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Vascular patterns in the normal and pathological human adrenal cortex

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Abstract The vasculature of the adrenal gland has been studied by microinjection techniques in a variety of species. While there is general agreement about the overall patterns, some uncertainty still exists over the structure of medullary arteries and the connections between the sinusoids of the cortex and medulla. We have taken a new approach to these problems by applying immunohistochemical techniques to the human adrenal gland, identifying overall vascular patterns by endothelial expression of CD34 and muscular channels by smooth muscle actin. We have also examined adrenal nodules, adenomas and carcinomas to see whether these can be differentiated on the basis of their vascular patterns. The general pattern in the normal gland was similar to that found in injection studies, but there appeared to be more connections between sinusoids of the zona fasciculata than previously reported. There was direct continuity between cortical and medullary sinusoids. Medullary arteries were demonstrated as thin-walled vessels. Immunopositivity for smooth muscle actin was present in sinusoids, apparently in endothelial cells, suggesting that they may express this protein and thus have a contractile function. Macronodules and adenomas could not be reliably distinguished, both showing a rich network of sinusoidal vessels. Carcinomas showed marked disorganization, with large-calibre vessels interspersed with irregular networks of vessels of very small calibre.

Key words Adrenal gland · Vascular supply · Immunohistochemistry · Adrenal neoplasms

Introduction

The vasculature of the adrenal gland has been studied in a number of species including man [3, 5, 7–9, 17]. There is general agreement that the arteries in the capsule divide to give rise to capsular capillaries, which are then in continuity with the sinusoids of the zonae glomerulosa, fasciculata and reticularis. The capsular arteries are also reported to branch into medullary arteries, which traverse the cortex before opening into capillaries in the medulla [8, 12]. The structure of medullary arteries has been poorly documented, and they have been described as both thick- [2] and thin-walled [15, 18, 24] vessels. There has also been some debate as to whether direct communications exist between the capillary plexuses of the cortex and medulla or whether the two circulations are separate [3, 8, 10, 12, 13]. Most of these studies have been performed using microinjection techniques, which have the potential disadvantage of inducing artefacts because of the pressures required to achieve perfusion.

It is now possible to visualize individual cell types in tissue sections using immunohistochemical staining of specific proteins. We have therefore revisited the human adrenal vasculature, attempting to elucidate some of these areas of debate by applying immunohistochemical techniques to outline vascular channels. We have identified endothelial cells by the expression of CD34 using monoclonal antibody QB-END/10 [19] to give an overall impression of all vascular channels. We have also used antibodies to smooth muscle actin [21] in an attempt to delineate capsular and medullary arteries, and venous channels. We applied these to standard thin sections, and also to thick sections in a technique we have previously used to study adrenal innervation [14]. This gives the opportunity of examining features of the vascular tree without the potential problems of injection techniques.

We have also applied the techniques to adrenocortical nodules, adenomas and carcinomas, to assess whether these might be differentiated on the basis of their vascular patterns. Nodule formation is not uncommon in the human adrenal cortex, being reported in up to 50% of unselected autopsy glands. They are more common with increasing age and in patients with evidence of vascular disease [4]. Their pathogenesis remains unclear, but there is evidence that they may be due to compensatory

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hyperplasia adjacent to an area of ischaemia [4, 20]. The histological differentiation of macronodules from non-functioning adenomas may be difficult, as may that of an adenoma from a small carcinoma with no obvious evidence of local spread or metastases at the time of presentation. Diagnosis is based at present on multifactorial histological analysis [11, 23, 24], but these systems do not always give absolute prediction. The need to establish alternative ways of distinguishing these lesions is increasing, because more nodules are being identified in vivo as a result of scanning for other intra-abdominal pathology, and an appropriate therapeutic approach depends on an accurate diagnosis.

