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The innervation of the adrenal gland. IV. Innervation of the rat adrenal medulla from birth to old age. A descriptive and quantitative morphometric and biochemical study of the innervation of chromaffin cells and adrenal medullary neurons in Wistar rats*

A. TOMLINSON AND R. E. COUPLAND

Department of Human Morphology, Queen's Medical Centre, University of Nottingham, Clifton Boulevard, Nottingham NG7 2UH

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INTRODUCTION

The preganglionic sympathetic nerve fibres of the splanchnic nerves have been implicated in the control of adrenal medullary catecholamine secretion since the late nineteenth and early twentieth centuries (Dreyer, 1899; Elliott, 1912, 1913; Feldberg, Minz & Tsudzimura, 1934). Subsequent work (see Coupland, 1965*a* for review; Schramm, Adair, Stribling & Gray, 1975) has favoured an ipsilateral innervation from neurons whose somata lie in the intermediolateral grey horn of the thoracic spinal cord with the majority of fibres arising from cells situated in the lower thoracic region.

Recently Kesse, Parker & Coupland (1988) using Fast Blue injected into the adrenal medulla, to label retrogradely the neurons, have demonstrated that in the adult rat the perikarya of preganglionic sympathetic neurons innervating the adrenal gland lie in spinal segments T1 and L1 with over 20% of preganglionic cell bodies being situated in segment T9 and over 60% in segments T7–T10. A similar distribution of retrogradely labelled neurons was observed in neonatal and young rats (Parker, Kesse, Tomlinson & Coupland, 1988) and once again the origin was strictly ipsilateral.

Electron microscopic studies on the rat (Coupland, 1965*a*, *b*) indicated that the majority of nerve fibres in the adrenal medulla are non-myelinated and that nerve endings are, in appearance, typical of cholinergic-type synaptic endings. They are usually observed on the normal surface of a chromaffin cell but are occasionally associated with invaginations of the plasmalemma.

Lewis & Shute (1969) reported that noradrenaline-storing (NA) cells had a denser innervation than adrenaline-storing (A) cells in the rat, based on the appearance of nerve fibres after staining for acetylcholinesterase, but presented no quantitative data to support this suggestion. Grynspan-Winograd (1974) described conformational differences in nerve endings on A and NA cells in the hamster.

Morphologically mature synapses were observed in the fetal rat adrenal medulla at E15·5 by Daikoku, Kinutani & Sako (1977) and at E20 by Ratzenhöfer & Müller (1967). Dalnock & Menssen (1986) reported a threefold increase in the innervation of the rat adrenal medulla during the first postnatal week.

During fetal life, extra-adrenal chromaffin tissue develops precociously (Coupland, 1965*a*) and is able to make a significant contribution to catecholamine secretion

* Reprint requests to Professor R. E. Coupland.

during fetal and neonatal life (Brundin, 1966; Hervonen & Körkala, 1972). The adrenal medulla is, however, relatively slow to achieve functional innervation. In the rat, splanchnic control of adrenomedullary function is absent at birth, appearing during the first week and becoming fully mature by 10 days after birth (Slotkin, Smith, Lau & Bareis, 1980; Slotkin, Chantry & Bartolome, 1982). According to Slotkin (1986) the unresponsiveness of adrenal chromaffin cells in the rat to neurogenic stimuli during the first postnatal week is probably due to deficiencies in neural connections with the target cells, as evidenced by the relatively few nerve terminals and the low levels of choline acetyltransferase activity at birth.

At the opposite end of the age spectrum it has been reported (Ito, Sato, Sato & Suzuki, 1986) that unitary adrenal sympathetic nerve activity increases in aged rats and is associated with an increase in adrenal catecholamine secretion.

The present work provides a descriptive and quantitative account of the innervation of rat adrenal chromaffin cells from birth to the age of 22 months with special reference to the appearance, size and number of synaptic endings on chromaffin cells; the morphological and morphometric data are correlated with the choline acetyl transferase (ChAT) and catecholamine content of the glands. ChAT activity provides a biochemical measure of the cholinergic innervation of the adrenal gland.

MATERIALS AND METHODS

Wistar rats, bred at the Joint Animal Breeding Unit, Sutton Bonnington, aged 0, 1, 2, 4, 8 and 26 days, 12, 17 and 22 weeks and 22 months were used. Five to 9 animals were used in each age group. They were housed under 12 hours light, 12 hours darkness at 21 °C and had access to Pilsbury 41B modified rat pellets (Heygates Ltd, Northampton) and water *ad libitum*.

Morphology and morphometry

The animals were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.; May & Baker Ltd, Dagenham, Essex). After thoracotomy, they were fixed by perfusion through the left ventricle (the right atrium having been previously opened) by (i) a pre-wash of veronal acetate-buffered (pH 7·4) physiological salt solution containing 0·4 mM dextran (mol wt 70000) and 4 mM procaine hydrochloride for 30 seconds in neonates and 1 minute in older animals, at a pressure of 100 mmHg and 37 °C; (ii) 2% glutaraldehyde (Taab Laboratories, Reading, UK) in 0·11 M cacodylate buffer (pH 7·2, 550 mosmol) at room temperature. The fixative was perfused at 120 mmHg for 5 minutes and then at 100 mmHg for a further 10 minutes.

The adrenal glands were then removed from the animals and cut with a razor blade on random axes into parallel slices approximately one millimetre thick, immersed in the above fixative for a further 3 hours at room temperature prior to washing in 0·1 M cacodylate buffer containing 3% sucrose and postfixing in Millonig's buffered 1% osmium tetroxide for one hour. They were dehydrated in ethanol and embedded in Araldite epoxyresin. Sections 1 μm or 70–80 nm thick were cut on a Reichert 0MU3 ultramicrotome. The thin sections were mounted on 200 mesh copper grids, stained with lead citrate and examined with a Philips 410 electron microscope.

Stereology

Numerical data relating to volumes, areas, lengths and numbers of medullary components were collected using systematic methods including the counting of specific profiles in randomly selected electron micrographs and fields and superimposition of

arrays of lines or points. As in a previous publication (Tomlinson, Durbin & Coupland, 1987), methods used included those of Weibel (1979) and Chalkley (1943), having regard for optimal sample design (Gundersen & Østerby, 1981) and sample size (Hally, 1964; Williams, 1977). Mean nuclear diameter was calculated and corrected using the Abercrombie (1946) formula and mean cell volume determined by the method of Williams (1977).

Quantitation of adrenal medullary and cell contents was performed on electron micrographs of randomly selected slices printed at final magnifications of $\times 4200$ and $\times 42000$ and measurements of synaptic contacts on chromaffin cells were made on prints at $\times 15000$–30000 as well as directly in the electron microscope.

Adrenal medullary volume was determined by planimetry using a Kontron MOPAMO3 analyser (Carl Leiss, Oberkochem, FRG) and point counting, adrenal weight and tissue density.

Estimation of synaptic profile density on chromaffin cells

Three randomly selected areas of adrenal medulla were examined from each specimen for synaptic contacts on chromaffin cells – the criteria of a synaptic contact being the presence of pre- and post-synaptic membranous thickenings together with the presence of clear and, in many instances, dense-cored vesicles in the pre-synaptic boutons. No nerve endings with exclusively dense-cored vesicles were observed. The area of the medulla examined was measured and volume density of chromaffin tissue determined by point counting. The number of nuclei of chromaffin cells was counted as a measure of the number of cells present. Synaptic profile density was converted to synaptic profiles per mean cell profile and to mean number of synaptic profiles per cell using the methods employed by Case & Matthews (1985), mean synaptic bouton diameter being calculated from the formula $d = \sqrt{ab}$ (Williams, 1977) where d = mean diameter of synaptic bouton, a = major axis of bouton profile, b = minor axis of bouton profile.

Biochemical determinations

Assay of choline acetyltransferase activity

Adrenal glands were rapidly excised from anaesthetised rats and dissected free of fat and connective tissue. They were then homogenised in Eppendorf tubes in ice-cold phosphate-buffered saline (0·1 M phosphate buffer, pH 7·85, 2·5% NaCl) containing 1 mM EDTA and 0·4% Triton X 100: final pH 7·5.

Enzyme activity was determined using the techniques of Fonnum (1975) as modified by Tomlinson, Moriarty & Mayer (1984).

Assay of adrenal catecholamines

Adrenal glands were rapidly excised as above and homogenised, using a teflon homogeniser, in one millilitre of a mixture of 1·0 M perchloric acid (PCA) and 40 mM sodium metabisulphite containing one millimetre 3,4-dihydroxybenzylamine hydrochloride (DHBA) as an internal standard (Aldrich Chemical Co Inc, Milwaukee, USA). Each homogenate was transferred to a plastic Eppendorf centrifuge tube on ice and the procedure repeated with a second 500 μl PCA. Samples were centrifuged at 39000 rpm for 30 minutes at 4 °C in a refrigerated centrifuge, and supernatants stored at −80 °C until assayed. Neonatal glands were homogenised with 2×250 μl of PCA solution.

Adrenaline, noradrenaline and dopamine were separated using an Ultrasphere ion-pair chromatography column 5 μl, 25 cm × 4·6 mm. Catecholamine levels were determined by the method described by Tomlinson *et al.* (1987).

Table 1. *Volumetric analysis of the adrenal medulla*

Age (n)	Adrenal gland volume (nm^3)	Medullary volume (mm^3)	Medulla as % total
Day 4 (4)	1·27±0·27	0·23±0·05	18·0±4·0
Day 8 (6)	1·64±0·27	0·26±0·04	15·8±2·3
Day 26 (6)	6·84±0·78	0·52±0·06	7·6±0·9
Week 12 (5)	14·1±2·1	1·34±0·20	9·8±0·3
Week 22 (5)	17·6±3·5	1·94±0·39	11·0±1·8
Month 22 (8)	27·0±2·4	3·30±0·29	12·2±1·2

Results expressed as mean±S.D.; n = number of animals.

Table 2. *Volume densities of medullary tissue components*

Age (n)	Chromaffin cells		Vascular tissue	Neuronal tissue	Interstitial tissue
Day 0 (6)	0·34±0·02		0·31±0·02	0·05±0·01	0·30±0·02
Day 4 (7)	0·36±0·03		0·25±0·04	0·05±0·01	0·34±0·03
	A cells	NA cells			
Day 8 (7)	0·44±0·05	0·09±0·03	0·23±0·03	0·04±0·01	0·21±0·02
Day 26 (8)	0·40±0·04	0·17±0·03	0·21±0·02	0·10±0·01	0·12±0·01
Week 12 (6)	0·51±0·02	0·12±0·02	0·20±0·02	0·05±0·01	0·12±0·01
Week 17 (9)	0·41±0·03	0·10±0·01	0·27±0·02	0·05±0·01	0·17±0·03
Week 22 (9)	0·48±0·04	0·08±0·01	0·23±0·09	0·08±0·03	0·13±0·02
Month 22 (8)	0·49±0·05	0·16±0·03	0·12±0·01	0·05±0·01	0·18±0·02

Results expressed as means±S.E.M. n = number of animals.

