The supernatants were neutralized with 0.1 N NaOH and used for the estimation of GnRH. The levels of FSH and LH in the sera were estimated by RIA using NIAMDD systems and expressed in terms of NIAMDD-Rat-FSH-RP-1 and NIAMDD-Rat-LH-RP-1 respectively. The GnRH was measured by RIA using the polyethylene glycol precipitation method, employing a kit obtained from Biodata, Hypolab, SA.

Results and discussion. Both testicular and ovarian inhibin suppressed serum FSH levels without affecting LH (table). The hypothalamic GnRH content of both these groups was significantly ($p < 0.001$) less than that of rats treated with saline. Since the content of hypothalamic releasing hormone and the serum FSH levels were lowered at the same time we conclude that inhibin acts by blocking GnRH synthesis. The unaltered levels of LH probably indicate that the threshold amount of GnRH required for LH release could be less than that for FSH.

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Prednisolone-binding proteins in the rat liver and gastrocnemius muscle

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Summary. Quantitatively, liver cytosol preparations were found to contain 2.3 times the number of prednisolone-binding proteins that were present per mg total protein in the rat gastrocnemius muscle. The liver proteins were larger molecules and, in a low ionic environment, were more chemically stable than the muscle proteins.

When prednisolone acetate was injected s.c. every day over a period of 8–10 days into male Wistar rats, there were losses of RNA and protein in the gastrocnemius muscle of the animals[2]. However the concentrations of these 2 cell constituents were increased in the liver[2].

These changes were shown to involve alterations in RNA turnover in the 2 tissues. In the liver, the rate of synthesis of ribosomal RNA was shown to be increased and its rate of breakdown decreased while, in the muscle, prednisolone caused net decreases in the rates of synthesis and breakdown of ribosomal RNA[2]. An increase in the activity of the RNA polymerase believed to be responsible for the synthesis of ribosomal RNA in the liver was also observed while, in the muscle, prednisolone treatment was shown to cause a decrease in the activity of this enzyme[2]. Thus, the alterations in the enzymic activities could explain the observed changes in RNA turnover in prednisolone-treated animals. In view of the opposite effects which prednisolone had in the 2 tissues on RNA turnover and RNA polymerase activities, it is difficult to visualize an identical mode of action of this steroid in the tissues under study. It is conceivable, however, that the responses differ because prednisolone-protein complexes in the respective tissues differ in physicochemical properties and, therefore, affect different regions of the DNA template. An extension of this concept would suggest that such differences might also account for the phenomenon of side effects which prolonged administration of corticosteroids produces in patients[3,4]. Since it is widely believed[5–7] that the phenotypic response of target tissues to a steroid begins with an initial binding of the steroid to cytoplasmic receptor proteins located in the target tissue, it seemed appropriate, in explaining the action of prednisolone, to investigate differences in the proteins which bind the steroid in the cytoplasm and which presumably translocate it to the nucleus.

Materials and methods. Wherever possible, Analar grade reagents were used without further purification. [6,7(n)-^{3}H] prednisolone was obtained from Radiochemical Centre, Amersham, England, in toluene/ethanol mixtures. For use, 0.1 ml unlabelled prednisolone in toluene/ethanol (9:1), containing 0.036 mg prednisolone was mixed with 0.4 ml ^{3}H-labelled prednisolone to give a final steroid concentration of 9.6 µM (sp. act. 173 µCi per mg prednisolone). The mixture was evaporated to dryness under nitrogen at 20 °C and redissolved in 0.5 ml absolute ethanol.

Preparation of cytosols and prednisolone-protein complexes. Gastrocnemius muscle, perfused in situ for 2 min with ice-cold 0.9% (w/v) saline was excised quickly from the animal and minced finely. The tissue was suspended in 4 times its volume of the homogenisation buffer (1,0 mM sodium phosphate buffer, pH 7.4, containing 0.5% (v/v) thiodiglycol) and homogenized using a Silverson homogenizer (Silverson Machines, Bucks, England) driven at top speed. This homogenisation procedure was carried out in 2 bursts each lasting 30 sec. The homogenate was immersed in ice throughout this procedure and a 2-min interval was interposed between each burst to maintain the temperature of the homogenate between 0 and 4 °C. Thereafter, the homogenate was centrifuged at 60,000 × g for 2 h at 2 °C. The supernatant constituted the cytosol fraction and was used within 24 h of preparation without further purification.