Materials and methods

Normal adrenal glands (*n*=8) were obtained at autopsy from patients who had no clinical adrenal dysfunction and in whom histological examination showed no evidence of nodularity. Sections were examined from head and body where medulla is present, and from tail, where it is absent. Autopsy cases (*n*=5) were also used for the assessment of micronodules. Macronodules were examined from two glands with nodular hyperplasia associated with Cushing's syndrome and a further autopsy case. Adenomas (*n*=6) and carcinomas (*n*=6) were removed at surgery. These were classified both on the basis of clinical behaviour and by the histological criteria of Weiss et al. [25].

Immunostaining was performed on both thick (100 μm) and thin (4 μm) sections. Thick sections were stained using a free floating method in glass Universal containers, with an indirect immunoperoxidase technique, using QB-END/10 (Novocastra Laboratories) or asm-1 (anti-smooth muscle actin, Bradshaw Biologicals) as primary antibody. Sections were pretreated with 0.1% trypsin for 15 min and incubated with primary antibody for 48 h at 4°C. Peroxidase conjugated goat anti-mouse immunoglobulin (Dako) was used as secondary antibody, again incubated for 48 h at 4°C, and sites of binding visualized with Vectorstain chromogen (Vector Laboratories). Sections were dehydrated and were mounted on glass slides using HSR.

Thin (4 μm) sections were mounted on glass slides coated with aminopropyltriethoxysilane. These were immunostained for α smooth muscle actin using asm-1. Sections with primary antibody omitted were examined as negative controls and immunostaining of thin sections of tonsil was performed as a positive control for QB-END/10 and of small intestine, for smooth muscle actin.

Results

In the normal gland, the general vascular pattern corresponded to that already described for the adult gland. Branching arteries were seen on the capsule. In the head and body, a sinusoidal plexus branched around the zona glomerulosa (Fig. 1). Centripetal sinusoids, which appeared to branch and interconnect, ran through the zona fasciculata (Fig. 2). A more complex plexus was present in the zona reticularis. Medullary arteries traversed the cortex and in some cases showed a spiral pattern within the medulla (Fig. 1). A sinusoidal network was present in the medulla. Direct continuity was seen between sinusoids of the zona reticularis and those of the medulla (Fig. 3). Smaller venous branches were seen entering the central vein. The pattern in the cortical cuff mirrored that