RESULTS

Morphometry of parenchymal elements

Before describing the appearance of neural elements, including supporting cells, in the adrenal medulla at different ages after birth the general composition of the adrenal medulla and the size and number of chromaffin cells during the postnatal period will be briefly described.

Throughout the period birth to old age the adrenal gland and medulla increased in size (Table 1). Up to Day 4 distinct A and NA cells could not be identified, all cells containing mixtures of A and NA granules. From Day 4 onwards the two cell types were distinguished along with a small number (about 1% or less) of small granule chromaffin cells (SGC). The granules of SGC cells contained storage granules which were highly electron-dense, suggesting NA storage.

The volume densities (Vv) of medullary components at different ages are indicated in Table 2 and the volume of medullary components in Table 3. The chromaffin cells formed some 34% of medullary contents at birth and increased to over 50% by Day 8. Thereafter chromaffin cells formed 50–65% of the adrenal medulla up to old age. The volumes of chromaffin, neuronal (nerve fibres, neurons and supporting Schwann cells), and of interstitial tissue at various ages is given in Table 3. The absolute volumes of chromaffin and neuronal tissues increased throughout life with most marked changes occurring in later adult life: between Week 22 and the age of 22

Table 3. *Volume of medullary tissue components (mm^3) (from Tables 1 and 2)*

Age	A cells	NA cells	Chromaffin tissue	Neuronal tissue	Interstitial tissue	Vascular tissue
Day 4	—	—	0·09	0·01	0·09	0·06
Day 8	0·11	0·02	0·14	0·01	0·05	0·06
Day 26	0·21	0·09	0·30	0·05	0·06	0·11
Week 12	0·68	0·16	0·84	0·07	0·16	0·27
Month 22	1·20	0·39	1·59	0·12	0·44	0·29

Table 4. *Nuclear diameters and cell volumes of chromaffin tissue*

Age (n)	Mean nuclear diameter (μm)		Mean cell volume (μm^3)	
	Chromaffin cells		Chromaffin cells	
Day 0 (6)	6·4		Approximately 500	
Day 4 (7)	6·0		Approximately 500	
	A cells	NA cells	A cells	NA cells
Day 8 (7)	6·5	6·1	620	490
Day 26 (8)	6·9	6·3	900	650
Week 12 (6)	7·3	6·9	1490	1100
Week 17 (9)	7·2	6·6	1450	950
Week 22 (9)	7·3	6·8	1600	1050
Month 22 (8)	7·5	7·4 (range 6·6–8·4)	1930	1730 (range 1010–2500)

Nuclear diameters are corrected according to the formula of Abercrombie (1946).

Table 5. *Diameters and numerical density (Nv) of amine storage granules in neonatal and adrenaline-storing chromaffin cells*

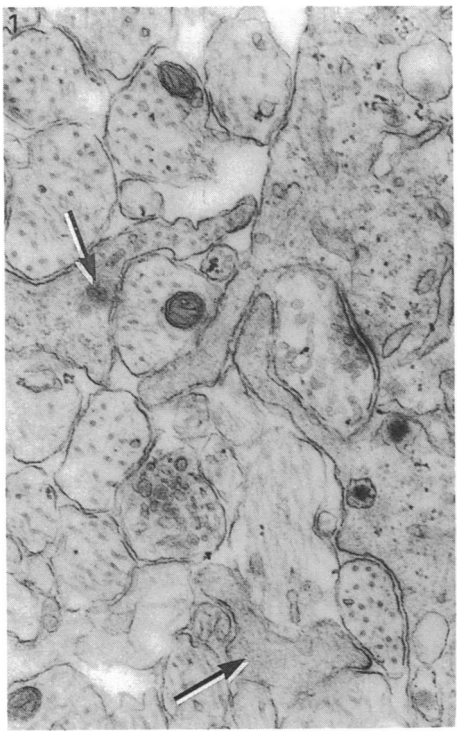
Age (n)	Mean diameter of granule (nm)	Nv per μm^3 cytoplasm
Day 0 (5)	161 ± 1·4 (184)	12·0 ± 4·0
Day 4 (6)	170 ± 2·0 (199)	16·0 ± 3·0
Day 8 (6)	189 ± 4·6 (211)	14·0 ± 3·0
Day 26 (6)	189 ± 5·1 (214)	34·0 ± 4·0
Week 12 (6)	187 ± 3·8 (227)	33·0 ± 5·0
Month 22 (6)	202 ± 4·9 (224)	32·0 ± 6·0

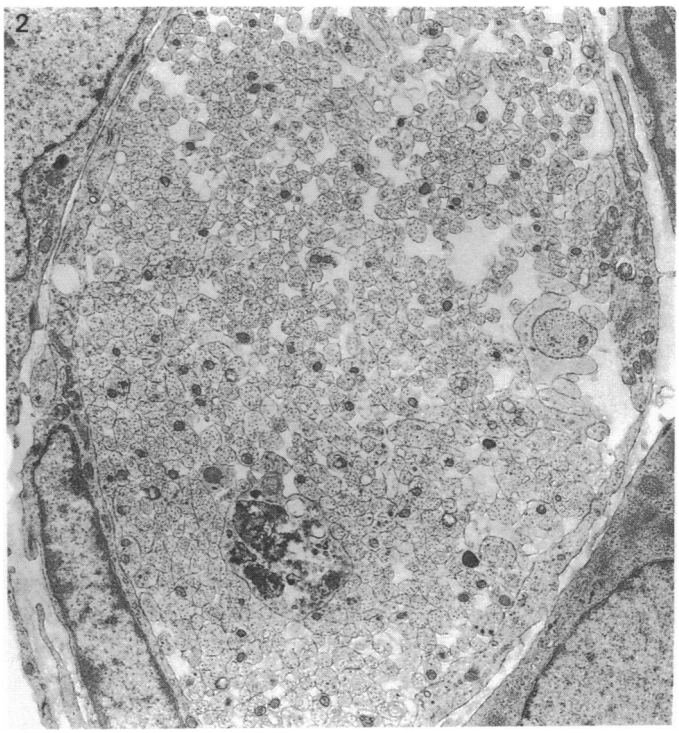
Figures for Day 4 and older specimens relate to A-storage granules. n = number of animals. Diameters in parentheses indicate mean diameter corrected by Saltykov (1958) conversion.

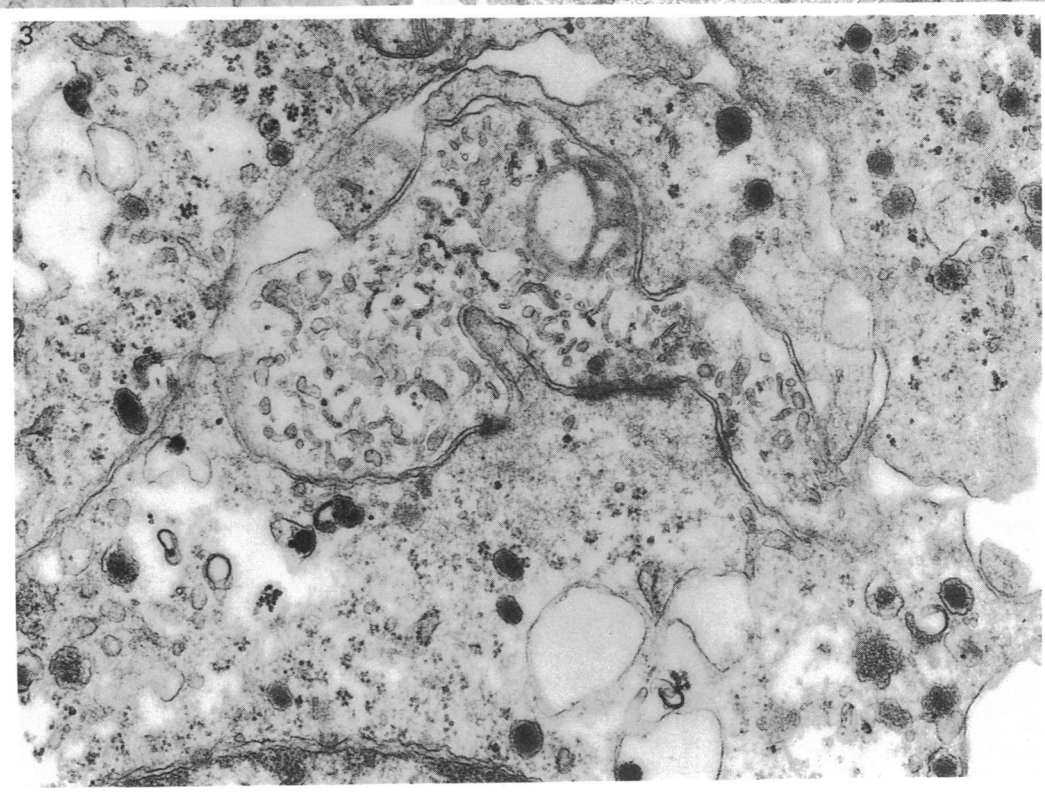
months interstitial tissue also more than doubled in amount though vascular tissue remained constant in volume.

Chromaffin cells showed an increase in volume from birth to young adult life (12 weeks) with A cells doubling in volume (Table 4).

Because of the distortion of NA granules by fixation in glutaraldehyde, meaningful estimates of sizes and numbers of chromaffin granules in NA cells cannot be made, hence mean diameters of storage granules and the numerical density of these at different ages given in Table 5 relate to neonatal and A granules only. The Nv of







adrenaline-storing granules at Day 26 was twice that of earlier specimens; thereafter it remained constant. Granule diameter showed an approximately 10% increase between birth and the age of 8 days, after which the size remained constant.

Morphology

Day 0–2

The future adrenal medulla contained some 20% cortical cells. Chromaffin elements were immature in form and size and contained a mixture of A and NA cell granules. Occasional pheochromoblasts, primitive sympathetic cells and neuroblasts were observed, each with typical cytoplasmic contents (Coupland & Weakley, 1968).

Individual axon profiles and small groups of axons were evident between medullary components. Some were associated with adjacent attenuated Schwann cell cytoplasm (Fig. 1). Occasionally axons were seen which had invaginated Schwann cell cytoplasm with early mesaxon formation. Occasional groups of up to about 1000 axon profiles occurred (Fig. 2) surrounded peripherally by attenuated Schwann cell cytoplasm. No myelinated axons were observed. The basement membrane that typically lies on the connective tissue face of nerve fibres and Schwann cells, and chromaffin cells in older specimens, was not evident. Axon profiles contained neurotubules and occasional mitochondria.

Synaptic nerve endings of widely different profile diameters and appearance were observed, together with zonal pre- and post-synaptic membrane thickenings. The differences in appearance were considered to reflect the degree of maturity of the endings.

Three stages or degrees of maturity were recognised. The most immature contain tubulovesicular profiles, some of which contain highly electron-dense material (Fig. 3). These appear to represent sections through pleomorphic convoluted tubules or irregular cisternae. They are quite distinct from neurotubules which may also be evident, but have a smaller diameter and a less distinct peripheral membrane. The surface of the chromaffin cell and the axon terminal in contiguity with it exhibited reciprocal projections, ridges or troughs which markedly increased the area of apposition; pre- and post-synaptic membrane densities occur at intervals in larger endings and singly in smaller synaptic profiles. Some densities appeared to be symmetrical and others asymmetrical.