Liver cytosol was prepared as described above for muscle, after perfusing the tissue in situ with cold saline through the portal vein. A portion of the cytosol preparation was diluted with the homogenisation buffer to give a solution containing 1.0 mg protein per ml, and 4.0 ml of the diluted cytosol was incubated with 0.2 ml of the ^{3}H-prednisolone, prepared as previously described, for 90 min at 2 °C. Thereafter, 0.5 ml of a suspension of activated charcoal (300 mg/ml in the homogenisation buffer) was added to the incubation mixture to adsorb unbound steroid and was agitated vigorously on a vortex mixer for 1 min. After standing for a further 5 min, the mixture was centrifuged at 1000 × g for 5 min at 2 °C and the supernatant was filtered through glass wool to remove any traces of charcoal.

Ion-exchange chromatography of prednisolone-protein complexes. A portion (3.0 ml) of the final filtrate from the preceding section was loaded onto a DE-52 cellulose ion-exchange column (25 cm high, 1 cm in diameter) packed under gravity and pre-equilibrated with 1.0 mM sodium phosphate buffer, pH 7.4. After an initial prewash with 60 ml of the same buffer, the column was eluted with a linear ionic gradient (prepared by mixing 60 ml each of 1.0 mM and 0.2 M sodium phosphate in a gradient former) at a flow rate of 1.0 ml per min. Fractions of the eluate were analyzed for protein by absorbance measurements at 280 nm and for radioactivity using the intertechnique liquid scintillation spectrometer (Nuclear Enterprises Limited, UK).

Sucrose density gradient analyses. A portion (1.0 ml) of the radioactive cytosol preparations (1.0 mg protein/ml) was layered over a 5–20% (w/v) sucrose density gradient and centrifuged at 60,000 × g for 24 h at 2 °C using a precooled 3 × 23 ml swing-out aluminium rotor (MSE Ltd, England). Thereafter, 25-drop fractions of the gradient were collected and counted for radioactivity. Bovine serum albumin was used as a molecular weight marker and sedimentation coefficients and apparent molecular weights were estimated by reference to this standard[8].

Results. A comparison of radioactivity level in the cytosol sample, before it was subjected to ion-exchange chromatography, and the sum of radioactivity levels in the eluate fractions, confirmed that no significant radioactivity remained bound to the column after the elution.

2 distinct peaks of radioactivity were observed following the elution of the samples prepared with the liver cytosol. The 1st and larger zone eluted in the prewash while the 2nd eluted at a phosphate concentration of 0.069 M (figure 1). Occasionally, a 3rd peak which showed extreme variability in amount was eluted at a phosphate concentration of 0.137 M and was presumed to be an artefact. 2 peaks of radioactivity were also observed on elution of the sample prepared with gastrocnemius muscle cytosol (figure 2). The elution profile obtained with these samples resembled the pattern obtained with the liver cytosol preparations but, quantitatively, only 43% of the radioactivity level observed in the liver cytosol was evident in the muscle cytosol.

In the absence of KCl, the liver prednisolone-protein complexes resolved into a single peak of radioactivity on centrifugation in the sucrose gradient (figure 3). The position of this peak, in comparison with the bovine albumin peak, indicated that these complexes had a sedimentation coefficient of 5.9 and a mol. wt of approximately 80,000. Agawaral et al.[9] and Beato and Feigelson[10] have reported similar values for corticosteroid receptors in the rat liver. A major proportion of the radioactivity present in the cytosol remained at the top of the gradient and would suggest considerable dissociation of the prednisolone-protein complexes during the lengthy centrifugation (24 h), as has been observed by Snart et al.[11]. A small peak of radioactivity was detected in the 3.9S region following sucrose gradient centrifugation of the gastrocnemius muscle preparation (figure 4). This peak increased in size by more than 2-fold when 0.3 M KCl was included, as recommended by Mayer and Rosen[12], in the homogenisation buffer used to prepare the cytosol and in the sucrose gradient on which the sample was loaded. The approximate mol.wt of the muscle predni-