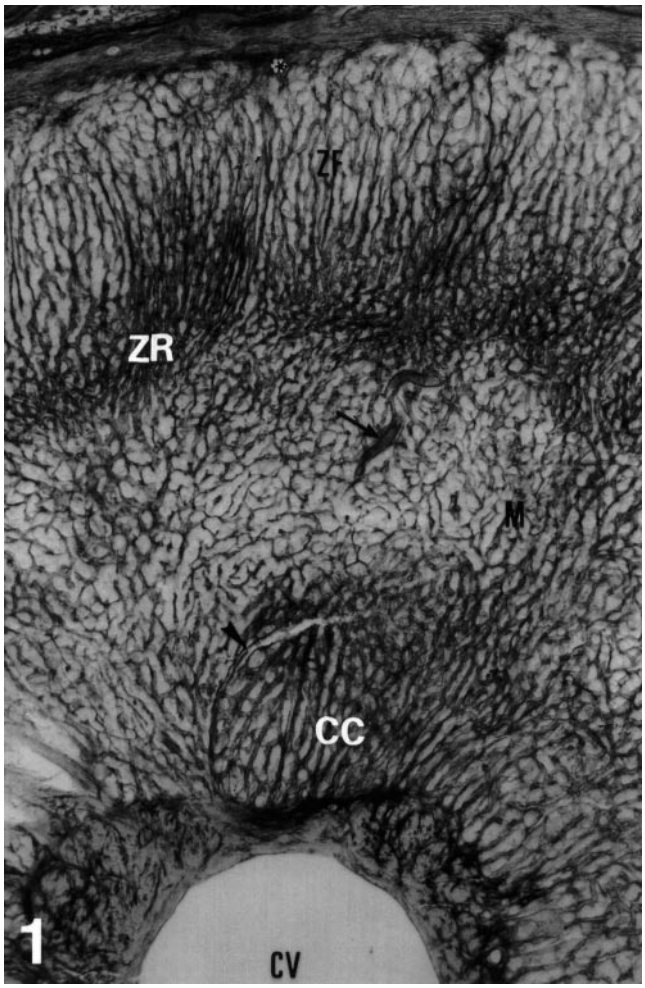

Fig. 1 Cross section, 100 μm thick, of the head of the normal adrenal gland immunostained for CD34 to demonstrate endothelial cells. Branching sinusoids are seen in the zona glomerulosa (*), zona fasciculata (*ZF*), zona reticularis (*ZR*), medulla (*M*), and in the cortical cuff (*CC*) surrounding the central vein (*CV*). A medullary artery (*arrow*) and venule (*arrowhead*) in the cortical cuff are also demonstrated

of the cortex. In the alae and tail the cortical pattern was similar to that in the head, but vessels ran along the raphe to join venous channels. In thick sections, immunostaining for smooth muscle actin was positive not only in capsular and medullary (Fig. 4) arteries and in central vein, but also extended through the zona fasciculata in the pattern of the sinusoids (Fig. 4). Staining of thin sections showed immunopositivity related to sinusoidal outlines within the cortex (Fig. 5a) demonstrated at higher power in Fig. 5b.

The overall pattern in micronodules was similar to that in normal cortex, but the sinusoids were subjectively further apart (Fig. 6). Between nodules, there appeared to be compression of vascular sinusoids, with an impression of loss of cells, although the number of vessels did not appear to be reduced

Macronodules showed marked vascularity, with thin-walled vascular channels appearing to surround small

