Distribution of contractile proteins and adrenergic nerves in the adrenal gland of guinea-pig, rat and ox as revealed by immunofluorescence and the glyoxylic acid technique[1]

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Summary. Myosin and actin were localized in the adrenal gland, using antibodies against these proteins which were isolated from chicken gizzard. Myosin and actin were preferentially located in vascular walls including endothelial cells and in the capsule. In rat and guinea-pig adrenal cortex, the amount of contractile elements in vascular walls corresponded well to the density of adrenergic nerves as revealed with the glyoxylic acid method.

Immunofluorescence studies have contributed considerably to detecting small amounts of actin and myosin in nonmuscle and smooth muscle-like cells. By using antibodies against highly purified actin and myosin from chicken gizzard[2,3], these contractile proteins have been located in the guinea-pig testis[4], in astrocytes of rat diencephalon[5], in the rat and cat ovary[6,7], in rat cornea, lens epithelium and in the retina[8], and in rat and human liver cells[9].

The present study was prompted by 2 categories of findings. 1. Recent biochemical studies have shown the presence of an actomyosin-like protein in the adrenal medulla[10–12]. Douglas[13] has pointed to analogies of stimulus-secretion coupling in adrenal medullary cells and excitation-contraction coupling in muscle and it has been suggested that the secretory process in chromaffin cells involves contractile elements[10]. An immunofluorescence study could detect the sites of actomyosin in the adrenal medulla, and establish whether or not the chromaffin cell possesses the prerequisites of an intracellular contractile system using actin and myosin.

2. Catecholamine histochemical and ultramorphological investigations in our laboratory have revealed considerable amounts of adrenergic nerves in the glomerulosa and reticularis zone of the guinea-pig adrenal cortex[14]. As judged from their localization, these nerves could affect either steroid-producing cells or vascular walls. The presence of actomyosin in the walls of non-arteriolar vessels would indicate the possibility for functional neuroeffector relations not only with steroidogenic cells, but also with pericytes and endothelial cells.

For immunohistochemistry, adrenal glands were quickly removed from 5 adult male guinea-pigs and 8 rats under ether anesthesia and frozen in liquid nitrogen or in a cryostat at −30 °C. 6 bovine glands were obtained from a local slaughterhouse and slices 2–4 mm thick were frozen in liquid nitrogen; 4–5 µm frozen sections were cut on a Dittes-Duspiva cryostat, air-dried for 1–2 h and incubated for 30 min in a moist chamber at room temperature with specific, γ-globulin-enriched rabbit antibodies and control sera as follows: a) antiserum (1 mg/ml) against purified myosin from chicken gizzard, b) antiserum (1–2 mg/ml) against purified actin from chicken gizzard, c) antigen-adsorbed antibody against chicken gizzard myosin and d) non-immune rabbit γ-globulin. After washing in PBS, the sections were incubated for 30 min with fluorescein-labelled goat antirabbit immunoglobulin (FITC). A few sections were incubated with the second antibody alone. After another wash and mounting in glycerol: 0.1 M glycine buffer, pH 8.6 (7:3), the sections were examined in a Zeiss fluorescence microscope fitted withe epi-illumination.

Fluorescence histochemistry of adrenergic nerves was performed on 15 µm thick cryostat sections adjacent to those incubated with the myosin antibody. The glyoxylic acid technique was used in the modification of de la Torre and Surgeon[15].

Electron microscopy was carried out on rat and guinea-pig adrenal glands, which had been fixed by perfusion with phosphate-buffered 3.5% glutaraldehyde and immersion in 2% aqueous OsO_4.

Low-power views of adrenal cortex and medulla revealed an intense myosin- and less intense actin-specific fluorescence mainly along blood vessels and in the capsule (figure 1). There were arteriolar vessels, which penetrated the cortex in an unbranched course (figure 1), and others which joined the cortical capillary network. Both types of arterioles emerged from capsular arterioles, which also gave rise to cortical capillaries. In guinea-pig (figure 1) and ox, there was a strong myosin-specific fluorescence bordering blood vessels in the glomerulosa and reticularis zone, which resulted from the fluorescence in both endothelial and perivascular cells. Capillaries in the fasciculata zone (figure 2) exhibited faint myosin-specific fluorescence within endothelial and occasional perivascular cells. Capillary contractile proteins in the rat adrenal cortex showed a rather uniform pattern, with only a few perivascular cells interposed between endothelium and endocrine parenchyma in the glomerulosa and reticularis zone.

