie van 'n geselekteerde antiserum met 'n skaap plasma ryk aan LH, tiroied stimulerende hormoon en follikel stimulerende hormoon was binne aanvaarbare perke. Willekeurig geteelde diere is klaarblyklik geskik vir die verkryging van antiserums met 'n hoë titer.

SUMMARY:

Antisera obtained from five rabbits, immunized aginst ovine luteinizing hormone (LH) were tested for their ability to combine with ^{125}I-labelled ovine LH. Thirty five days after immunization commenced only one rabbit produced an antiserum which on dilution (1:20 000) could precipitate approximately 50% of the ^{125}I-LH added. Subsequent immunization resulted in only one animal yielding an antiserum which could not be diluted at least 1:50 000, while in the case of three rabbits, dilution 1:100 000 was possible. The cross-reaction of a selected antiserum with an ovine plasma high in LH, thyroid stimulating hormone (TSH) and follicle stimulating hormone (FSH) was within accepted limits. Random-bred animals would appear to be suitable for the production of high-titre antisera.

In the development of a successful immunoassay system the production of a suitable antiserum is of paramount importance. (Hurn & Landon, 1971; Odell, Abrahams, Skowsky, Hescox & Fisher, 1971).

The response to a given antigen varies both between, and within, species although at present data in this connection is limited, expecially when the glyco-protein hormones are used as antigens. The results in the present communication indicate the manner in which a small number of random-bred rabbits responded to repeated immunization with ovine LH. The selection of an antiserum suitable for use in quantitating LH levels in sheep plasma is also described.

Procedure

On days 1, 11 and 21 each of five, random-bred rabbits were injected subcutaneously with 1,0 mg NIH-LH-S16 emulsified in 1,0 cm^3 0,14 M NaCl and 1,0 cm^3 Freund's complete adjuvant. Two weeks after the last LH injection (day 35) approximately 15 cm^3 blood was drawn from the ear vein and allowed to clot. The serum was decanted, centrifuged and an aliquot of 100 µcm^3 removed for testing. The remaining serum was snap-frozen and stored at -15°C for future use. The serum to be tested was diluted 1:400 with 0,05 M phosphate-buffered saline-EDTA (EDTA-PBS), pH 7,0. Further dilutions (up to 1:40 000) were made, using 0,05M EDTA-PBS, pH 7,0 containing 0,25 percent normal rabbit serum.

Booster injections of 0,5 mg LH in saline and adjuvant were given on days 47, 71, 116 and a final injection of hormone in saline medium on day 128. Blood was again collected on day 136 and treated as before.

The ability of the antisera to precipitate trace amounts of ^{125}I-LH was tested in the double-antibody immunoassay system for ovine LH developed by Niswender, Reichert, Midgeley & Nalbandov (1969). Purified ovine LH (Papkoff) was iodinated according to the method of Greenwood Hunter & Glover (1963) as modified by Niswender *et al.* (1969). The anti-rabbit gamma globulin used to precipitate the LH-antibody complex was produced in adult male castrate sheep as described by Aono, Goldstein, Taymore & Dolch (1967). Following the second bleeding, and initial testing, the sera from three rabbits were selected for further examination. Various quantities of standard LH (NIH-LH-S16) were incubated with the anti-serum at a dilution which bound approximately 50 percent of the ^{125}I-LH, viz., 1:100 000 in all three cases.

The antiserum selected for use in routine assays was further tested with a plasma high in LH and also examined for cross-reaction with ovine FSH and ovine TSH.

Results

The percent ^{125}I-LH bound by increasing dilutions of the sera obtained from the five rabbits is presented in Fig. 1A. From this figure it is evident that, of the sera obtained at the first bleeding, only that produced by rabbit no. 3 resulted in acceptable precipitation of the radio-iodinated LH (approximately 50%) when the anti-serum was used at a dilution exceeding 1:20 000. However, subsequent immunization resulted in a dramatic improvement in all sera, particularly in the case of rabbit no. 4 (Fig. 1B). This consideration, together with the appearance of the standard-dose response curves suggested that the anti-serum from this particular rabbit could be successfully used for routine assay purposes.