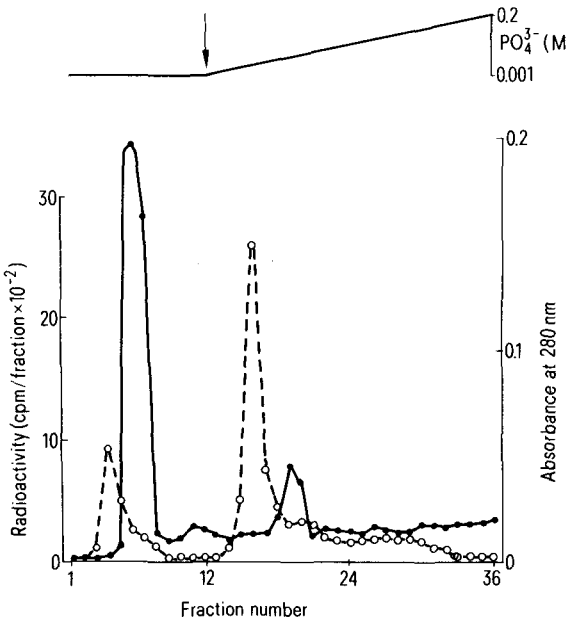

Fig. 1. DE-52 cellulose ion-exchange chromatography of prednisolone-binding proteins in rat liver cytosol. Liver was processed for chromatography as described in the text. After initial prewash, gradient elution was begun at the arrow: ●——●, radioactivity; ○- - -○, absorbance at 280 nm.

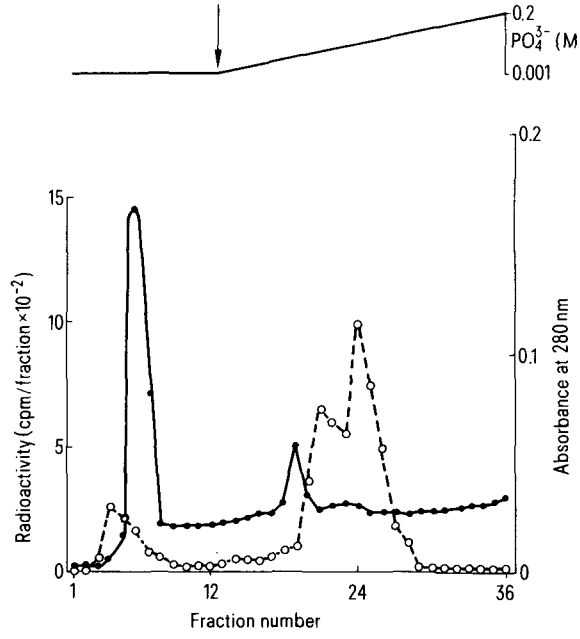

Fig. 2. DE-52 cellulose ion-exchange chromatography of prednisolone-binding proteins in rat gastrocnemius muscle cytosol. The muscle was processed for chromatography as described in the text. After initial prewash, gradient elution was begun at the arrow: ●——●, radioactivity; ○----○, absorbance at 280 nm.

solone-protein complexes was estimated to be 50,000. In the presence of 0.3 M KCl, liver prednisolone-protein complexes sedimented in the 6.5 S region (figure 3). Thus, the ionic environment was a factor that influenced the behaviour of the proteins that bound prednisolone in the two tissues.

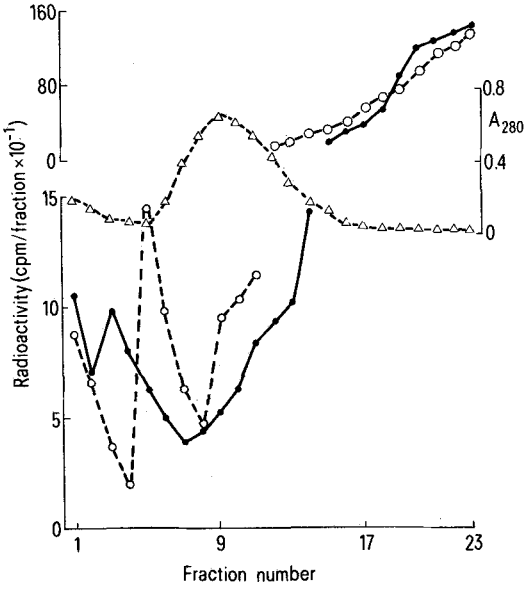

Fig. 3. Sedimentation profile of rat liver cytosol prednisolone-binding proteins in 5%–20% (w/v) sucrose gradients: Centrifugation was performed at 60,000 × g for 24 h at 2 °C, as described in the text. O----O, gradient containing no KCl; ●——●, gradient containing 0.3 M KCl. The sedimentation peak of bovine albumin (△——△) was identified by UV absorption measurements at 280 nm.