Glyoxylic-acid treated section revealed a striking correspondence (figure 1 and 3) in the density of adrenergic nerves and contractile cells along capillaries in rat and guinea-pig adrenal cortex. In the latter, blood vessels of the glomerulosa and reticularis zone were well supplied by numerous beaded green fluorescent nerve fibres (figure 3). In the rat, the adrenergic nerve supply was

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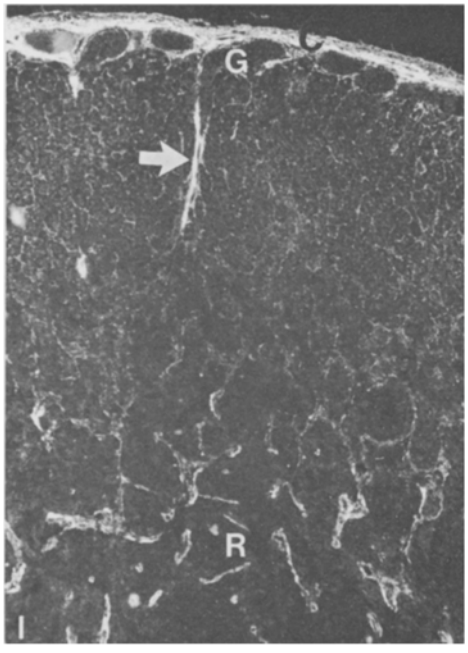
scarce and mostly confined to the arteriae corticis. No glyxylic-acid treatment was performed on bovine adrenal sections.

In the adrenal medulla of rat and guinea-pig, specific antibody staining was observed along veins and their tributaries both in the endothelium and in perivascular contractile cells. Actomyosin-containing cells also formed in thin sheath around groups of medullary cells (figure 4). Along vessels these cells were not arranged in a continuous layer; rather they appeared concentrated where capillaries or small veins joined larger ones, being arranged in a longitudinal or spiral fashion. No attemps were made to assess systematically the arrangement of contractile cells in the bovine medullary vascular tree.

As to the endocrine cells of adrenal cortex and medulla, incubation with antimyosin did not yield fluorescence beyond that in sections treated with the antigen-absorbed antibody or with FITC (figure 5). However, treatment with an actin antibody revealed the presence of actin in medullary cells in a thin-layer underneath the cell membrane. Ultrastructurally, actomyosin-containing cells, which surrounded groups of chromaffin cells, resembled both fibroblasts and smooth muscle cells. Golgi elements and the bulk of rough ER and free ribosomes were stored in the perinuclear area, whilst thin filaments (4–6 nm), plasmalemmal inpocketings (figure 7) and attachment sites (figure 6) were predominant in the cell periphery.

The present study demonstrates a distribution of myosin-containing elements preferentially along blood vessels of adrenal cortex and medulla. Our results show that even capillaries of the fasciculata zone have a myosin-specific fluorescence. There is increasing evidence that capillaries may contain actomyosin. Thus, Owman et al.[16] have localized myosin and actin in brain capillaries of rat and cat employing identical antibodies to those used in this study. It appears noteworthy that these very same antibodies will not stain fibroblasts and endothelial cells in primary cultures, and at present we have no explanation for this. Becker[17] has obtained positive results with endothelial cells from various sources using immuno-