Profiles of intermediate maturity (Fig. 4) contained irregularly-shaped tubulovesicular elements together with occasional discrete rounded vesicular profiles about 100 nm in diameter which had moderately electron-dense contents. These resembled the neuropeptide-containing vesicles of mature cholinergic nerve endings.

Synaptic profiles of mature appearance (Fig. 5) occurred infrequently at this stage. They contained many small clear vesicles of about 50 nm diameter some of which were rounded in shape while others appeared to be sections through prolate spheroids or through larger spherical vesicles (about 100 nm) which had moderately electron-dense

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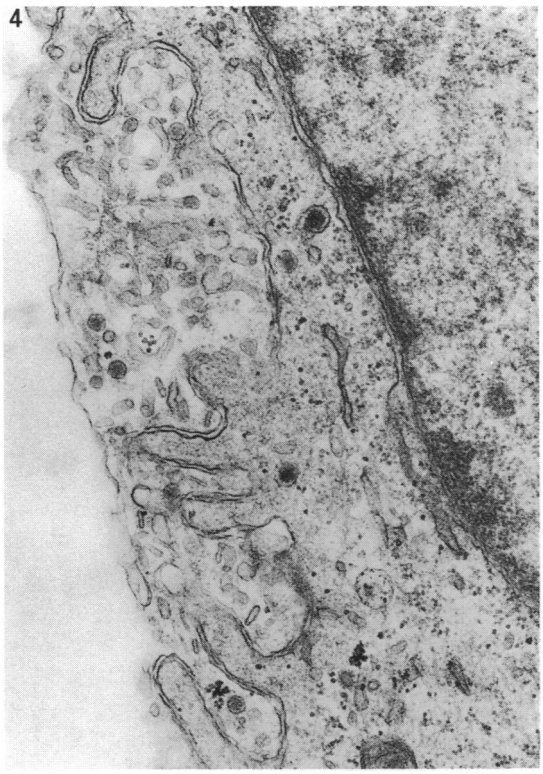
Fig. 1. Day 0 rat adrenal medulla showing a synaptic ending invaginated into a chromaffin cell and numerous axons some of which are associated with processes of Schwann cells (arrows). ×26000.

Fig. 2. Day 0 rat adrenal medulla. Large collection of axons surrounded by attenuated Schwann cells. ×6000.

Fig. 3. Day 0 rat adrenal medulla. Immature synaptic nerve ending on a chromaffin cell which contains a mixture of A and NA granules. Note highly electron-dense content of some of the branched tubulovesicular neural contents and relatively few obovate or flattened clear vesicular profiles. ×26000.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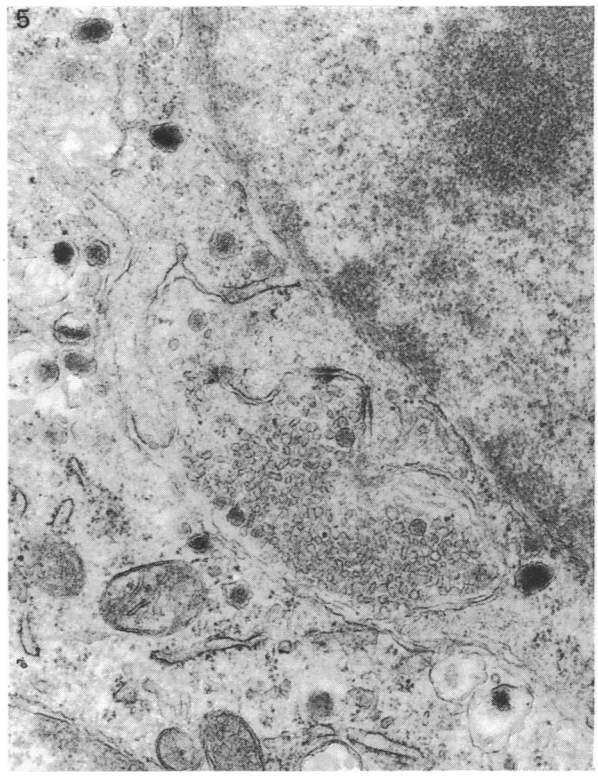
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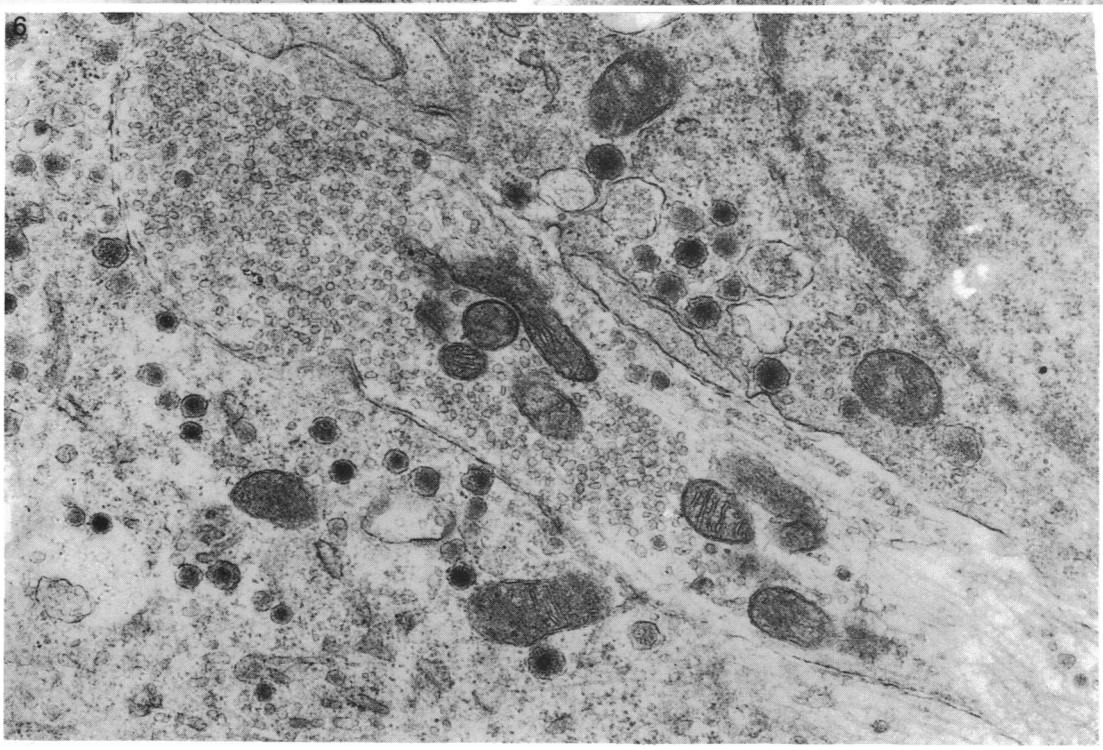
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Table 6. *Synaptic profile density in chromaffin tissue and numbers per chromaffin cell*

Age (n)	Synaptic profile density number/100 μm^2 chromaffin tissue		Mean number of chromaffin synaptic profiles/cell profile		Mean number* of synapses/chromaffin cell	
Day 0 (5)	0·22±0·03		0·14		2·2	
Day 4 (6)	0·35±0·03		0·22		2·9	
Day 8 (4)	0·26±0·12		0·12		1·7	
					A cells	NA cells
Day 26 (4)	0·28±0·3		0·24		4·4	3·6
	A cells	NA cells	A cells	NA cells		
Week 12 (5)	0·24±0·03	0·30±0·03	0·29	0·34	5·4	5·4
Month 22 (6)	0·12±0·01	0·18±0·04	0·18	0·20	4·7	5·4

* Calculated by method used by Case & Matthews (1985).

granular contents. The preterminal region contained small mitochondria and neurotubules. Synaptic membrane densities were variably asymmetrical or symmetrical.

The mean number of synapses per chromaffin cell was 2·2 (Table 6).

Days 4–8

Distinct adrenaline (A) and noradrenaline-storing (NA) chromaffin cells were evident in the adrenal medulla and together they accounted for some 46% of medullary volume (Table 2). In absolute terms neuronal tissue had a volume of 0·01 mm^3 at Days 4 and 8 (Table 3), while the volume of chromaffin cells was 0·09 mm^3 at Day 4 and 0·14 mm^3 at Day 8.

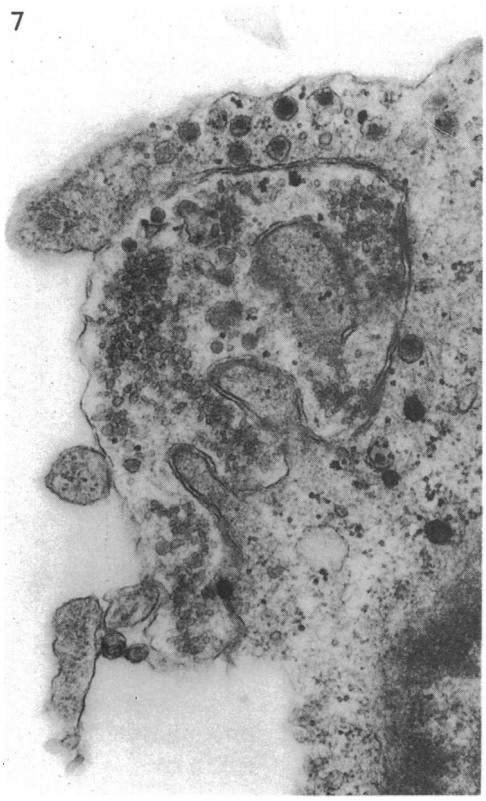
Axon profiles were again non-myelinated though early mesaxon formation was occasionally evident. Associated Schwann cells were in more intimate proximity to nerve fibres than in earlier specimens and the attenuated cytoplasmic processes of Schwann cells were often observed enveloping individual axons within axon bundles.

