sites within the central nervous system. Such a possibility is supported by the recent findings of HALE and SYMINGTON[32] who studied the effects of DA on FSH release by isolated pituitaries in the presence of whole hypothalami, when release was increased, while in the presence of median eminence tissue release was decreased.

There is considerable evidence that central α-adrenoceptor stimulation induces the release of IRGH in higher species. In dogs, phentolamine, administered intraventricularly, abolished the release of IRGH induced by the NE precursor L-dopa (GANONG [9]). In conscious cats similar results were obtained with phenoxybenzamine but not with the dopamine receptor blocking agent pimozide (RUCH and GALE, unpublished observations). BROWN and CHAMBERS[8] reported that systemic administration of clonidine and DL-threodops (the immediate precursor of NE) induced an increase of IRGH in rhesus monkeys. TOIVOLA and GALE[6] demonstrated that microinjection of NE into or in the vicinity of the ventromedial nucleus of baboons induced a release of IRGH.

In humans, IRGH release following insulin hypoglycemia was attenuated by phentolamine (BLACKARD and HEIDINGSFELDER[1]). More recently, it was demonstrated that clonidine increased IRGH in human subjects (LAL et al.[4]).

Our findings provide evidence that α-adrenergic stimulation is one important mechanism in the regulation of GH secretion in the rat, as already postulated by MÜLLER et al.[14]. It seems, therefore, that the control of GH secretion through an α-adrenergic mechanism in this species is probably similar to that found in higher species, including primates.

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Chromatographic Conditions in the Expression of Corticosteroid Receptor Specificity

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Summary. It is shown that cytosol preparations bound with various concentrations of a steroid are necessary to reveal physicochemically distinct, heterogeneous and polymorphic receptors present in the hormone specific target organ, that these cannot be fully appreciated in one-shot experiments at suboptimal steroids levels, and that they escape detection by equilibrium binding and Scatchard analysis alone.

The first step in the mechanism of corticosteroid hormone action is currently believed to consist of the binding of the steroid molecule with its appropriate intracellular receptor leading, thereafter, to timely, sequential and selective genetic modulation[1]. Contrary to the usual association-dissociation studies used to reveal the presence of high affinity hormone-specific binding sites in the target tissue, in 1970 we pioneered partial purification of corticosterone binders in rat liver and demonstrated that the receptor moieties exist subdivided into physicochemically distinct subpopulations which, naturally, cannot be revealed by Scatchard analysis alone[2]. More recently, these same techniques were adapted to revealing a mineralocorticoid (aldosterone) specific receptor present only in the target tissue kidney, and absent from the liver[3]. In the present report we describe the saturation characteristics and chromatographic behaviour of these latter in comparison with the glucocorticoid receptors that appear to be identical in most tissues studied. We chose to employ DE-52 gels for this purpose since they appear to be ideally suited as compared to a number of other chromatographic procedures tested[4].

Material and method. Male, Wistar rats (150–200 g) were bilaterally adrenalectomized 2–3 days prior to sacrifice by exsanguination under light ether anaesthesia.

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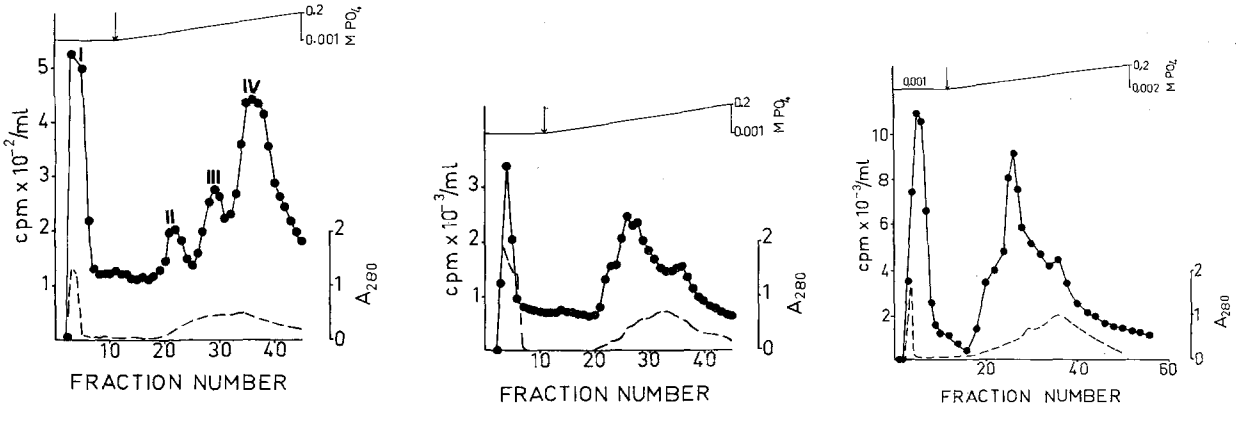

