

acetone 92:5:7:5). Six major radioactive spots were separated; the immunoreactive compound isolated in this way was submitted to t.l.c. in systems 1 and 2 (chloroform/ethyl acetate 95:5) where it behaved as a single peak. (Chromatographic controls demonstrated no chemical damage or loss of activity after 30 days.) The new tracer was compared in a RIA with both the tritiated and tyrosine-iodinated (TI) antigen. The standard inhibition curve obtained with the new tracer can almost be superimposed on the curve obtained using  $^3\text{H}$ -NET. This suggests that the immunoreactive portion of the molecule maintained its characteristics. The sensitivity of the new method is 3 pg; this is approximately 5 times that reached with the tritiated antigen and in the same range of that obtained with the TI antigen.

**48. Measurement of anabolic steroids by radioimmunoassay**  
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The aim of this study was to develop a radioimmunoassay capable of detecting anabolic steroids in blood and urine. Antiserum was raised in rabbits against  $17\alpha$ -methyltestosterone linked *via* the 3-oxo group to human serum albumin. The specificity was in theory thus directed towards the structural features of the steroid D ring (i.e.  $17\alpha$ -methyl,  $17\beta$ -hydroxy). Many of the anabolic steroids have this common feature (Dianabol, Stanozolol, Oxymetholone etc.). This specificity was found to be such that anabolic steroids with a  $17\alpha$ -methyl group did crossreact, whereas those with a  $17\alpha$ -ethyl group did not. However, testosterone, the natural male hormone, crossreacted to the extent of 5.0% which was unacceptably high. Testosterone acetate did not crossreact at all and this fact was used to eliminate the effect of testosterone in the assay by conversion to the 17-acetate. Organic solvent extracts of blood or urine were therefore acetylated before assay. The  $17\alpha$ -alkylated anabolic steroids are not acetylated and remain unchanged. A coated tube (solid phase) radioimmunoassay was developed using a steroid- $^{125}\text{I}$ -histamine conjugate as label. Using this "group specific" antiserum, anabolic steroids with a  $17\alpha$ -methyl group have been readily detected in both blood and urine samples from healthy males after an oral dose of the steroid. (This work was supported by a grant from the British Sports Council).

**49. Antisera for radioimmunoassay of mestranol and ethynylestradiol**  
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Antisera for the synthetic estrogens, mestranol and ethynylestradiol, have been prepared by immunizing rabbits with

6-(0-carboxymethyl) oxime-bovine serum albumin conjugates prepared from 6-oxo-mestranol and 6-oxo- $17\alpha$ -ethynylestradiol, respectively. The antisera for mestranol showed a titer of 50% binding of 50 pg of mestranol- $9\alpha$ ,  $11\xi$ - $^3\text{H}$ ) at a dilution of 1:5,000 and the antisera for  $17\alpha$ -ethynylestradiol- $(9\alpha, 11\xi$ - $^3\text{H})$  at a dilution of 1:25,000. Individual rabbit sera vary greatly in their spectrum of sensitivity and cross-reactivity between mestranol and EE and other known metabolites of these steroids. These sera are compared to antisera obtained by others which have been coupled at the C-7 position.

**50. The use of  $^{125}\text{I}$  labelled tracers for the radioimmunoassay of oestradiol- $17\beta$  and norethindrone**  
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Previous reports on iodinated tracers for steroid radioimmunoassay have described the conjugation of the steroid to a protein or to a tyrosyl methyl ester followed by radioiodination of the steroid conjugates. In steroids with phenolic ring A the introduction of iodine into the A ring has been shown to destroy immunoreactivity. In the present study this has been avoided by first iodinating tyrosine or histamine which was then conjugated to the steroid. Oestradiol- $17\beta$ -6-(0-carboxymethyl)oxime was conjugated to  $^{125}\text{I}$ -tyramine by the mixed anhydride method. A maximum specific activity of 6.2 mCi/ $\mu\text{g}$  oestradiol- $17\beta$ -6-(0-carboxymethyl)oxime was obtained. The high specific activity enabled a dilution of 1 ml of the antiserum to oestradiol- $17\beta$ -6-(0-carboxymethyl)oxime-bovine serum albumin of 1:12,000,000 sufficient for 120,000,000 assay units. When using 2,4,6,7- $^3\text{H}$  oestradiol- $17\beta$  as tracer this antiserum was used in a dilution of 1:100,000. The high dilution of the antiserum in combination with the iodinated tracer made possible the use of radioimmunoassay systems detecting levels below 1 pg of oestradiol- $17\beta$ . Norethindrone-3-(0-carboxymethyl)oxime was conjugated to  $^{125}\text{I}$ -histamine by the mixed anhydride method. Specific activities of about 1 mCi/ $\mu\text{g}$  was obtained. This tracer was used in a radioimmunoassay system with an antiserum to norethindrone-3-(0-carboxymethyl)oxime-bovine serum albumin. The antiserum was diluted 1:50,000 in combination with the iodinated tracer. Five pg of norethindrone was found to give a reduction of 15% of bound counts (O pg = 100%). When using 6,7- $^3\text{H}$  norethindrone as tracer this antiserum could only be diluted 1:500 and 250 pg of norethindrone could be read off the standard curve as significantly different from zero.  $^{125}\text{I}$ - as compared to  $^3\text{H}$ -tracers resulted in assay systems with increased sensitivity and specificity.