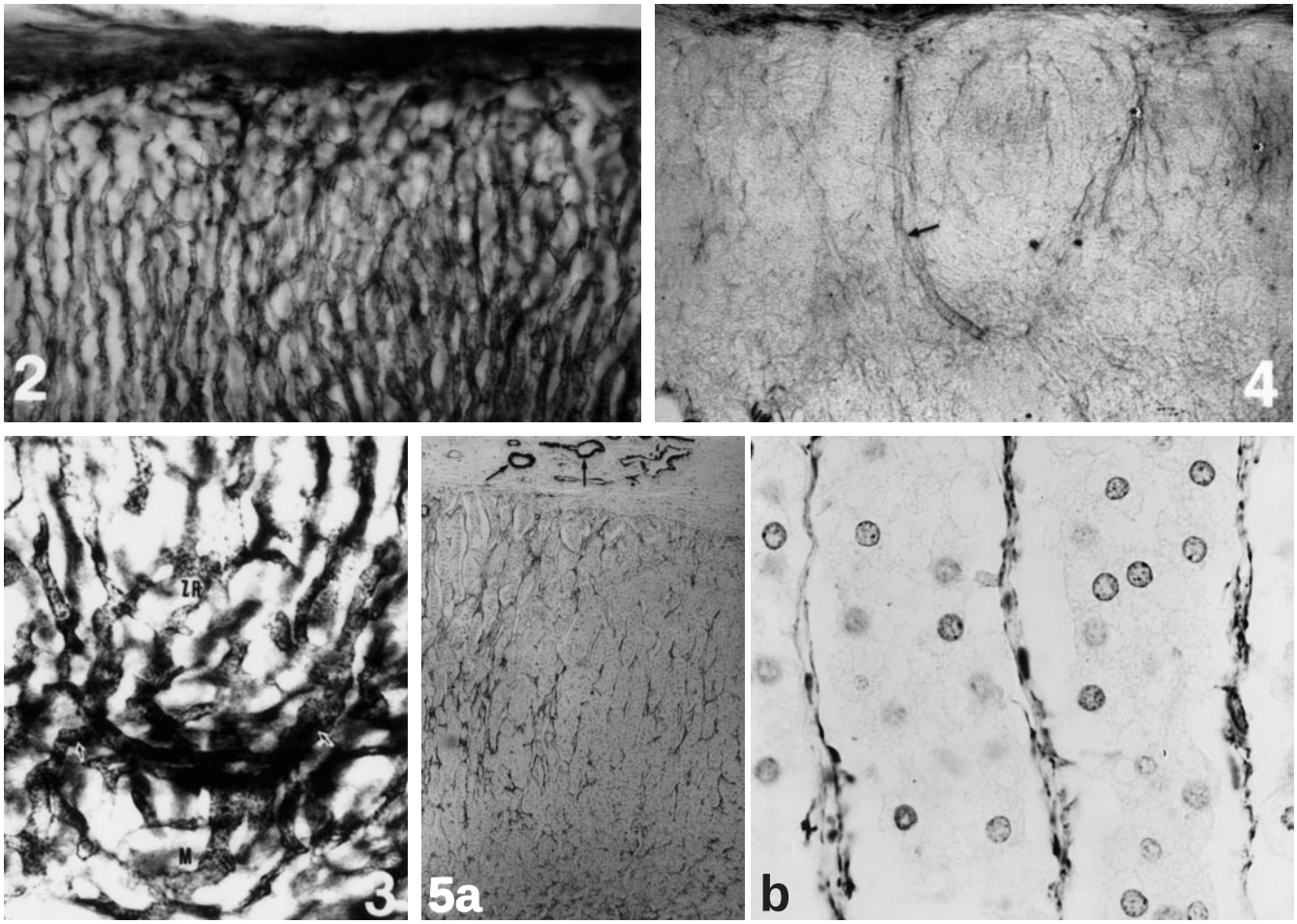

Fig. 2 Normal adrenal cortex (CD34) showing complex branching of the sinusoids of the zona fasciculata

Fig. 3 Sinusoids are seen crossing the zona reticularis (*ZR*) to the medulla (*M*) (*arrows*). CD34 immunostaining

Fig. 4 Normal adrenal (100 μm thick) immunostained for smooth muscle actin. Positivity is seen in a thin-walled medium-calibre vessel traversing the cortex (*arrow*), consistent with a medullary artery. There is other, less distinct, immunopositivity (*asterisks*)

Fig. 5 a Normal adrenal (4 μm thick) showing positivity for smooth muscle actin in capsular arterioles (*arrows*) and widespread positivity related to sinusoidal endothelial cells. **b** High-power view showing variable positivity for smooth muscle actin in relation to sinusoidal walls

groups or individual cells (Fig. 7). In places there was an appearance of multiple nodule formation. In some areas appearances similar to micronodules were seen, with compression of adjacent vessels (Fig. 8). Immunopositivity for smooth muscle actin was present in vessel walls

The patterns in adenomas were similar to those in macronodules (Fig. 8). Focally, branching larger calibre vessels could be seen. In some areas there seemed to be continuity with vessels of the adjacent cortex, whereas in others the two seemed to run parallel to each other around the junction. Immunostaining for smooth muscle actin was also seen in relation to sinusoidal walls.

Carcinomas showed extremely variable patterns. Some showed mainly large-calibre vessels, while others also showed networks of disorganized, very-small-calibre vessels (Fig. 9). Few vessels were seen in relation to areas of necrosis.

Discussion

The expression of CD34 has proved to be a useful marker for endothelial cells [19] and superior to factor VIII-related antigen, which may not identify small-calibre vessels [16]. We confirmed this in the present study, where little staining of sinusoids was seen with factor VIII-related antigen (data not shown). We were able to demonstrate all types and calibres of vessel with CD34 immunostaining, in both thin and thick sections. Examination of the latter gave some appreciation of the three-dimensional arrangement of these vessels.

Our results were generally in agreement with previous studies on the overall organization of the vasculature of the human adult adrenal. However, the degree of branching and interconnection of sinusoids within the zona fasciculata was more marked than previously reported [5, 20]. This could reflect alterations produced by the injection techniques in those studies, which may have damaged interconnecting branches. Staining with smooth