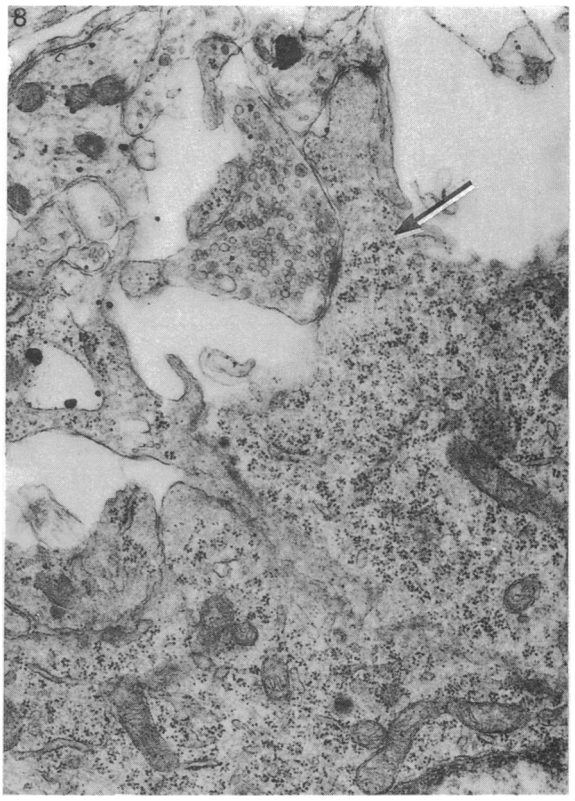
Synaptic profiles usually resembled the more mature type of 0–2 days old specimens (Fig. 6) and only rarely were they less mature with small vesicular profiles having variable shapes and occasionally containing moderately electron-dense material (Fig. 7). The majority of endings contained round small clear synaptic vesicles with a diameter of about 40 nm; some larger vesicles were cored with moderately electron-dense contents and were 80–120 μm in diameter. A few varied in size from 50 to 200 nm and in shape (rounded or flattened) and were either clear or possessed slight to

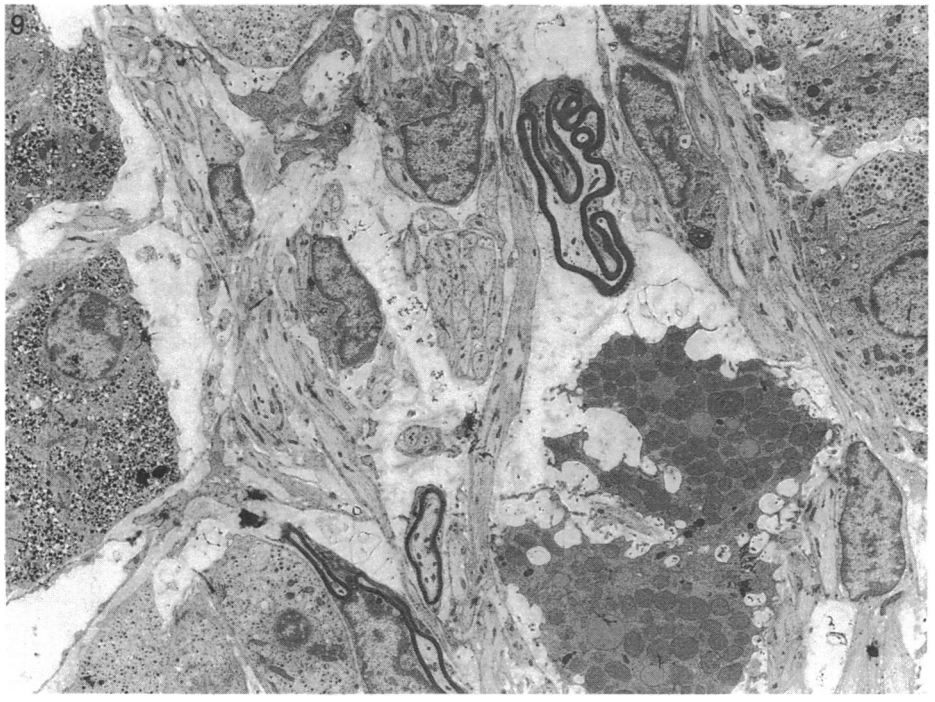
Fig. 4. Day 0 rat adrenal medulla. Synaptic profile of intermediate maturity. Tubulovesicular profiles are without electron-dense contents and larger rounded (about 100 nm) granular vesicles with moderately electron-dense contents are evident. ×26000.

Fig. 5. Day 0 rat adrenal medulla. Synaptic ending of mature appearance on a chromaffin cell. Small clear synaptic vesicles vary in shape but most are round or obovate and larger granular vesicles are evident. ×19000.

Fig. 6. Adrenal medulla of 8 days old rat showing a large synaptic profile of mature appearance on an A cell. The small clear synaptic vesicles are mainly round and the relatively few larger granular vesicles have moderately electron-dense contents. The preterminal region (to the right) contains small mitochondria and neurotubules. ×26000.







moderately electron-dense contents. No differences were observed in the structure or maturity of ending on A and NA cells. Some endings showed multifocal synaptic thickenings and an abundance of small clear vesicles with small mitochondria and neurotubules particularly in the pre-terminal axon.

At Days 4 and 8 the calculated number of nerve profiles per cell was 2·9 and 1·7 respectively (Table 6).

Although neuronal profiles were identified in earlier specimens no distinct synaptic endings on these were observed – possibly because of chance sectioning. In 4–8 days old specimens cholinergic-type preganglionic endings on dendrites were observed (Fig. 8); they contained many small clear vesicles (about 40 nm diameter) and fewer larger (about 100 nm) granular vesicles, which had moderately electron-dense contents.

Day 26

The general appearance of the adrenal medulla was similar to that of the mature animal. The mean cell volumes of A and NA cells were 900 μm^3 and 650 μm^3 (Table 4) respectively and chromaffin tissue comprised some 57% of the medullary components (Table 2).

Many non-myelinated and occasional myelinated nerve fibres were seen (Fig. 9) in association with Schwann cells, the unmyelinated fibres being some 100 times as numerous as myelinated fibres in the fibre bundles. At this age (Table 2) neuronal tissue formed some 10% of the adrenal medulla and in absolute terms had a volume of 0·05 mm^3 (Table 3).

Synaptic endings of mature appearance, similar to those in adult specimens, were evident on A and NA cells (Figs. 10, 11). The area of contiguity between axons and chromaffin cells was sometimes a shallow depression on the plasmalemma of the A and NA cell with a single site of membrane thickening. The opposed membranes sometimes showed more marked folds, depressions and projections. Extended endings, possibly trail in character with multifocal synaptic thickenings, were also occasionally observed. The mean length of the zone of contiguity was 2·4 μm on both A and NA cells with range 0·5–5·0 μm and the endings had a mean perpendicular dimension (minor axis) of 1 μm. No immature synaptic endings were observed. The mean number of synapses per chromaffin cell was 4·3 and 3·6 for A and NA cells respectively (Table 6).

Typical medullary neurons were present and one such profile showed a large axosomatic ending (Fig. 12).

Medullary neurons, when grouped, usually presented a close packed appearance with neuron somata lying in close contact or being separated by attenuated extensions of Schwann cell cytoplasm and associated dendritic spines or axonal processes (Fig. 13). Axodendritic synapses at the periphery of the neuronal somata were frequently observed (Fig. 14). The external faces of the neuronal somata were usually related to axonal and dendritic processes and associated Schwann cell cytoplasm (Fig. 13); however, in some regions, they were separated from adjacent chromaffin cells by only a narrow connective tissue space (Fig. 13).

Fig. 7. Adrenal medulla of a 4 days old rat. The synaptic profile on an A cell is of late-intermediate maturity. The zone of continuity between axon and A cell is markedly folded and the clear synaptic vesicles round or obovate. × 26000.

Fig. 8. Adrenal medulla of a 4 days old rat. The section passes through the soma of an adrenal medullary neuron. Note the axodendritic synapse on a dendritic spine (arrow). × 19000.

Fig. 9. Adrenal medulla of a 26 days old rat. Note the occasional myelinated and the many non-myelinated nerve fibres lying between loosely aggregated chromaffin cells and a cortical cell. × 2600.

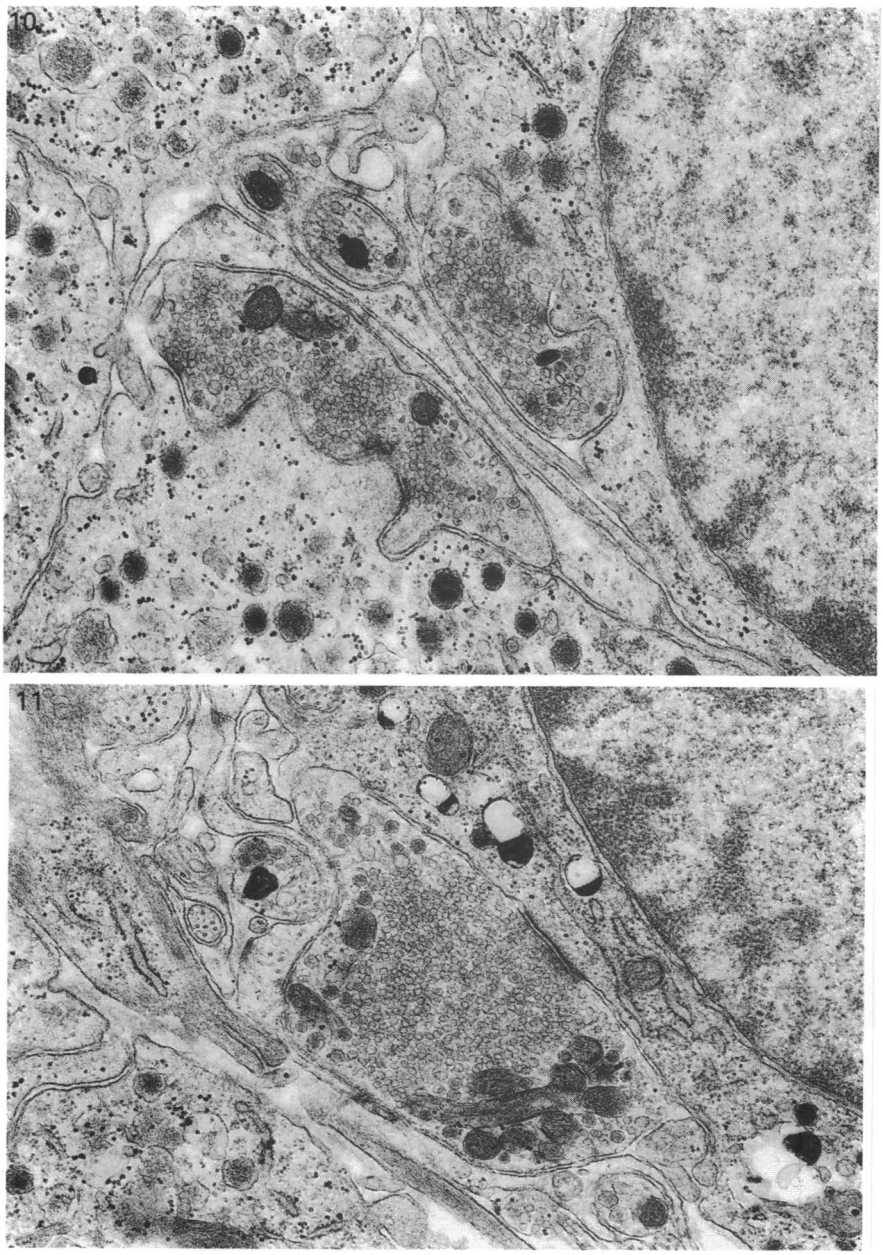
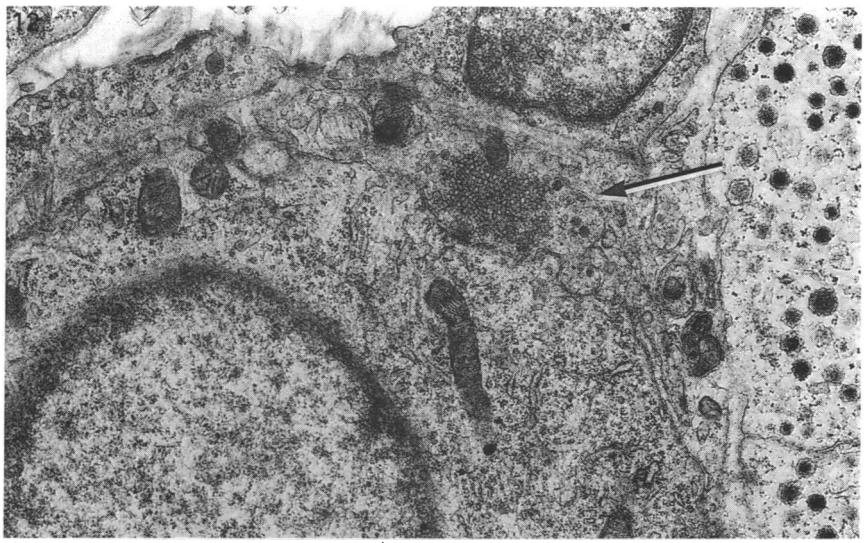


Fig. 10. Adrenal medulla of a 26 days old rat. Synaptic nerve endings of mature appearance on A cells with single or multifocal membrane densities. ×22000.