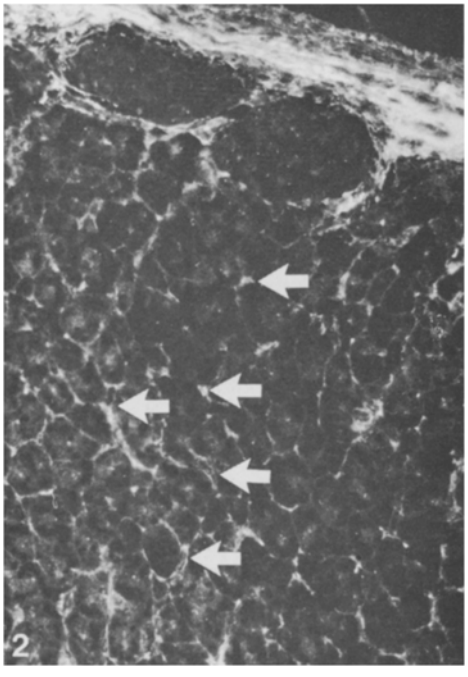


Fig. 1 and 2. Myosin-specific immunofluorescence in the guinea-pig adrenal cortex is predominant in the capsule (C) and along blood vessels in the glomerulosa (G) and reticularis (R) zones. Cortical artery (arrow, see also figure 3). Figure 2 clearly demonstrates that there is also a specific fluorescence associated with the walls of capillary vessels (arrows) in the fasciculata zone, which, however, is faint compared to that in the glomerulosa and reticularis zones.

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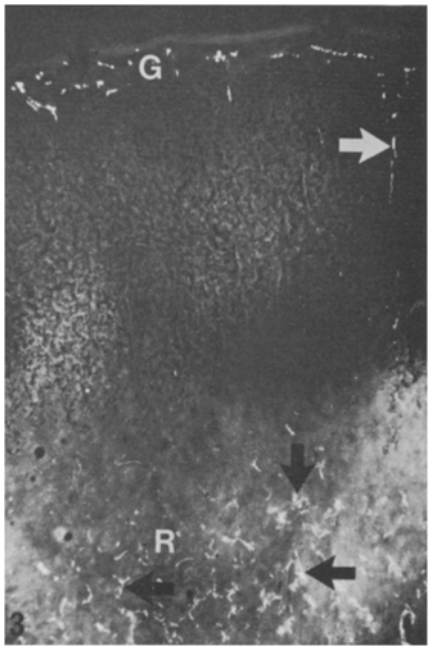

Fig. 3. Adrenergic nerve fibres in the guinea-pig adrenal cortex as revealed by the glyoxylic acid technique. Note that fluorescent nerves (black arrows) are concentrated in the glomerulosa (G) and reticularis (R) zones. Adrenergic nerves along a cortical artery are marked with a white arrow. Irregular light and dark patches are due to a technical artifact (low magnification combined with a dark field condersor). Figures 1 and 3 × 50. Figure 2 × 200.

fluorescence and immunoperoxidase techniques. In vivo observations of endothelial contraction in rat mesentery vessels[18] also support the concept of contractile properties of capillaries. Contractile properties may also be attributed to perivascular cells, which lack the complete set of typical features of smooth muscle as judget by electron microscopy. These cells were abundant along capillary vessels in the glomerulosa and reticularis zone of the guinea-pig adrenal, but not very prominent in rat adrenal cortex. In the adrenal medulla of the 3 species studied, these cells bordered sinusoid vessels in an irregular array and surrounded groups of chromaffin cells.

The presence of actin and myosin in the walls of all types of adrenal blood vessels suggests that a regulation of adrenal blood flow may occur not only at the main gates (cortical and medullary arterioles, medullary vein; see Coupland[19], review) but also at various levels in between. The prominent supply of adrenergic nerves to vessels in the reticularis zone of the guinea-pig suggests a mechanism controlled by intrinsic nerves, which allows variations in the amount of corticosteroid hormones feeding the medulla. This it particulary interesting in view of the relative importance of glucocorticoids for the methylation of noradrenaline[20]. Species differences as