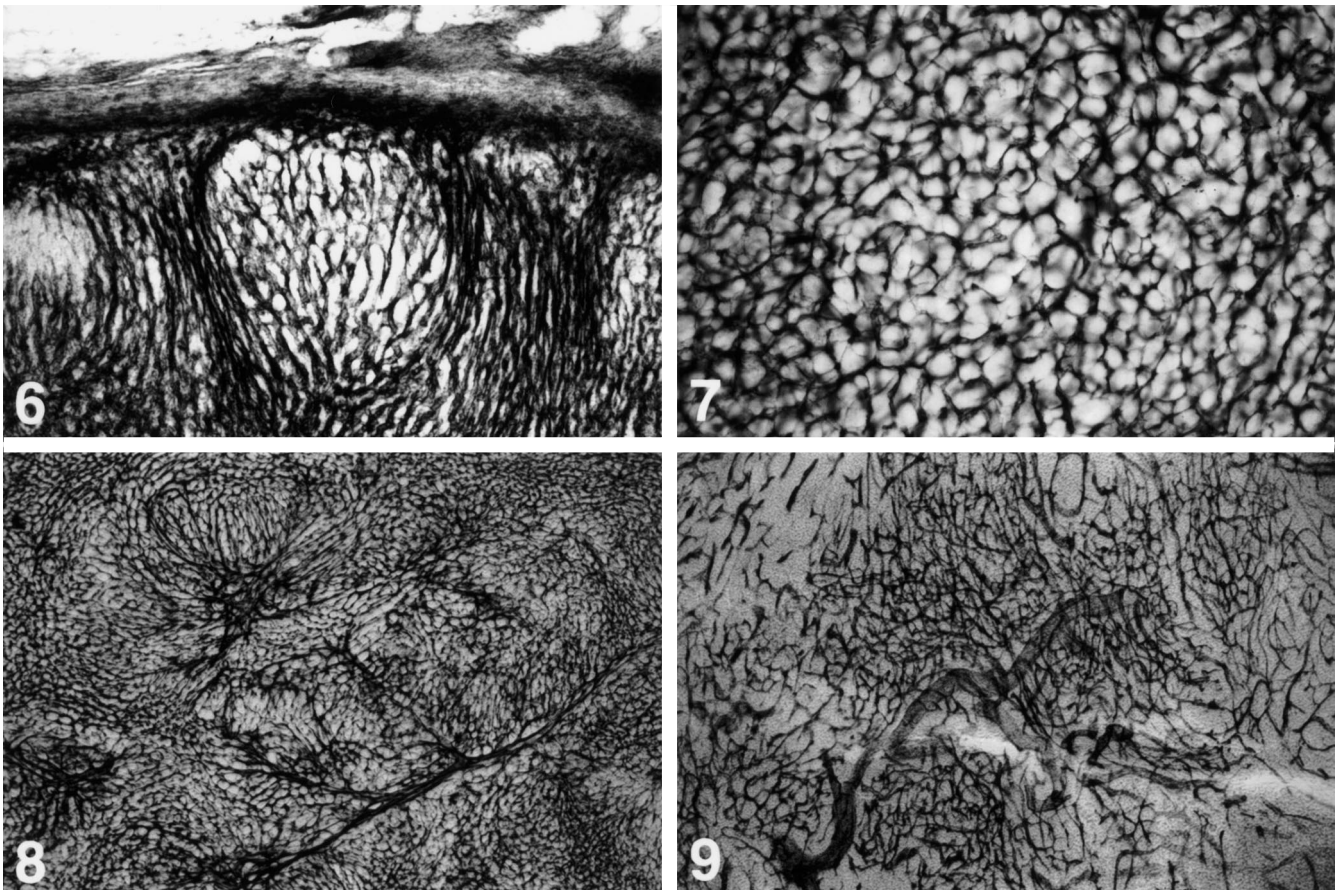

Fig. 6 Adrenal micronodule (CD34) showing vascular pattern similar to normal, but with increased distance between the sinusoids. There is compression of adjacent sinusoids, with apparent loss of cortical cells

Fig. 7 Adrenal macronodule (CD34) showing complex vascular arrangement in which most cells appear to be in contact with sinusoids

Fig. 8 The vascular appearances in adenomas were similar to those in macronodules. Some larger calibre vessels are also seen running through the tumour (CD34)