Fig. 1. Dose dependant separation of aldosterone binding proteins in rat kidney.

After perfusion with the initial buffer (0.001 M PO_4, pH 7.5), 4 ml of a 40% supernatant (105,000 g) was incubated, for 60 min at 4 °C, each with 2×10^{-9}, 5×10^{-9}, 10^{-8}, 2×10^{-8}, 10^{-7} and 10^{-6} M ^{3}H-aldosterone (only 3 experiments are shown here). The free radioactivity was thereafter removed by additional incubation in presence of 100 mg/ml cell sap of activated charcoal (Sigma C-5260) for 10 min, centrifugation at 3000 g and passage through glass wool, in that order. The cytosol was loaded onto DEAE-cellulose-52 column (1×25 cm) that had been packed and equilibrated at 4 °C with 0.001 M $NaPO_4$, pH 7.5, as described previously[3]. After passage of 60–70 ml initial buffer (fraction vol. 6–7 ml), protein was eluted with a linear gradient between 60 ml each of 0.001 M and 0.2 M Na phosphate, pH 7.5, at a flow rate of 60 ml/h (fraction vol. 3–5 ml). Aliquots (1 ml) were mixed with 10 ml Unisolve (Koch Light) and counted in a Packard Tricarb Liquid Scintillation Spectrometer with corrections for quenching, spilling and background. 1,2,^{3}H-aldosterone (batch 57072, 26 Ci/mM) was a product of Saclay (France); all other reagents were high purity reagent grade.

Data presented in Figure 1a reveal that aldosterone (5×10^{-9} M) is bound to 4 sorts of components in rat kidney cytosol of which the most abundant (I) in the low ionic prewash, and the least abundant (II), eluting in 0.006 M PO_4 region, represent mineralocorticoid specific receptors by a number of criteria including competitive binding followed by chromatography. This is compatible with the fixed time assay value of $KD_{37°C}$ 5×10^{-9} and the levels circulating in vivo[5], although 10^{-8} M aldosterone is apparently required to saturate all the binding sites under these conditions[3]. Peaks III and IV (Figure 1a) have been established to represent the glucocorticoid specific receptor and transcortin, respectively, in double labelling experiments[3,4]. It is remarkable that this clear separation is lost as the concentration of aldosterone is raised from 5×10^{-9} to 2×10^{-8} M (Figure 1b) such that the peaks II and IV are reduced to mere humps of peak III with 10^{-7} M (Fig. 1c) aldosterone (the concentration at which the glucocorticoid receptors begin to get saturated). At certain concentrations, peaks III and IV appear to elute as one peak[3], whereas at less than half saturation concentrations (2×10^{-9} M) of aldosterone, no bound radioactivity could be eluted anywhere (data not shown). It is thus amply clear that chromatography over a wide concentration range is required to provide unequivocal demonstration of the protein in question since erroneous and even negative results may be obtained if one's efforts are limited to quick one-shot experiments.

