PRO EXPERIMENTIS

A Direct Plasma Testosterone Radioimmunoassay

A radioimmunoassay for plasma testosterone without chromatographic purification has been developed. Other methods[1-3] have been reported previously which are accurate, sensitive and precise but require some type of chromatography for purification of the extract. Recently FURUYAMA et al.[4] developed a radioimmunoassay method for plasma testosterone determination which included one step column chromatography; ISMAIL et al.[5] published a procedure for radioimmunoassay of testosterone without chromatography which employed ammonium sulfate for separation of testosterone binding globulin fraction from serum, followed by an extraction step. Both of these techniques are accurate and specific; however, they are laborious and time consuming, and thus are impractical for routine clinical laboratory application.

A simple and reliable radioimmunoassay method which can be used with plasma or serum, male or female, is herein described in which a single step of solvent extraction is used for purification. The results of this procedure are comparable to column chromatography as well as other procedures.

For comparison of methods disposable glassware rinsed with methanol: methylene chloride solution (1:1) was used. The Al_2O_3 microcolumns and their purification have been previously described[4]. Testosterone-3-oxine and its conjugate to bovine serum albumin (BSA) was prepared according to the method of ERLANGER et al.[6].

Antisera was produced by immunizing 3 New Zealand rabbits with periodical toe pad injections. Satisfactory antisera was obtained from all rabbits after 8 months and used to a final dilution of 1:60000. For direct radioimmunoassay (RIA), after pre-extraction with hexane (0.1 ml male, 0.5 ml female serum), 2 ml of 2% ethanol in hexane were pipetted into each male sample tube, mixed on a vortex for 1 min, and allowed to stand for 1 min. Then 0.25 ml was withdrawn directly into a counting vial for the determination of recovery of labelled testosterone. Duplicate aliquots were pipetted into 2 RIA tubes. For female samples, 2.5 ml of hexane and ether (6:4) mixture were used to extract the serum twice and the extracts pooled and mixed. The total volume of extract was reduced to 2 ml with an air blowing device[7], and 0.5 ml aliquots of extract were transferred into a counting vial and 2 RIA tubes. To prepare the standard curve, 0.1 ml of standard solution of different concentrations of testosterone were pipetted in triplicate into RIA tubes, together with 0.1 ml of labelled testosterone solution of about 2100 c/min into each tube.

Twenty-five µl of anti-testosterone serum diluted to 1:60000 with BSA borate buffer was added to each tube and mixed with a vortex mixer. Tubes were covered and incubated 1 h at 4 °C. To separate free and bound testosterone, 0.25 ml saturated ammonium sulfate was added to each tube. Tubes were mixed and centrifuged at 3,500 rpm for 20 min at 4 °C. An aliquot of 0.25 ml of supernatant was transferred into a counting vial with an autodiluter followed by 10 ml scintillation fluid. Counting vials were shaken and counted in a scintillation spectrometer.

The standard curve was constructed on semilog paper by plotting percent free of labelled testosterone as a function of the mass of unlabelled testosterone in picograms (Figure). Testosterone concentration in the sample was calculated according to FURUYAMA et al.[4]. Recovery of labelled testosterone improved from 68.4 ± 5.3 (S. D.)% to 79.9 ± 4.8 (S. D.)% when column chromatography was omitted.

Accuracy was checked by adding 100-200-500 pg of testosterone to ten 0.1 ml aliquots of a female plasma pool and repeating the experiment. No systematic error in the procedure could be found. The inter and intra assay precision was examined by analyzing the results of accuracy study. The coefficient of variation was 8.4%. At the 95% confidence limit the percent free for 10 pg was signi-

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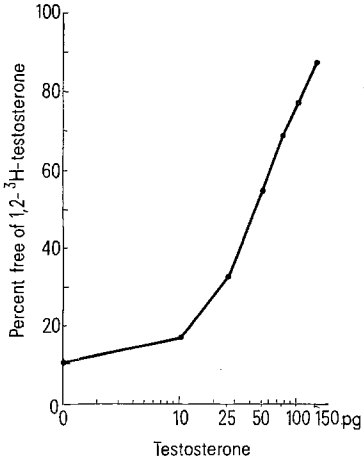

Standard curve of testosterone concentration.

Table I. Percent cross reactivity of testosterone anti-sera

	Cross reactivity (%)
Testosterone	100
5α-Dihydrotestosterone	27.3
5α-Androstane-3α, 17β-diol	2.2
5-Androsten-3β-ol-17-one	< 0.01
5-Androsten-3β, 17β-diol	0.36
4-Androsten-3, 17-dione	0.7
1,4 Androstedien-17β-ol-3-one	46.5
4-Androsten-3β 17β-diol	2.6
5-β-Androstan-17β-ol-3 one	6.2
1 (5α) Androsten-17β-ol-3 one	18.5
5α-Androstan-3β-ol-17-one	< 0.01
5α-Androstan-3α-ol-17-one (androsterone)	< 0.01
(Etiocholanolone) 5β-androstan-3α-ol-17-one	< 0.01
5β-Androstan-3β, 17β-diol	0.20
Estradiol-17β	< 0.01
Estrone	< 0.01

ficantly different from that for 0 pg of testosterone. The coefficient of variation of each standard curve point assayed in triplicate was always less than 7%.