Fig. 9 Adrenal carcinoma (CD34) showing a mixture of large calibre vessels and a disorganised network of small-calibre vessels

muscle actin demonstrated the branching of capsular arteries and arterioles. We did not demonstrate arteries looping back to the capsule, but this may be a problem of sampling. We confirmed the presence of medullary arteries, and also the spiral twisting within the medulla reported by some [20]. The pattern of staining suggested that these are fairly thin-walled vessels, more in keeping with the findings of Pauly [18] and Merklin [15] than those of Bennet [1] and Bennet and Kilham [2], who reported vessels with a pronounced muscular wall. These findings concur with those of Vinson et al. [24] in the rat adrenal, indicating that the structure of the wall of the medullary arteries is not consistent with these vessels having significant control of blood flow. An unexpected finding was the immunopositivity of sinusoidal walls with the smooth muscle actin antibody, which appeared to be related to endothelial cells. There have been no reports of pericytes or other perisinusoidal cells that might express smooth muscle actin within the adrenal gland. This raises the possibility that sinusoidal endothelial cells might have some contractile role and exercise local regulation of tone, although it is unlikely that major regulation of blood flow could be achieved in this way. Features consistent with myoid differentiation have also been demonstrated in endothelial cells lining high endothelium venules in the spleen [22]. It would be of interest to investigate this further when other antibodies reactive with smooth muscle actin in paraffin sections become available, or by in situ hybridization for specific messenger RNA.

Our findings support the idea of a dual blood supply for the medulla, in that in addition to the supply from medullary arteries, direct connections were also demonstrated between cortical and medullary sinusoids. This permits direct exposure of phaeochromocytes to steroids. In keeping with the majority of reports [3, 12, 24], we did not see evidence of a portal system.

Changes in vascular patterns were demonstrated in adrenal nodules and tumours. In micronodules (i.e. nodules visible only on microscopic examination) there was some evidence of increased distance between sinusoids, but the overall arrangement of these appeared similar to that in normal cortex. In agreement with Dobbie [4] and Sasano et al. [20], we demonstrated crowding of sinu-

soids adjacent to nodules consistent with atrophy of cortical cells. The numbers of sinusoids did not, however, appear to be reduced. We were unable to examine the relationship between these atrophic areas and capsular arteriopathy with the material available.

Macronodules (visible to the naked eye) and adenomas showed similar disorganization of vascular patterns, with a dense, mainly sinusoidal, pattern in keeping with the abundant blood supply proposed by Sasano et al. [20]. In places, this pattern suggested that these lesions expand by continued nodular proliferation and expansion. Some have suggested that macronodules arise as a result of coalescence of micronodules. An alternative is the expansion of clones within the nodule. The larger branching vessels we identified may correspond to those seen on angiography by Ekström et al. [6]. In contrast to Dobbie [4], we found connections between the sinusoidal vessels of the adjacent cortex and those of the nodule/adenoma, albeit only in limited areas. The high degree of vascularity of these lesions and the continuity of vascular supply with that of the normal gland suggest that their failure to respond to normal stimuli is related to defects in cellular signalling pathways rather than to lack of exposure to adrenocorticotrophic hormone or other humoral factors. Again, the expression of smooth muscle actin in sinusoidal endothelial cells may play some part in regulating blood supply, but this requires further investigation. On the basis of our observations, it appears that adrenal macronodules and adenomas cannot be distinguished by their vascular patterns.

The distinction of carcinoma from other nodular lesions is of the greatest importance from a therapeutic viewpoint. In a study based on angiography, Ekström et al. [6] reported that in carcinoma the vessels varied in density throughout the tumour. Our findings of a mixture of large-calibre vessels and foci of very dense small-calibre vessels are similar to those illustrated in the angiograms of that study. Such changes were seen even in a small (28 g) tumour defined as potentially malignant by the histological criteria of Weiss et al. [25] and proven so on clinical follow-up. These changes in vascular patterns most probably reflect the secretion, by malignant tumours, of angiogenic factors that differ from those regulating the normal gland. The presence of abnormal vascular patterns might therefore provide additional information that would be useful in the distinction between benign and malignant adrenocortical tumours.