Fig. 11. Adrenal medulla of a 26 days old rat. Synaptic nerve ending of mature appearance on a NA cell. The clear synaptic vesicles are mainly round and the membrane densities asymmetrical. ×22000.

Adult animals aged 12–22 weeks

Between 26 days and 12 weeks there was a marked increase in cell volume of A and NA cells (Table 4) and the medullary volume more than doubled (Table 1). The proportion of the medulla composed of neuronal tissue decreased from 10% to 5% (Table 2) and in absolute terms was 0·07 mm^3 at 12 weeks.



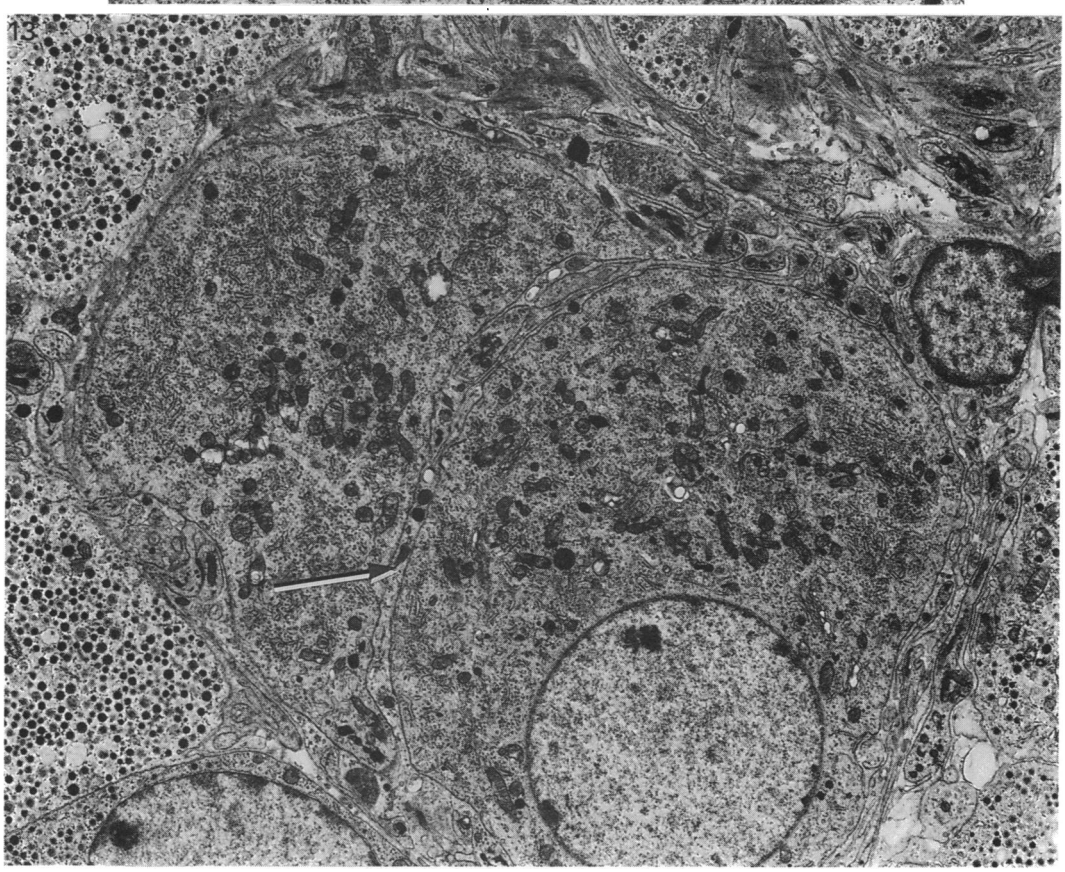
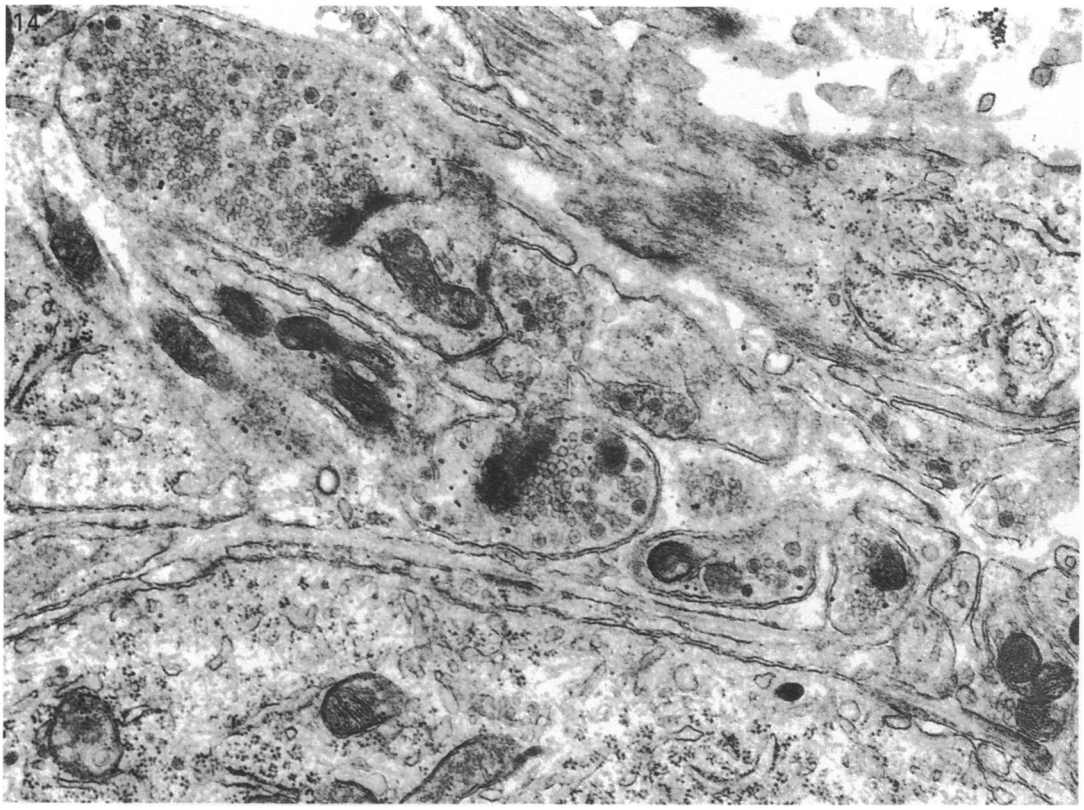


Fig. 12. Adrenal medulla of a 26 days old rat. Section through the soma of an adrenal medullary neuron and an adjacent A cell. Note the supporting Schwann cell (*S*) and the large axo-somatic synapse on the neuron (arrow). × 14000.

Fig. 13. Adrenal medulla of a 26 days old rat. Section through a group of adrenal medullary neurons showing close packing together with Schwann cell cytoplasm which extends (arrow) between the somata of two neurons. × 5600.



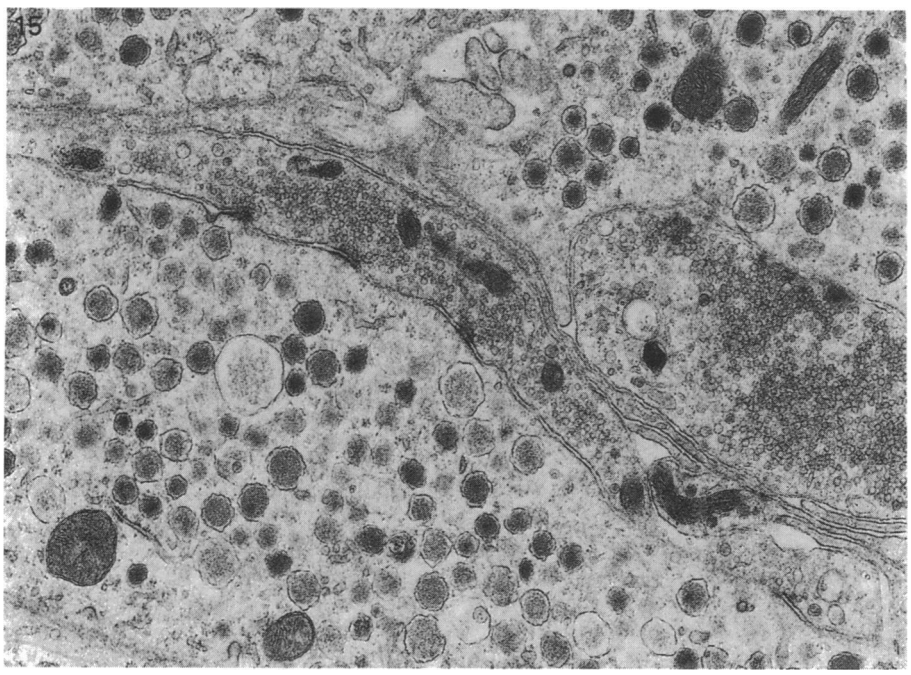


Fig. 14. Adrenal medulla of a 26 days old rat showing obliquely sectioned axo-dendritic synapses. The neuronal soma lies below. ×23500.

Fig. 15. Adrenal medulla of a 12 weeks old rat. A relatively compact synapse is associated with the upper A cell and a trail-like ending with the lower A cell. ×18000.

Synaptic endings contained both small clear and larger granular vesicles and, as at all other ages, the granular contents of the latter were of moderate electron density. The mean length of contiguity between the synaptic ending and chromaffin cell (the major axis of the synapse) was 2 μm but they ranged from 0·5 to 6 μm with a mean perpendicular height (minor axis of ending) of 1 μm. The synaptic endings with extended contact zone resembled trail endings and showed multifocal asymmetrical membrane thickenings (Fig. 15). The mean synaptic bouton diameter, calculated from the formula of Williams (1977), was 1·4 at this and all other ages. The mean number of synapses per cell at 12 weeks increased to 5·4 on both A and NA cells. Most synaptic profiles were associated with a slight depression of the plasmalemma of the chromaffin cell. Up to 5 individual asymmetrical synapses have been observed in a section through an individual chromaffin cell; two or three of these were clustered and the others were widely separated and often on different faces of the chromaffin cell (Figs. 16, 17).