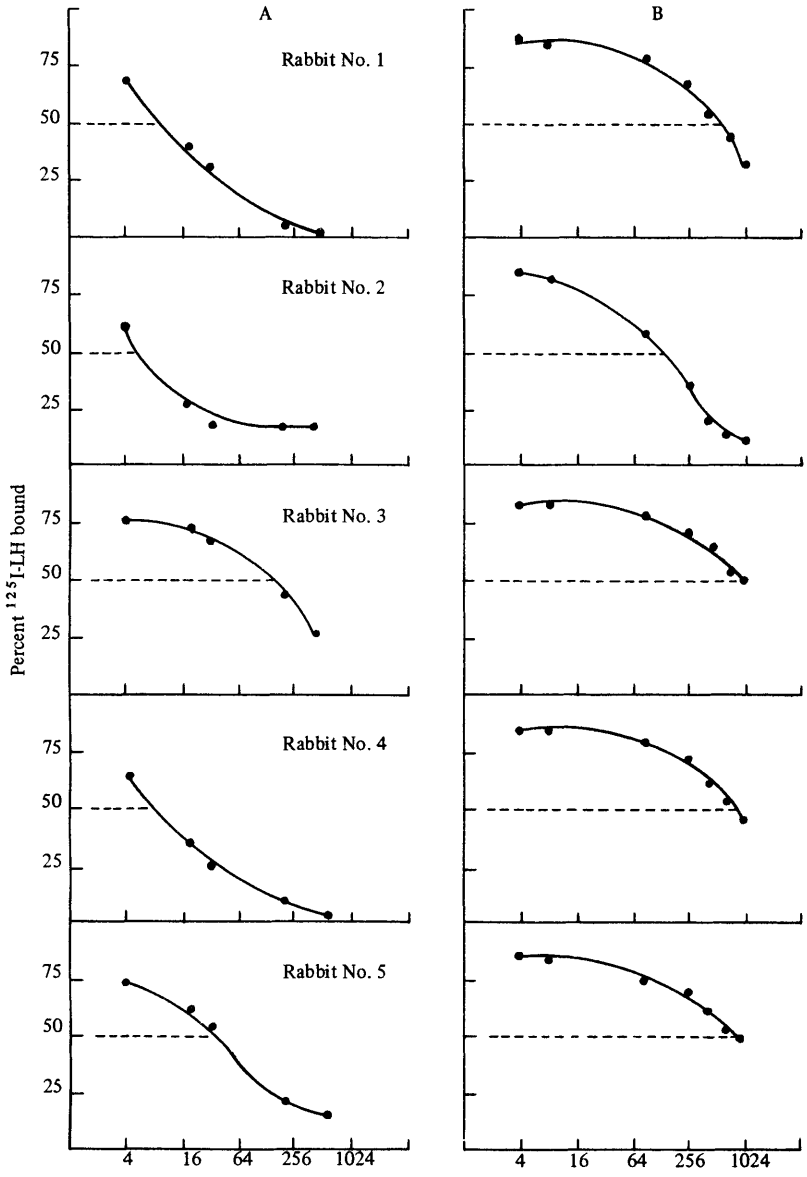


Fig. 1. *Anti-serum dilution curves of sera obtained from five rabbits following a preliminary immunization schedule (A) and after booster injections of antigen had been given (B).*