Specificity of this method was evaluated by investigation of blanks, antibody specificity, and comparison of results obtained with column chromatography with those obtained without. The specifity of our antiserum was tested by direct incubation with 15 other steroids. Percent cross reaction was calculated according to the method of ABRAHAM[8]. Table I shows the cross reactivity of our antibody to different steroids. Recovery of labelled testerone without column chromatography was improved. Pre-extraction with hexane was still a necessary step.

Table II. Pooled plasma testosterone values obtained after radioimmunoassay with and without column chromatography (mean $\pm$ S.D. ng/100 ml)

	Column used	Column omitted
Male	$538 \pm 59\ N = 56$	$528 \pm 30\ N = 40$
Female	$40 \pm 8\ N = 35$	$41 \pm 7\ N = 9$

Normal testosterone values for male and female are shown in Table II. These results agree with other methods. Thus, samples may be assayed without the use of column chromatography; this column omission procedure is simple, accurate, sensitive, specific, and suitable for clinical use.

Résumé. On a développé und méthode simple pour déteminer sans chromatographie en colonne le testostérone du plasma. La purification a été faite par préextraction avec l'hexane. Un antisérum contre le testostérone a été produit chez des lapins inoculés avec des conjugués de testostérone-3-oxine et d'albumine de sérum bovin.

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Simultaneous Measurement of Velocities of Adjacent Sacromere Length Changes in Single Muscle Fibres

Light, phase contrast, interference and polarizing microscopy have been applied to the study of structures of living muscle fibres. To measure dynamic changes during muscle contraction and relaxation, various transducers have been used to record tension; whereas changes in striation pattern of muscle fibres are recorded either by photoelectric devices with markers applied onto the muscle fibres, or by means of high-speed cinematography[1-4]. Recently changes in sarcomere length during isometric contraction of isolated frog muscle fibres were studied using laser diffraction techniques. The diffracted light was then projected on a screen for photography[5]. The marker techniques however do not indicate accurately the individual sarcomere lengths, and analysis of cinematography has the disadvantage of being tedious and time consuming. A method for measuring the different velocities of sarcomere length changes during contraction and relaxation of single striated muscle fibres has been developed.

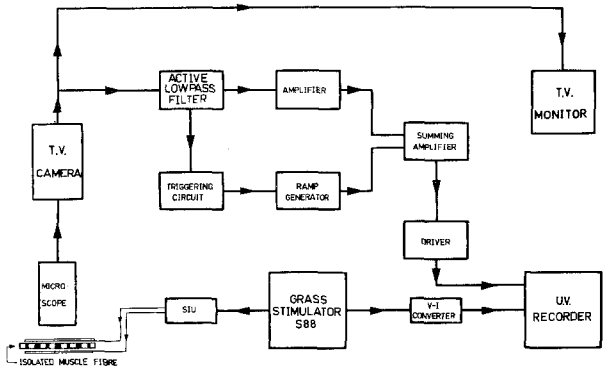

Fig. 1. Block diagram for measurement of sarcomere length and velocity of contraction in single striated muscle fibre. SIU, stimulus isolation unit.

Materials. The accessory muscle located in the coxatrochanteric joint of the Asiatic horseshoe crab, *Tachypleus gigas* is used. This muscle is essentially a receptor muscle with sensory neurons attached to it for the detection of joint movement[6]. The broad striations (2–12 μm) and small diameters (2–6 μm) of these fibres are very favourable for signal identification and separation. Morever, electron microscopic studies have revealed this muscle to be devoid of H zones[7,8].

Method. Isolated single muscle fibres are fixed at one end by a stainless steel spring, clipped onto the exoskeleton and bathed by oxygenated filtered sea water in a lucite chamber. The tendon end of the muscle fibres is attached by means of a stainless steel microhook to a special micromanipulator constructed in our laboratory. The isolated single fibres could then be stretched by the micromanipulator to varying degrees of the resting length. The muscle fibre is then observed under a phase contrast microscope (Olympus, Vanox NH $40\times/0.65$ or NH $100\times/1.30$). The image is also displayed through a closed circuit T.V. system (ITC. CTC-5000 T.V. camera) on a monitor (Pye Model 59). An adjustable slit is placed on top of the photo eye-piece (Olympus P-15$\times$) to limit the

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