Occasional axonal profiles with a content of mainly small mitochondria were seen adjacent to chromaffin cells or lying between these and nerve fibres or blood vessels (Fig. 18). The contents also included a few small clear or larger moderately electron-dense granular contents and often no evidence of local synaptic membrane density. Others showed a focal synaptic membrane thickening and more synaptic vesicles (Fig. 19).

Groups of medullary neurons often showed a close packing of somata that were occasionally separated by small intercellular canaliculi (Fig. 20). Some of the latter also contained fine axonal and dendrite profiles.

Aspects of neurons facing chromaffin tissue or connective tissues showed many small dendritic spines which were intimately associated with axons and supporting Schwann cell cytoplasm and axo-dendritic synapses were observed (Fig. 21). The faces of neurons and satellite Schwann cells facing connective tissue elements were always covered by a granular basement membrane, as were chromaffin cells in similar situations; no basement membrane was observed between Schwann cell cytoplasm and either adjacent chromaffin cells or neurons.

Profiles of SGC cells were either polyhedral or were elongated; all showed an abundance of rough endoplasmic reticulum and possessed mainly small amine storage granules (less than 150 nm core diameter) with highly electron-dense contents typical of NA storage: some also contained occasional larger NA storage granules. Nerve endings on these cells resembled those on A and NA cells.

No nerve terminals or profiles containing synaptic vesicles were observed to be specifically related to the vasculature of the adrenal medulla in this or any other age group.

17–25 months

During the period between young adult life and 17–25 months of age, rats show a marked increase in the volume of chromaffin tissue (Table 3) which is accounted for in part by cell hypertrophy (Table 4) and in part by hyperplasia since from data presented in Tables 3 and 4 it can be calculated that the chromaffin cells have mean numbers of $6{\cdot}23 \times 10^5$ A cells and 2·25 NA cells in older animals. During this period the Vv of neuronal tissue remains proportionally similar at 5% of medullary contents.

As compared with young adult animals the adrenal medulla of older rats showed a marked increase in collagenous fibrils in interstitial tissues and changes in the appearance of organelles in some chromaffin cells – especially those that appeared to be hypertrophic – involving lysosomes, endoplasmic reticulum and storage granules. These have been described in detail previously (Coupland & Tomlinson, 1989).

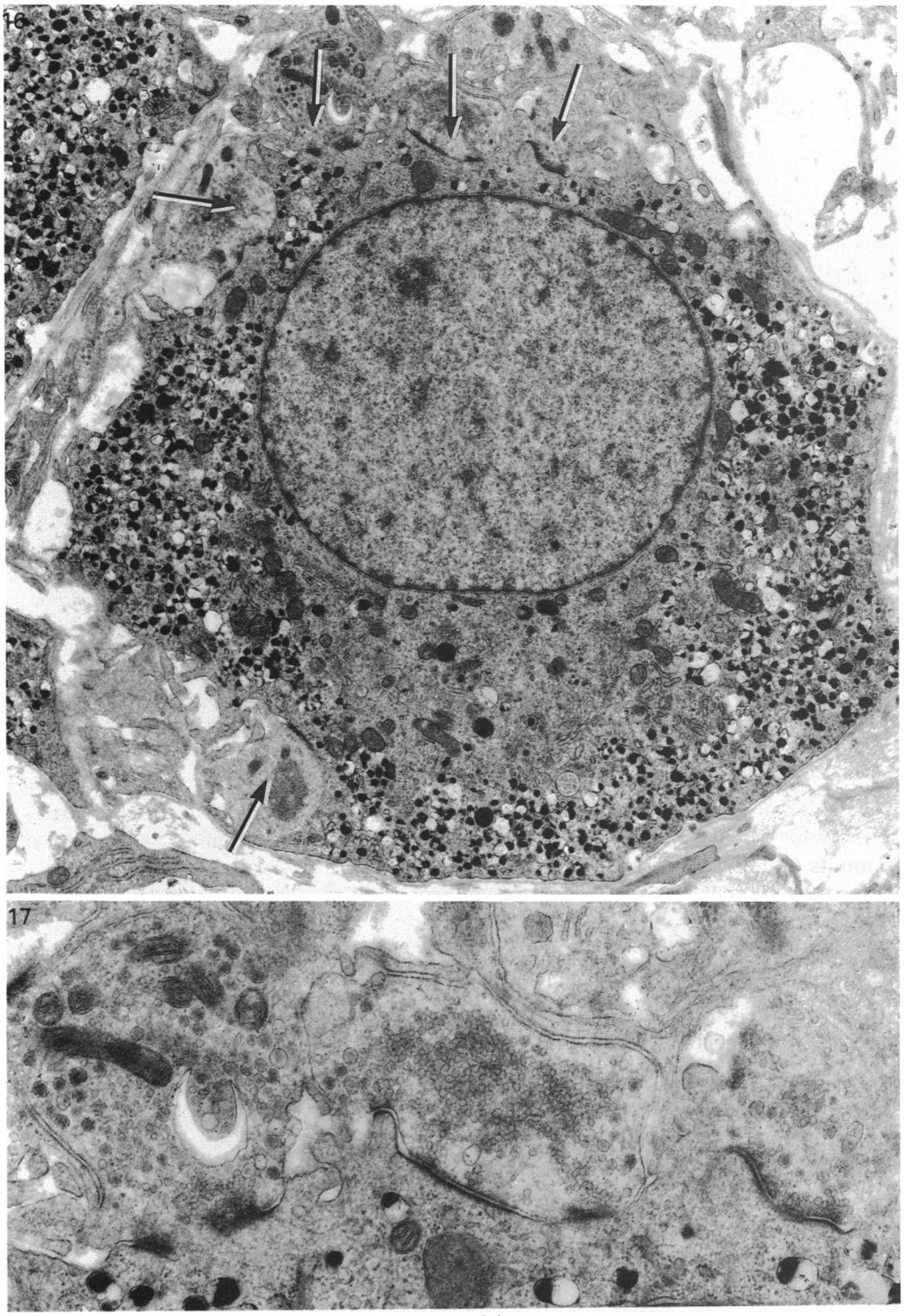
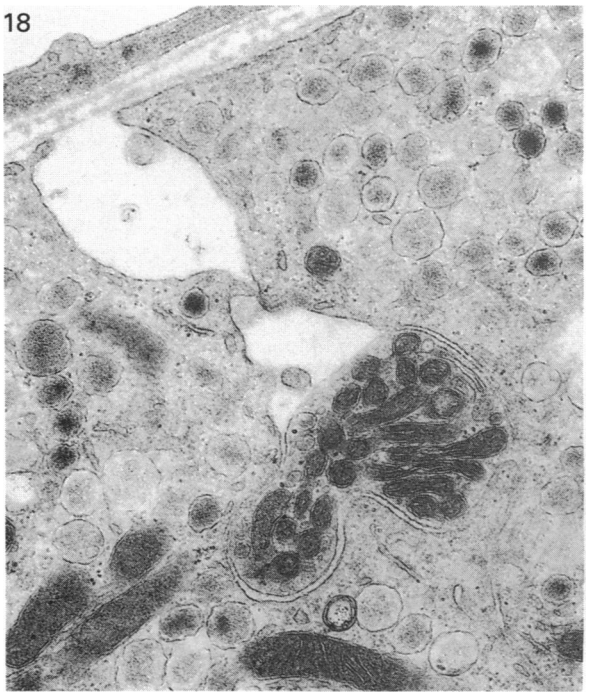


Fig. 16. Adrenal medulla of a 12 weeks old rat. Multiple synapses on an NA cell (arrows). ×8500.

Fig. 17. Adrenal medulla of a 12 weeks old rat. Upper group of synapses in Figure 16 at higher magnification to show marked variation in the relative number of small clear and larger granular synaptic vesicles in the three synaptic profiles. ×24000.



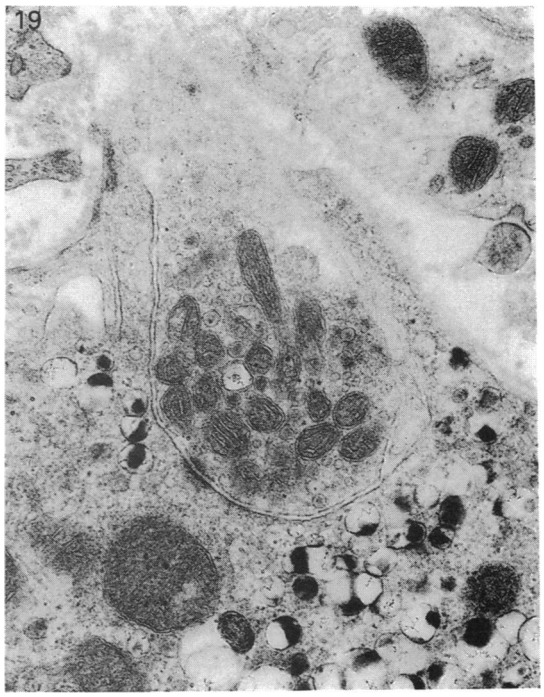


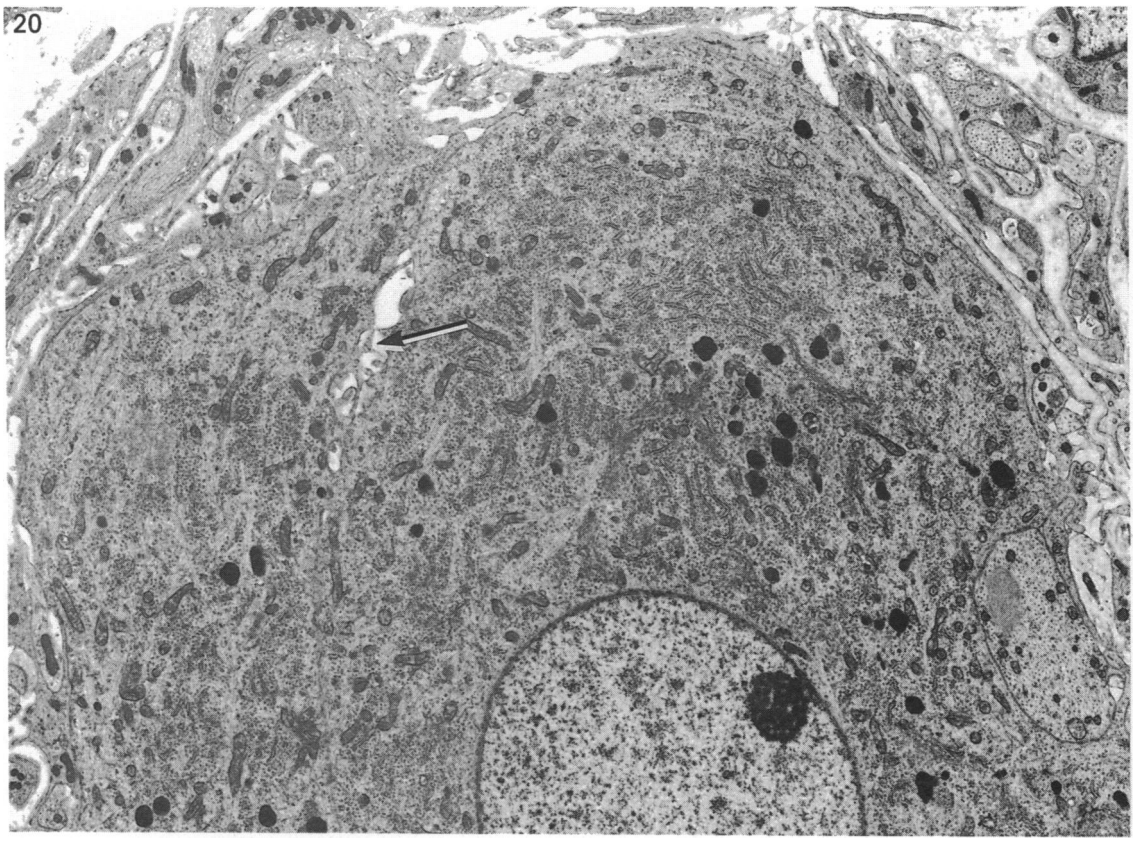
Fig. 18. Adrenal medulla of a 12 weeks old rat. An axonal profile packed with small mitochondria and with very few clear or larger granule synaptic vesicles lies in an intercellular canaliculus between two A cells. ×23000.