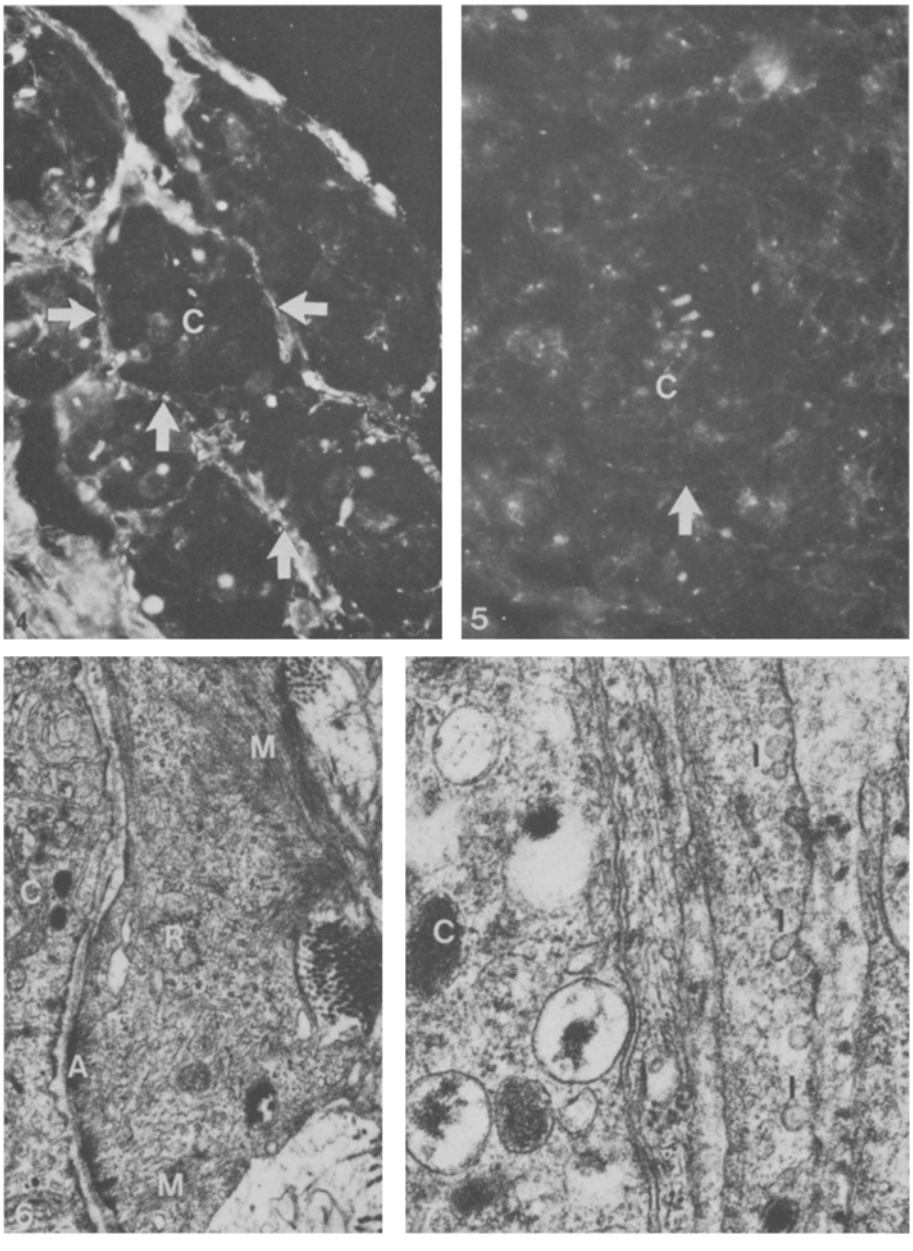

Fig. 4 and 5. Guinea-pig adrenal medulla incubated with antismooth muscle myosin (figure 4) and the same antibody previously adsorbed to the antigen (figure 5). Groups of chromaffin cells (C) are surrounded by cells (arrows), which display a myosin-specific immunofluorescence. A specific fluorescence is absent from chromaffin cells. ×510.

Fig. 6 and 7. Electron micrographs showing features of both smooth muscle and fibroblast in the cells, which form a peripheral layer around groups of chromaffin cells (C). These cells may exhibit plasmalemmal attachment sites (A), inpocketings (I) and microfilaments (M) as well as an abundance of free ribosomes (R). Figure 6 ×18,000, figure 7 ×54,000.

shown here to occur between rat and guinea-pig indicate that several mechanisms for regulating the medullary blood supply may exist. In fact, it has been argued that contraction of the medullary vein is an essential factor in determining the amount of corticosteroids reaching chromaffin cells.