In Figure 2 are shown chromatographic characteristics of the glucocorticoid specific binders in rat kidney. At 5×10^{-9} M (Figure 2a), corticosterone reveals only transcortin (intracellular?) since 10^{-7} M steroid are required to saturate the glucocorticoid specific binders which elute in the 0.001 M and 0.02 M PO_4 regions in keeping with levels circulating in vivo[5] and which are clearly visible as peaks I and II in Figure 2b. However, even at 10^{-6} M, corticosterone did not reveal the peak at 0.006 M PO_4 that could be observed with aldosterone even at 5×10^{-9} M (Figure 1a). The elution profile convincingly demonstrates that the peak in the 0.001 M prewash is not an artifact of the washing procedure (compare Figure 1a and b), as suggested by others, using only one concentration of the steroid[6]. Indeed it is evident that both gluco- and mineralo- corticoid specific receptors elute in this prewash region with great abundance in a dose-dependant manner.

Lastly, liver glucocorticoid receptors (peaks I and II in Figure 2c) appeared to be saturated with 10^{-7} M corticosterone and eluted in the same position (0.001 M and 0.02 M PO_4) as in the kidney, followed by a transcortin peak (III) at 0.06 M PO_4. No radioactivity could be found in the 0.006 M PO_4 region in the liver (compare Figures 1a and 2c), where the mineralo-corticoid specific receptors elute in the kidney, and this was true even when 10^{-6} M aldosterone was employed to equilibrate the liver cytosol (data not shown).

The results described here confirm and extend our previous observations that corticoid specificity (gluco- vs mineralo-) indeed resides in recognition of the signal by physicochemically distinct macromolecules present in the appropriate target organ(s). It is amply evident that they are resolved into well-defined heterogeneous polymor-

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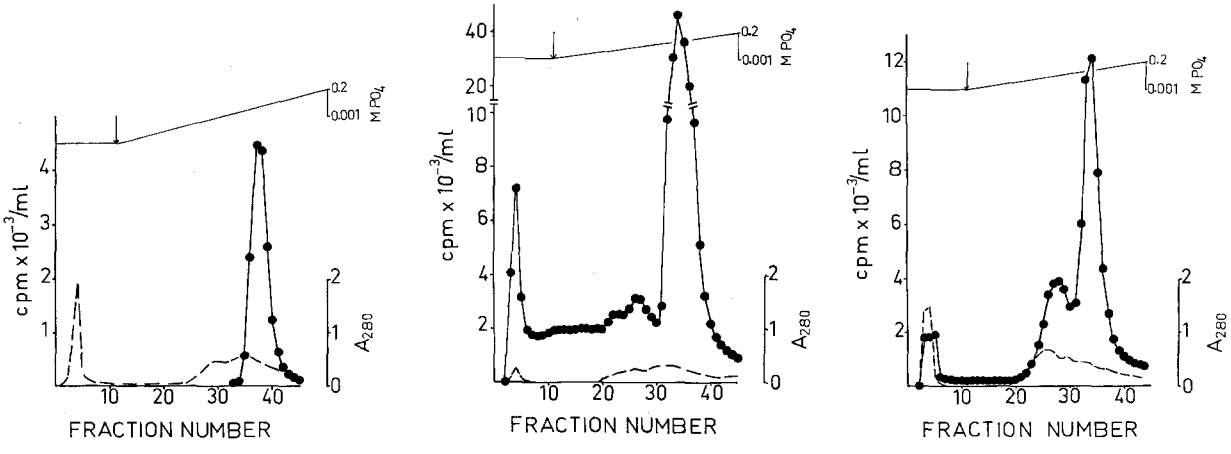

Fig. 2. Dose dependant separation of corticosterone binders in rat liver and kidney.
All details as in Material and method except that corticosterone was used in place of aldosterone at all those concentrations previously stated; only 3 experiments are shown here.

phic subpopulations if appropriate assay procedures are employed[3,4]. The single most important contribution of these findings is the observation that chromatography must be performed with various concentrations of the steroid vs the receptor to avoid negative or even erroneous conclusions such as have already appeared in other reports[6]. It is to be hoped that these considerations may lead to better understanding of the mechanism regulating steroid-target organ specificity. The most frustrating aspect, however, remains the technical limitations that have hitherto prevented unequivocal demonstration of a direct relationship between receptor activity and physiological action of the hormone in question.