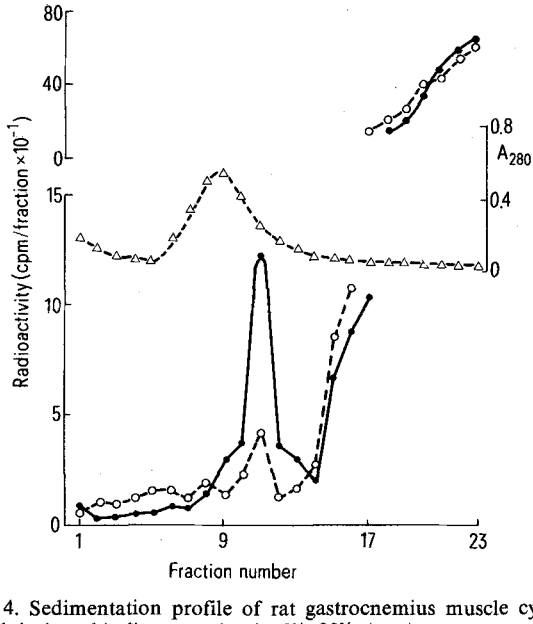

Fig. 4. Sedimentation profile of rat gastrocnemius muscle cytosol prednisolone-binding proteins in 5%–20% (w/v) sucrose gradient. Centrifugation was performed at 60,000 × g for 24 h at 2 °C, as described in the text. O---O, gradient containing no KCl; ●---● gradient containing 0.3 M KCl. Bovine albumin peak (△---△) was identified by UV absorption measurements at 280 nm.

Discussion. The finding that less than half the quantity of prednisolone bound in the liver cytosol was bound in the gastrocnemius muscle cytosol would suggest that there were fewer prednisolone-binding sites in the muscle than existed in the liver. Since there is a dose-related response to steroids[13], differences in the amount of prednisolone-protein complexes formed in the tissues under study may be sufficient to determine the response of each tissue to the steroid. It is recognized that the differences observed in this study could reflect differences in the distribution of prednisolone-binding proteins between the cytoplasm and the nucleus. However, it is generally accepted that it is the proteins that bind the steroid in the cytoplasm that are transferred and translocated to the nucleus[14-16]. Thus, the binding of the steroid to protein in the cytoplasm enhances nuclear binding and changes in the capacity of the cytoplasm to bind a steroid closely resemble changes in the nuclear binding capacity[17]. The quantity of prednisolone-protein complexes formed in the liver and gastrocnemius muscle cytosol is therefore a relevant consideration and may be significant for the different responses observed in these tissues.

The appearance of a single peak of radioactivity on sucrose gradient centrifugation of the prednisolone-protein complexes from each of the 2 tissues under study may suggest that a single class of proteins of the same approximate molecular size was present in each case; the appearance of more than 1 peak in the ionic gradient elution profile may be explained by the gradual disaggregation of the complexes as the ionic strength of the eluant was increased[18]. It is recognized, however, that other proteins could be present near the top or under the sucrose gradient peak in each case. The sucrose gradient analyses also indicated that the proteins which bind prednisolone in the rat liver were bigger molecules than those which bind the steroid in gastrocnemius muscle, a factor which could influence the relative accessibilities of the prednisolone-protein complexes in the nucleus and, ultimately, the response of the target tissue. The physicochemical differences in the prednisolone-binding proteins may also determine both their specificities and affinities for binding sites on the genetic apparatus. Thus, the manner in which prednisolone modulates genetic transcription in a target tissue may depend on the physicochemical properties of the cytoplasmic proteins that bind the steroid in that tissue.

1 The authors gratefully acknowledge the advice and encouragement of Dr G.A.J. Goodlad.

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