In addition to a local regulation of blood flow, contractile mechanisms in the vascular wall, and especially in endothelial cells, could also serve to regulate vascular permeability by either widening intercellular gaps os or transendothelial channels[21]. In this investigation, actomyosin could not be detected in the endocrine cells of adrenal medulla or cortex. This does not exclude the presence of contractile proteins in these structures, but the amount may have been too low to be detected with the methods applied here. While this paper was in preparation Creutz[22] presented immunofluorescence pictures from bovine adrenal medulla, suggesting that chromaffin cells contain myosin. Since his approach to the problem of immunocytochemical localization of myosin was somewhat different from ours – he used myosin from bovine adrenals as an antigen – the results cannot be directly compared.

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Induction of developmental anomalies in mice by maternal stress[1]

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Summary. The short-time restraint of pregnant mice on day 8 of gestation led to a significant increase of the anomaly rate in fetuses. This effect may be due to stress factors of endocrine origin.

Teratological studies using ionizing radiation at low dose rates require relatively long exposure times for the pregnant animal. When only a part of the body is to be exposed, or the field size of the radiation beam (85% isodose) just covers the animal, the movements of the animal have to be prevented either by anesthesia or by restraining in a 'snug-fitting' cage.

Since anesthesia may be an effective agent in radioprotection[2], or some narcotic drugs can themselves be teratogenic (like Epontol, in publication) we used the restraining method to avoid chemical interactions. In our earlier experiments[3], using 200 kV X-rays at high dose rates, no harmful effects could be observed in sham-irradiated animals, which were restrained only for a short period. However, in recent work with negative pions at low dose rate, or with protracted 140 kV X-irradiation, the prolonged restraint represents a stress situation which is teratogenic in mice. This so-called 'cage effect' has been seen also in experiments using Lucanthone as a possible

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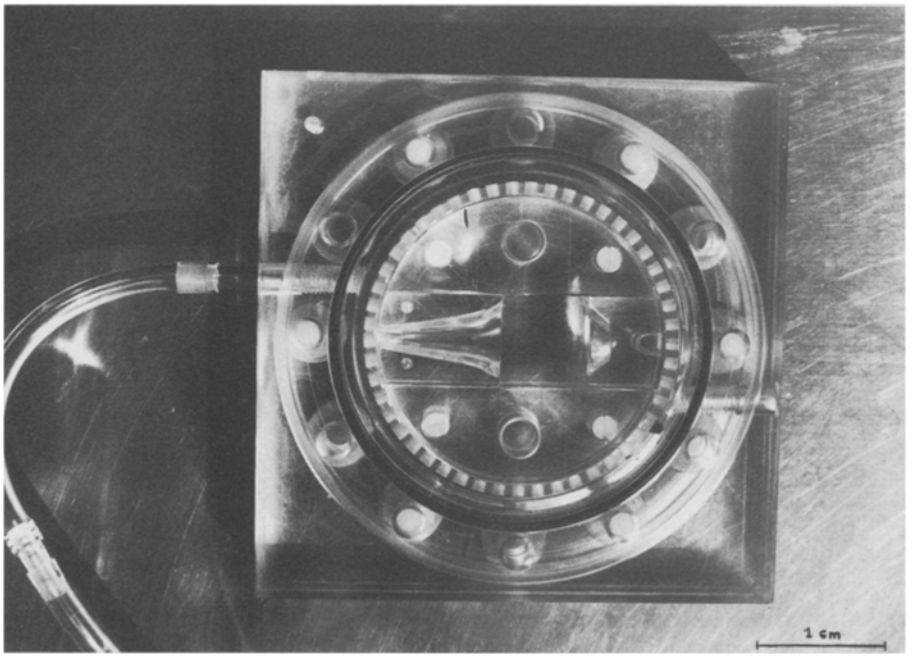

Plexiglas cage with air supply used in this study. The restraining chamber in the centre of the cage.