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PRO EXPERIMENTIS

A Preparation for the Study of Muscle Metabolism During Rest and Activity

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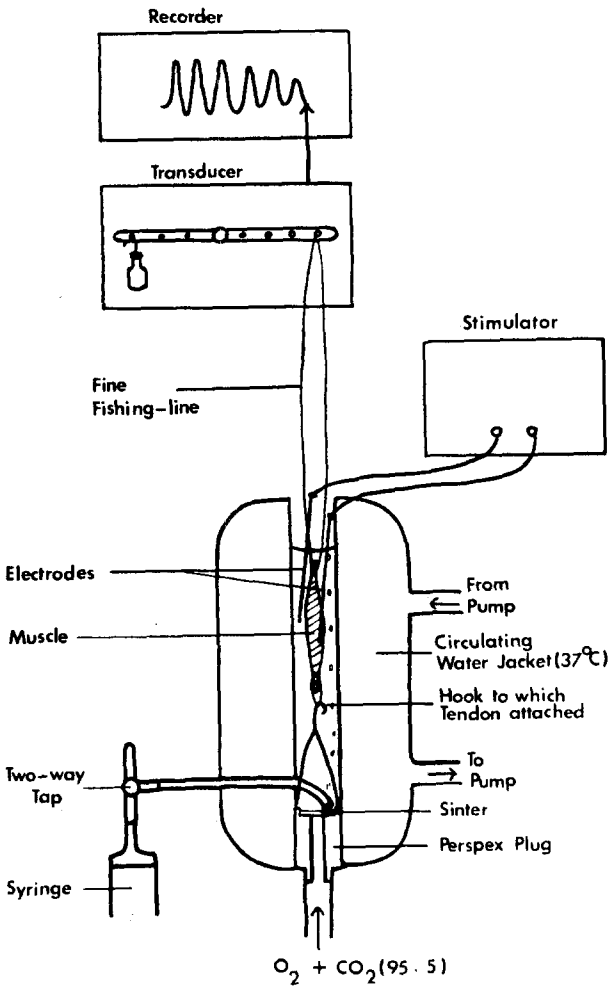
Summary. A method for the investigation of metabolism in the isolated rat extensor digitorum longus muscle has been described. This muscle was shown to be suitable for investigations of skeletal muscle metabolism during rest and activity.

In mammals skeletal muscle makes up about 40% of the body weight. Yet investigations into the biochemical properties of the muscle at rest and during exercise are comparatively rare. It has often been considered that whole muscles incubated in physiological solutions are not sufficiently thin to rely on diffusion of substrates and oxygen from the medicine. It is also difficult to catheterize the vessels of small laboratory animals if perfusion is considered as an alternative. The rat diaphragm, which is widely used for insulin assay[2], is clearly not representative of the bulk of skeletal muscle[3].



Apparatus to study isolated skeletal muscle metabolism.

The present study was undertaken to explore the suitability of the isolated rat extensor digitorum longus (EDL) muscle for studies of muscle metabolism during rest and activity. This muscle has many features which make it particularly suitable for in vitro experimentation: it is thin and cylindrical therefore allowing easy diffusion, the fibres are longitudinally arranged and there are long well-defined tendons at each end which can be ligatured without interfering with any of the muscle tissue, and as each animal yields 2 muscles a paired control technique can be used.

Materials and methods. Male Wistar rats weighing 240–260 g were killed by cervical fracture, and with minimal handling the EDL muscle was quickly removed and weighed. A small cotton loop was fixed to the distal tendon and a 10 cm length of fishing line (breaking strength 0.5 kg) fixed to the proximal tendon. Use of fishing line avoided the shrinkage found when cotton was placed in the perfusate. The muscle was transferred to the incubation chamber (Figure) containing 4 ml Krebs bicarbonate buffer[4]. A one-gram load was attached on the recording arm as a counterbalance and the resting muscle was adjusted so that it was slightly stretched by the load. In order to study muscle metabolism during rest, activity and post-activity periods the following incubation procedure was employed: 15 min preincubation, 30 min rest, 30 min stimulation and 30 min post-stimulation. After each period the medium was withdrawn and fresh medium added. In experiments involving measurement of intramuscular metabolites, 2 muscles were incubated in separate chambers and the metabolite concentrations could be determined in one muscle at the beginning of the period, and in the other at the end of the period.

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