References

1. Bennett HS (1940) The life history and the secretory cycle of the cells of the adrenal cortex of the cat. Am J Anat 67:151–227
2. Bennett HS, Kilham L (1940) The blood vessels of the adrenal gland of the cat. Anat Rec 7:447–471
3. Coupland RE, Selby JE (1976) The blood supply of the adrenal medulla : a comparative study. J Anat 122:539–551
4. Dobbie JW (1969) Adrenocortical nodular hyperplasia : the ageing adrenal. J Pathol (Lond) 99:1–18
5. Dobbie JW, Symington T (1966) The human adrenal gland with special reference to the vasculature. J Endocrinol 34:479–489
6. Ekström T, Iremack B, Lagergren C (1968) The vasculature of the adrenal gland in neoplasia and hyperplasia. Virchows Arch [A] 343:189–196
7. Flint JM (1900) The blood vessels, angiogenesis, organogenesis, reticulum and histology of the adrenal. Johns Hopkins Hosp Rep 9:153–229
8. Gersh I, Grollman A (1941) The vascular pattern of the adrenal gland of the mouse and rat and its physiological response to changes in glandular activity. Contrib Embryol Carnegie Inst 29:112–125
9. Harrison RG (1951) A comparative study of the vascularisation of the adrenal gland in the rabbit, rat and cat. J Anat 85:12–23
10. Harrison RG, Hoey MJ (1960) The adrenal circulation. Blackwell, Oxford
11. Hough AJ, Hollifield JW, Page DL, Hartmann WH (1979) Prognostic factors in adrenal cortical tumors. Am J Clin Pathol 72:390–399
12. Kikuta A, Murakami T (1982) Microcirculation of the rat adrenal gland : a scanning electron microscope study of vascular casts. Am J Anat 164:19–28
13. Lazorthes G, Gaubert J, Poulhes J, Roulleau J, Martinez-Cobo C (1959) Note sur la vascularisation de la surrenale. C R Assoc d'Anat 45:489
14. McNicol AM, Richmond J, Charlton BG (1994) A study of general innervation of the human adrenal cortex using PGP9.5 immunohistochemistry. Acta Anat 151:120–123
15. Merklin RJ (1962) The arterial supply of the suprarenal gland. Anat Rec 144:359–372
16. Miettinen M (1983) *Ulex europeaeus* I lectin as a marker for tumors derived from endothelial cells. Am J Clin Pathol 79:32–36
17. Neville AM, O'Hare MJ (1982) The human adrenal cortex. Springer, Berlin Heidelberg New York
18. Pauly E (1957) Morphological observations on the adrenal cortex of the laboratory rat. Endocrinology 60:247–264
19. Ramani P, Bradley NJ, Fletcher CDM (1990) QBEND 10, a new monoclonal antibody to endothelium : assessment of its diagnostic utility in paraffin sections. Histopathology 17:237–242
20. Sasano N, Takezawa Y, Sato H, Horikawa N (1971) Micro-angiography of normal and pathologic human adrenals in prenatal and aging course. Tohoku J Exp Med 104:129–141
21. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G (1986) A monoclonal antibody against alpha smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 103:2787–2796
22. Toccanier-Pelte MF, Skalli O, Kapanci Y, Gabbiani G (1987) Characterization of stromal cells with myoid features in lymph nodes and spleen in normal and pathologic conditions. Am J Pathol 129:109–118
23. Van Slooten H, Schaberg A, Smeenk D, Moolenar AJ (1985) Morphologic characteristics of benign and malignant adrenocortical tumors. Cancer 55:766–773
24. Vinson GP, Pudney JA, Whitehouse BJ (1985) The mammalian adrenal circulation and the relationship between adrenal blood flow and steroidogenesis. J Endocrinol 105:285–294
25. Weiss LM, Medeiros LJ, Vickery AL Jr (1989) Pathologic features of prognostic significance in adrenocortical carcinoma. Am J Surg Pathol 13:202–206