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SHORT COMMUNICATION

EPISODIC RELEASE OF STEROID ANTIBODIES

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The routine preparation of steroid antisera in rabbits is carried out in many laboratories. These antisera find use in the immunoassay of individual steroids in biological fluids. Most authors have used one of two general injection regimes; either one-time multiple site intradermal injections or more commonly, a scheme involving monthly booster injections. In the latter case antiserum is normally harvested at or around ten days after an injection [1–10]. Although single measurements of antibody titre have been made after each of several monthly booster doses [1, 11] there has been, to our knowledge, only one brief investigation of precisely when in the month this blood should be taken [1]. High titres are desirable not only for economy but also because they result in the dilution of material present in the antiserum which may affect the immunoassay. We have therefore undertaken a detailed study of the immune response in rabbits after a single booster injection.

The following steroid derivatives, 4-(4′-carboxyphenylazo)oestetrol (4-E4), 3β-hydroxy-5-androsten-19-al-17-one 19-(O-carboxymethyl)oxime (19-DHA) and 11α-hydroxytestosterone 11-hemisuccinate (11-T) were prepared as previously described [10, 12, 13]. The steroid acids were then coupled to bovine serum albumin (BSA) and the conjugate purified [10]. The molar steroid:protein ratios were determined spectrally to be 34, 29 and 36 respectively. These antigens were injected into male New Zealand White rabbits (2.5–3.0 Kg.) as previously described [10]. The number of monthly injections given prior to the present experiment were as follows: 4-E4, five; 19-DHA, four and 11-T, two. After a further single booster injection, blood samples were removed from the marginal ear vein into heparinized tubes, at regular intervals. Serial dilutions of the antisera were prepared [14] and their binding capacity using oestetrol-6,7-[^{3}H] (S.A. 50 Ci/mmol; 10,000 c.p.m.), dehydroepiandrosterone-7-[^{3}H] (S.A. 16.6 Ci/mmol; 5,000 c.p.m.) and testosterone-1,2,6,7-[^{3}H] (S.A. 92 Ci/mmol; 5,000 c.p.m.) determined as the dilution that bound 50% or 25% of the tracer (all radioactive tracers were obtained from the Radiochemical Centre, Amersham). The change of antibody titre with time is shown in Fig. 1.

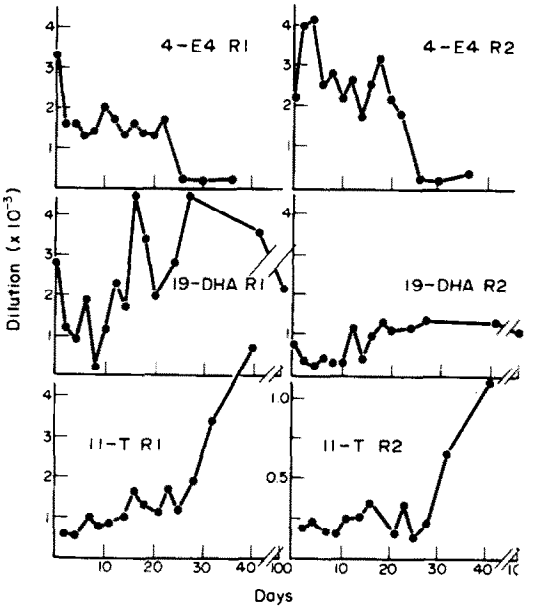

Fig. 1. Dilution of antisera, collected on various days, which gave 50%† binding of a tritiated steroid tracer. 4-E4, 19-DHA and 11-T refer to antisera raised against oestetrol-4-BSA, dehydroepiandrosterone-19-BSA and testosterone-11-BSA conjugates respectively. Each antigen was injected into two rabbits and in each case these are referred to as R1 and R2. (†In the case of 11-T, 25% binding was used).

The first and most obvious conclusion to be drawn from Fig. 1 is that the period around ten days after a booster injection cannot be relied upon to give the highest titre. In fact in only one animal (4-E4 R1) was there any peak in titre on day ten. For five of the animals studied (4-E4 R1, 19-DHA R1, 19-DHA R2, 11-TR1 and 11-T R2) the initial period following immunization was characterized by relatively low titres. It is unlikely that this low binding was caused by changes in the numbers of antibody-producing cells since it has been demonstrated that the numbers of such cells rise rapidly following a secondary immunization [15, 16]. A more probable explanation is that the pre-existing circulating antibodies are partly saturated by the relatively large amounts of antigen which would be released from the injection site during this period [17].

In all the rabbits the antibody titres fluctuated in a cyclical pattern with changes in titre of 2–3 fold occurring at short intervals (4–6 days). This pattern is consistent with a hunting mechanism in the negative feed-back control on antibody levels, as proposed by Britton and Muller [17]. It was suggested that antigen stimulates the production of antibody secreting cells which in turn produce antibody. These antibodies then combine with the antigen rendering it immunogenically impotent. In the absence of further stimulation antibody-producing cells and antibodies decay. This subsequently leaves the antigen free to initiate a second cycle. The overshoot of antibody production is probably due to the delay between antigen challenge and antibody secretion [16].

From the results in Fig. 1 it would appear that no single interval can be generally recommended for harvesting steroid antisera from rabbits. We therefore suggest that in cases where low titres (or no titres at all) are obtained that a temporal study of the immune response should be undertaken.

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