Fig. 19. Adrenal medulla of a 12 weeks old rat showing a synaptic profile containing many mitochondria on a NA cell. Both small clear and larger granular synaptic vesicles are evident but not numerous. Note the partial covering of the synapse by processes of NA cell cytoplasm. ×23000.

Myelinated and non-myelinated nerve fibres associated with Schwann cells occurred in the same proportions as in younger adult rats and were surrounded by extensive amounts of collagenous fibrous tissue.

Synaptic endings resembled those of younger adult rats varying from endings with extended contact and multifocal asymmetrical densities to small discrete endings with a rounded or obovate profile and a single asymmetric membrane density usually associated with a slight depression of the plasmalemma of the chromaffin cell. The zones of contiguity ranged from 0·5 to 4·5 μm. Occasionally synaptic endings were observed associated with a local invagination of the plasmalemma. The calculated mean number of synapses per chromaffin cell was 4·7 for A cells and 5·4 for NA cells. Up to 3 distinct endings were observed on a single chromaffin cell. Cells which showed evidence of hypertrophy of endoplasmic reticulum and granule depletion were normally innervated (Fig. 22) as were the SGC cells.

Small granule chromaffin cells had polyhedral or elongated profiles: the latter often exhibited tapering extensions or processes which ended adjacent to other chromaffin cells, desmosomes being occasionally seen between the two cell types. Cytoplasmic organelles of SGC cells included all those seen at younger age groups but in addition they contained small round vesicles of about 40 nm diameter which possessed electron-dense contents and resembled adrenergic synaptic vesicles. These often occurred in clusters and were occasionally seen in the peripheral cytoplasm adjacent to the plasmalemma (Fig. 23). Where these occurred towards the end of the cell



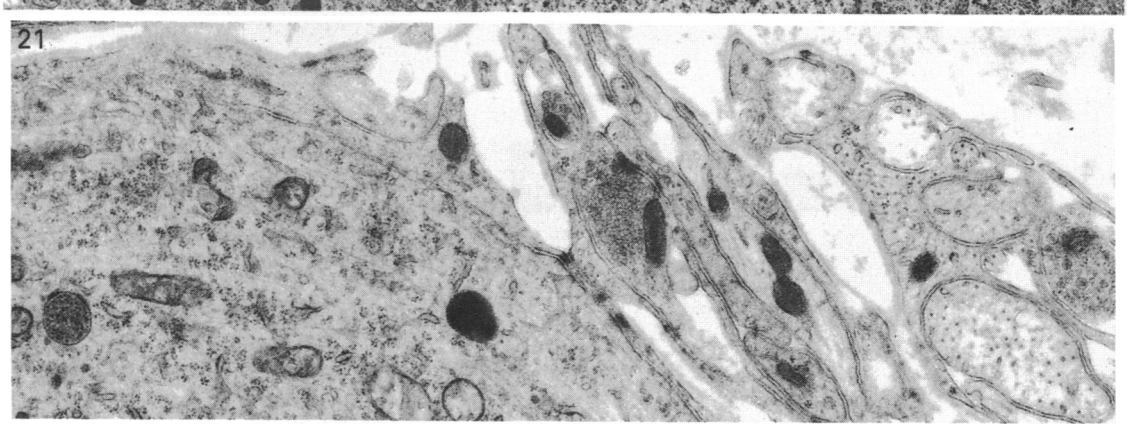


Fig. 20. Adrenal medulla of a 12 weeks old rat. Section through adrenal medullary neurons. Note the intercellular canaliculus (arrow) between the apposed soma of two neurons and the peripheral groups of axonal and dendritic profiles associated with Schwann cell cytoplasm. × 5500.

Fig. 21. Adrenal medulla of a 12 weeks old rat showing a typical axo-dendritic synapse. × 15000.

Fig. 22. Adrenal medulla of a 22 months old rat. The hypertrophic NA cell shows an increase in rough endoplasmic reticulum, enlarged lysosomes and a cytoplasm largely depleted of chromaffin granules. Note the synaptic endings (arrows) on two NA cells. × 9500.

Fig. 23. Adrenal medulla of a 22 months old rat. Section through the process of an SGC cell showing typical medium-sized amine storage granules and a cluster of SSV (arrow). The mitochondria are typical of those of SGC cells. × 16500.

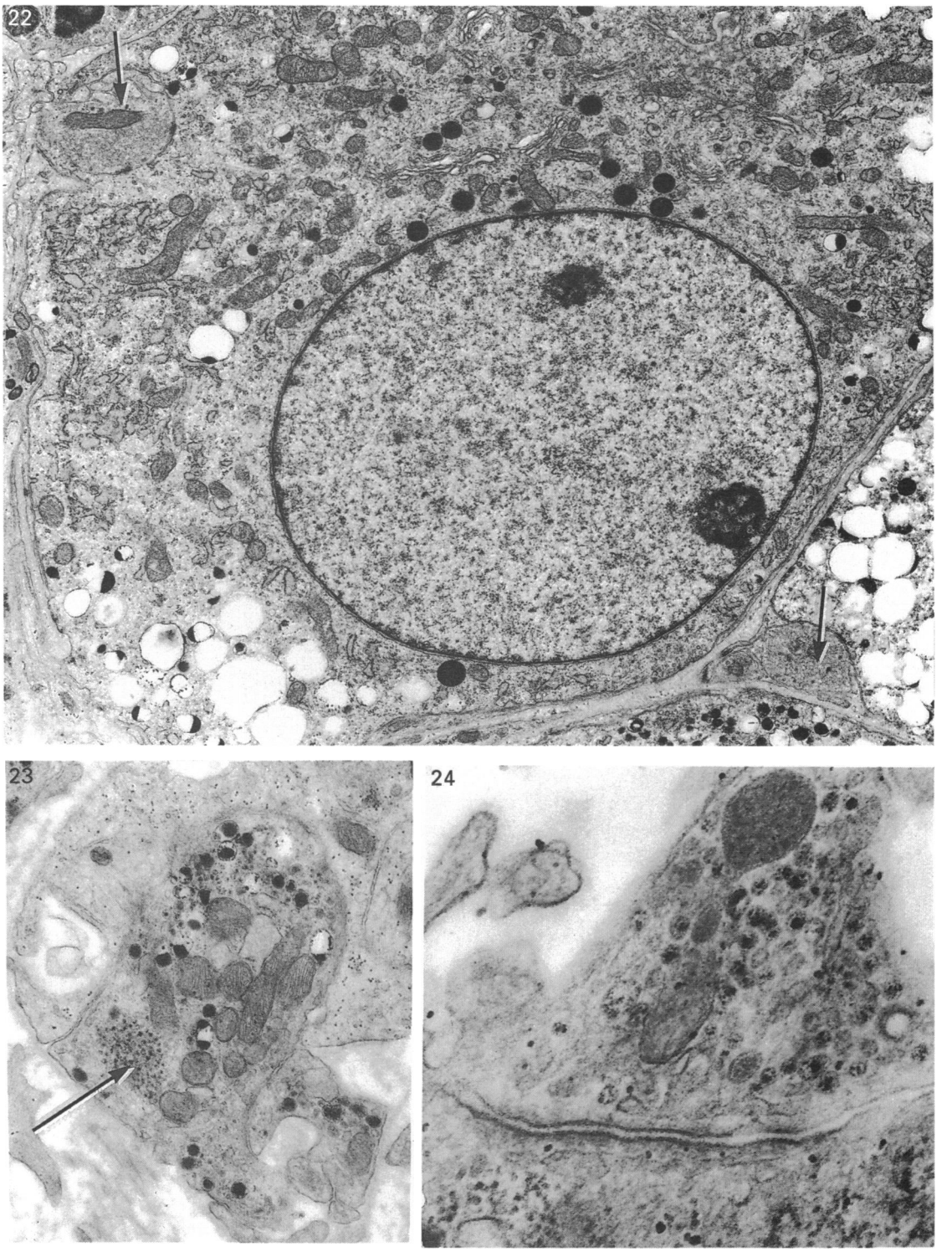


Fig. 24. Adrenal medulla of a 22 months old rat. A process of an SGC cell which contains an aggregate of SSV abuts on an adjacent NA cell; at the site of apposition symmetrical membrane densities are evident. × 52700.

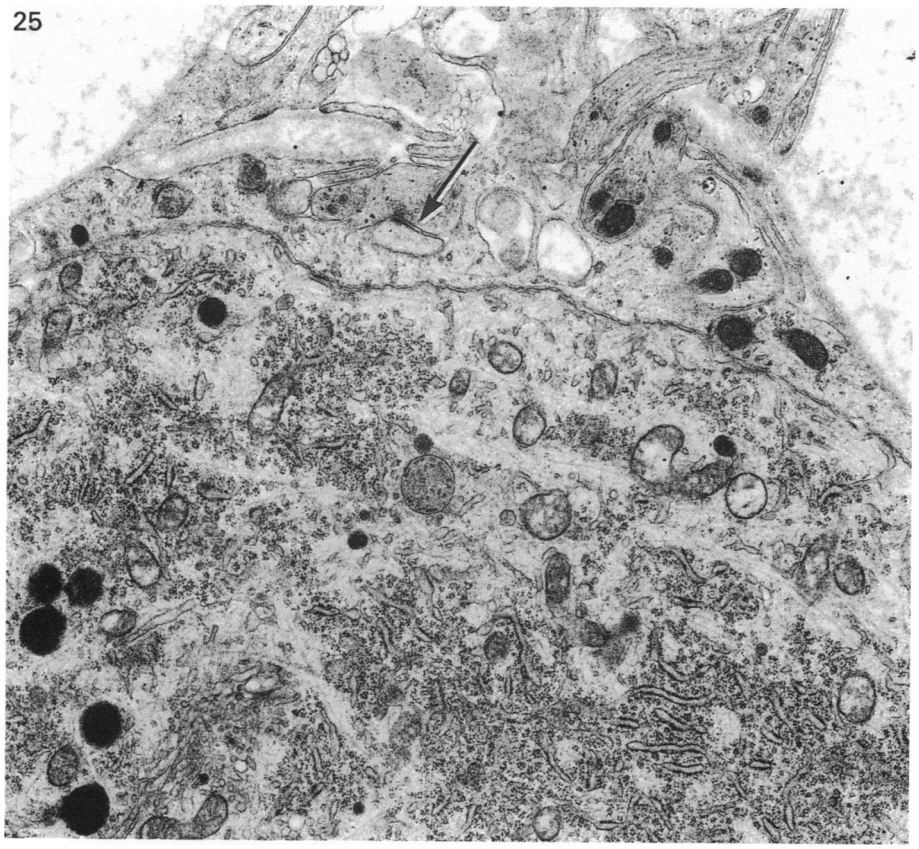


Fig. 25. Adrenal medulla of a 22 months old rat. The adrenal medullary neuron contains lipofuchsin granules. Note the axodendritic profiles and Schwann cell cytoplasm above, with an axodendritic synapse (arrow). × 14000.

processes they could easily have been mistaken for the contents of adrenergic nerve fibres, though they could be distinguished from the latter by the presence of mitochondria of the same dimensions as chromaffin and SGC cells and SGC-type amine storage granules. Very occasionally a synaptic arrangement was suggested by desmosomal symmetrical densities of plasma membranes at the contact zone between SGC cell process and an adjacent NA chromaffin cell (Fig. 24).