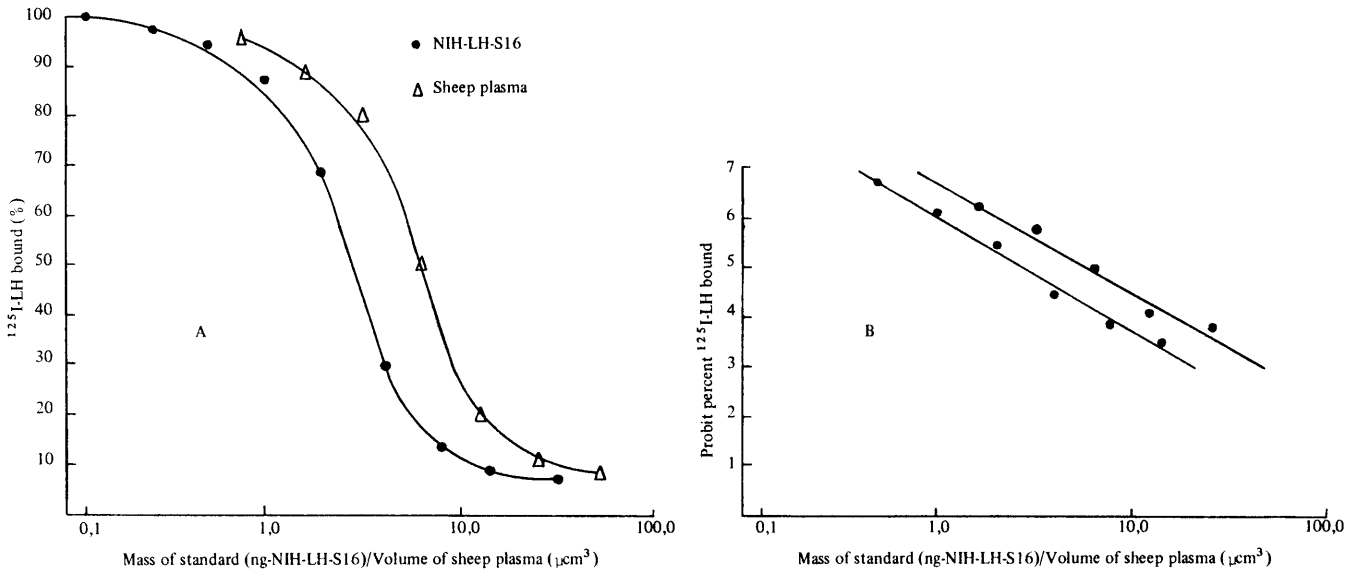
When the anti-LH serum obtained from rabbit no. 4 was used to compare the dose-response curve of a plasma previously shown to contain a high level of LH with that obtained for the standard LH preparation (NIH-LH-S16) it appeared that the two curves were parallel. The percentage ^{125}I-LH bound ("Y" axis) was expressed as a probit and the semi-logarithmic sigmoid response-curve converted to a straight line. A line was then fitted to the points between 0,5ng/cm^3 and 16,0ng/cm^3 of standard LH (Fig. 2). Tests of linearity and parellelism indicated that there was no significant difference between the two lines.

The results obtained after testing of the anti-serum for cross-reaction with ovine FSH and ovine TSH are shown in Fig. 3. From the lines fitted using the probit transformation the cross-reaction with TSH at 50 percent ^{125}I-LH bound was calculated to be 15,94 percent. The cross-reaction of FSH with the LH anti-serum was negligible.

Discussion

Although the general nature of the immune response is understood (Parker, 1971) the exact procedure to be followed in attempting to generate antisera of high titre and avidity remains equivocal. Factors such as the route of administration (Hurn & Landon, 1971), immunization schedule, molecular size and degree of "foreign-ness' of the antigen have all been implicated (Odell *et al.*, 1971). In addition, the viewpoint has developed that success involves a considerable amount of good fortune (Hurn & Landon, 1971).

Typically, antibody titre improves as the period of immunization increases while animals which do not react to the antigen at a fairly early stage in the immunization program usually do not warrant further testing. The data obtained in this study generally agree with these conclusions,



Mass of standard (ng-NIH-LH-S16)/Volume of sheep plasma (μcm^3)

Fig. 2. *Dose response curves for ovine NIH-LH-S16 and an ovine plasma sample. The amount of ^{125}I-LH bound in the absence of unlabelled LH was set equal to 100 percent.*

A – semilograthmic
B – probit

but the marked improvement in the antiserum produced by rabbit no. 4 after day 35 was not consistent with the expected pattern.

