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}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 17α-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. 17α-methyl, 17β-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 17α-methyl group did crossreact, whereas those with a 17α-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 17α-alkylated anabolic steroids are not acetylated and remain unchanged. A coated tube (solid phase) radioimmunoassay was developed using a steroid-^{125}I-histamine conjugate as label. Using this "group specific" antiserum, anabolic steroids with a 17α-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-17α-ethynylestradiol, respectively. The antisera for mestranol showed a titer of 50% binding of 50 pg of mestranol-9α, 11ξ-^{3}H) at a dilution of 1:5,000 and the antisera for 17α-ethynylestradiol-(9α, 11ξ-^{3}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}I labelled tracers for the radioimmunoassay of oestradiol-17β 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-17β-6-(0-carboxymethyl)oxime was conjugated to ^{125}I-tyramine by the mixed anhydride method. A maximum specific activity of 6·2 mCi/μg oestradiol-17β-6-(0-carboxymethyl)oxime was obtained. The high specific activity enabled a dilution of 1 ml of the antiserum to oestradiol-17β-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}H oestradiol-17β 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-17β. Norethindrone-3-(0-carboxymethyl)oxime was conjugated to ^{125}I-histamine by the mixed anhydride method. Specific activities of about 1 mCi/μg 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}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}I- as compared to ^{3}H-tracers resulted in assay systems with increased sensitivity and specificity.