Samli & Geschwind (1967), Geschwind & Dewey (1968), Goding, Catt, Brown, Kaltenbach, Cumming & Mole (1969) and Scaramuzzi, Caldwell & Moor (1970) have discussed the implications of the cross-reaction of anti-LH sera with TSH. The cross-reaction of 15,94 persent reported here agrees closely with the theoretical figure of 16 per cent, as calculated by Scaramuzzi *et al.* (1970).

With respect to FSH, Baron, Terterin & Jutisz (1967) and Geschwind & Dewey (1968) concluded that this hormone did not cross-react in their assay for LH. Scaramuzzi *et al.* (1970) observed that as much as 10,0 ng of FSH was required to cause notable precipitation of LH. In a later study, Scaramuzzi, Blake, Papkoff, Hilliard & Sawyer (1972) showed that up to 50,0 ng FSH did not cause significant displacement of ^{125}I-LH from the antibody. The present findings also indicate negligible cross-reaction and are compatible with those published by other workers.

The problems associated with the production of effective antisera cannot be over-emphasised. Therefore, the present findings in which the antiserum of only one rabbit could not be successfully diluted to at least 1:50 000 (Fig. 1B) must be regarded as a notable achievement.

In attempting to account for this high rate of success it should be noted that the response to an immunogen is genetically determined and inbred strains may produce significant quantities of antibodies only to a particular antigenic determinant (Green, Paul & Benacerrat, 1969). It is therefore recommended that in attempting to produce antisera, random-bred animals should be used (Hurn & Landon, 1971). Our findings support this recommendation.

Acknowledgments

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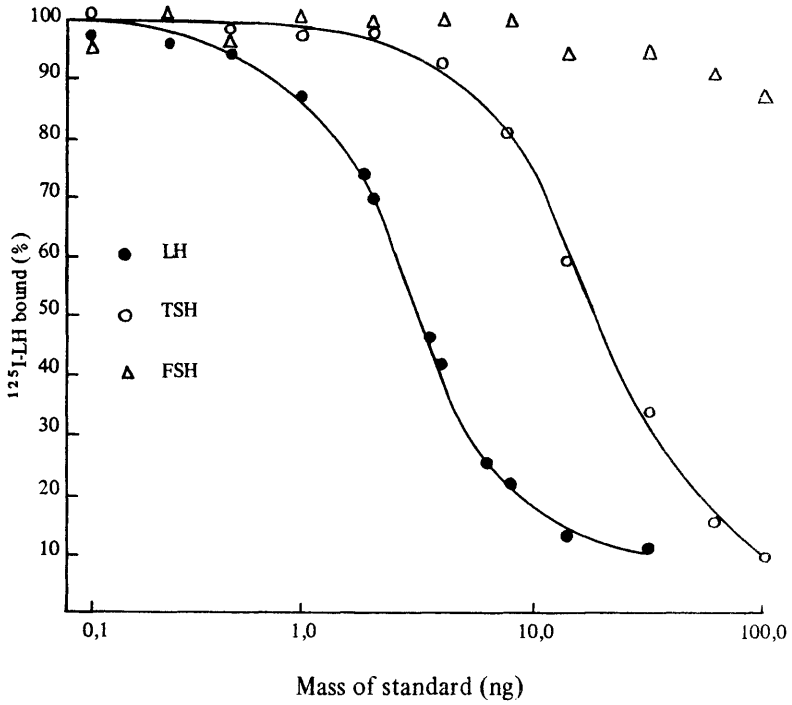

Fig. 3. *Dose response curves for ovine LH, ovine FSH and ovine TSH using an anti-serum to ovine NIH-LH-S16.*

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