Medullary neurons were occasionally observed and these, in general form and in their relations, resembled those of young adult animals. These differed from neurons of young adult rats in the presence of numerous lipofuchsin granules in the cytoplasm (Fig. 25). Axodendritic synapses (Fig. 25) were frequently observed.

Choline acetyltransferase (ChAT) activity

This was expressed as enzyme activity per gland and hence included activity in the richly innervated medulla together with that of the relatively sparsely innervated cortex.

ChAT activity was low up to the end of the first postnatal week and then rapidly increased to adult levels by Day 26 (Table 7). Between Week 22 and old age there was a further increase of about 30%.

The ChAT activity correlated well with the total number of synapses on chromaffin cells except at Day 26.

Table 7. *Choline acetyltransferase activity and synaptic endings on chromaffin cells in the adrenal gland*

Age	ChAT activity nmol Ach/hr per gland	Chromaffin cells $\times 10^5$		Total number of synapses $\times 10^{5*}$
		A cells	NA cells	
Days 4–5	0·73 ± 0·08 (4)	1·8		3·9
Days 7–8	0·65 ± 0·08 (3)	1·78	0·41	6·4
Day 26	3·59 ± 0·35 (5)	2·33	1·38	15·0
Weeks 12–22	3·51 ± 0·21 (6)	4·56	1·45	32·4
22–25 months	4·39 ± 0·07 (4)	6·23	2·25	41·5

Results expressed as mean ± S.E.
Number of animals in parentheses.
* Calculated from data in Tables 3, 4 and 6.

Table 8. *Catecholamine content of the adrenal glands of neonatal, adult and aged Wistar rats*

Age (n)	Adrenaline	Noradrenaline	Dopamine
Day 2 (7)	1·08 ± 0·14	0·53 ± 0·06	0·04 ± 0·01
Day 4 (6)	3·12 ± 0·30	0·68 ± 0·04	0·06 ± 0·01
Day 7 (6)	4·78 ± 0·30	1·01 ± 0·03	0·04 ± 0·02
Day 14 (5)	9·70 ± 0·64	3·13 ± 0·32	0·20 ± 0·04
Day 26 (11)	17·81 ± 1·00	6·43 ± 0·54	0·33 ± 0·03
Week 12 (10)	98·71 ± 2·48	29·74 ± 1·80	NA
Week 17 (6)	112·55 ± 4·70	21·11 ± 1·26	0·51 ± 0·05
Week 22 (6)	139·00 ± 3·27	26·08 ± 1·76	0·91 ± 0·06
Month 22 (11)	184·70 ± 22·20	60·00 ± 29·50	2·40 ± 1·60

Results expressed in nmoles per adrenal gland, means ± S.E.M.
Number of animals in parentheses.

Catecholamine content of the adrenal gland

The catecholamine content of the gland increased throughout life (Table 8) and showed a fivefold increase between 26 days and 12 weeks of age and doubled between 12 weeks and old age. In adult animals dopamine formed less than 1% of total catecholamines and in younger animals was less than 2% except in the neonate. The relative proportions of adrenaline and noradrenaline reflect reasonably well the cellular composition of the gland (Table 9). An increase in Nv of amine storage granules in A cells preceded (Table 5) the marked increase in catecholamine storage observed between Day 26 and Week 12 (Table 8). There was little change in the amine content of an A granule (Table 10) during adult life.

DISCUSSION

The present findings indicate that at birth and during the first week of postnatal life the numbers of synaptic profiles per chromaffin cell (Table 6) is only some 50% of that of adult animals. From birth to Day 2 the majority of synapses have an immature appearance characterised by marked folding of the region of contiguity between nerve fibre and chromaffin cell and the presence of tubulovesicular profiles, some of which

Table 9. *Relative amounts of catecholamines and of A and NA cells at different ages*

Age	Catecholamine (%)		Cellular composition (%)	
	A	NA	A	NA
Day 7–8	80·8	17·3	84·6	15·4
Day 26	72·5	26·2	70·0	30·0
12 weeks	77·0	23·0	81	19
22 months	74·7	24·3	75·5	24·5

Table 10. *Catecholamine content of A cells and granules*

Age	Amine content of an A cell ($\times 10^{-6}$ μmol)	Amine content of a NA cell ($\times 10^{-6}$ μmol)	Amine content* of an A granule ($\times 10^{-12}$ μmol)
Day 4	0·02	0·02	3·0
Day 8	0·03	0·03	3·9
Day 26	0·08	0·06	4·4
Week 12	0·22	0·21	5·2
Week 22	0·24	0·17	5·2
22 months	0·30	0·27	5·5

* Based on calculations using Nv granules.

show branching and some of which contain electron-dense material (Fig. 3). Zonal synaptic membrane densities are already evident and some of these appear symmetrical in the immature endings though the obliquity of a section often makes symmetry difficult to determine.

By the end of the first week after birth the majority of synaptic endings contain small round clear vesicles and a variable number of obovate clear vesicles. They also contain a small number of larger granular vesicles (about 100 nm in diameter). The latter are usually absent in the most immature synaptic profiles but are a constant feature, particularly in the presynaptic part of mature synaptic endings, where they are often associated with small axonal mitochondria (about 250 nm in transverse diameter) and occasionally neurotubules (Fig. 6).

From Day 8 onwards the many synaptic profiles on chromaffin cells (Fig. 6) are similar to those in adult specimens and are typical of the cholinergic nerve endings previously described (Coupland, 1965*b*).

Between Day 8 and 26 days there is a fivefold increase in the volume of neuronal tissue (Table 3) and at the same time it achieves a Vv of 10% of medullary contents – higher than in any other age group examined. These changes are associated with the appearance of myelinated nerve fibres and a marked increase in Schwann cell cytoplasm, which is intimately associated with the many axonal profiles, with medullary neurons and often with chromaffin cells. Close cellular contact, free from basement membranes, is achieved between all these tissue components. It is likely that the increase in Vv of neuronal tissue at 26 days is due, at least in part, to the increase in size and complexity of supporting Schwann cells, including the myelination of a proportion of nerve fibres; it must also relate to the increase in the mean number of

synapses per cell. The marked rise in ChAT activity which reaches adult levels at 26 days cannot be explained simply on grounds of the increase in the number of synaptic profiles in the adrenal medulla (Table 7) and could represent an increased activity per ending, the onset of activity in newly forming endings necessary to innervate the increase in number of chromaffin cells (which doubles between 26 days and 12 weeks, Table 7), or increased activity in adrenal medullary neurons or in axon terminals associated with them.

The number of chromaffin cells in the adrenal medulla increases throughout life and in adult rats between 12 weeks and 22 months it increases by some 60%. Throughout this period neuronal tissues remain proportionally similar in volume to chromaffin cells. Thus new synaptic endings are formed as the cells multiply though there is no evidence of hyperinnervation of the hyperplastic chromaffin cells, even though the latter show bizarre cytoplasmic changes including granule depletion, marked hypertrophy and the formation of phagolysosomes (Coupland & Tomlinson, 1989). These latter changes may result from the increased adrenal sympathetic nerve activity in aged rats reported by Ito *et al.* (1986).

The morphological changes observed in synaptic nerve endings during the first 8 days of life are in keeping with the mainly pharmacological observations of Slotkin and co-workers (see Slotkin, 1986) on the unresponsiveness of adrenal chromaffin cells in the rat to neurogenic stimuli during the first week after birth. They are also in keeping with the physiological findings of Smith, Slotkin & Mills (1982), Smith, Evoniuk, Poston & Mills (1983) and Mills & Smith (1986) that the rate-limiting step in sympathetic neurotransmission is the maturation of ganglionic synaptic transmission. Tonic sympathetic preganglionic impulse frequency and response to centrally acting stimuli such as hypoglycaemia are already comparable with those in adult rats though tonic postganglionic sympathetic activity in the cardiovascular system is barely detectable.

The presence of highly electron-dense material in some of the tubulovesicular profiles of the immature synapses raises the question: do the preganglionic cholinergic neurons innervating chromaffin cells, in common with neurons innervating rat sweat glands (Leblanc & Landis, 1986) and primary sensory neurons (Katz & Black, 1986), express a transient catecholaminergic trait as they develop?

It is now generally accepted that the moderately electron-dense granular vesicles in synaptic nerve endings contain neuropeptides and in the case of synaptic endings on adrenal chromaffin cells metenkephalin has been localised by Kobayashi, Ohashi, Uchida & Yanaihara (1983). It is interesting to note, therefore, that the appearance of these granular vesicles during the first four days after birth coincides with the first appearance of metenkephalin in nerve endings in the rat adrenal medulla (Kent & Coupland, 1989).

Although the presence of neurons in the adrenal medulla has been recognised for many years and can always be demonstrated in serial sections of many common laboratory and domestic animals (Coupland, 1965*a*) no ultrastructural studies of these elements appear to have been published. The neuronal somata and their processes give a strongly positive reaction for acetylcholinesterase (Coupland & Holmes, 1958; Coupland, 1965*a*) and for vasoactive intestinal peptide (VIP; Holzwarth, 1984). Furthermore the findings of Holzwarth (1984) suggest that the VIP-containing nerve fibres in the adrenal medulla and outer parts of the adrenal cortex arise, at least in part, from the medullary neurons.

In the present work small groups of adrenal medullary neurons have been identified in specimens of all age groups. They are closely packed with neuronal somata

separated by an intercellular gap of only 15–20 nm in some situations and by a similar close relationship with the plasmalemma of Schwann cells in others. Where neurons are apposed intercellular canaliculi similar to those which exist between chromaffin cells (Coupland 1965*c*) have been observed (Figs. 13, 20). The medullary neurons are innervated by occasional axosomatic and numerous axodendritic synapses, the synaptic endings containing small clear synaptic vesicles that are occasionally closely packed (as in the axosomatic synapse of Figure 12) and more commonly loosely aggregated together with a variable number of larger granular vesicles (about 100 nm) which have moderately electron-dense contents. Groups of neurons and associated Schwann cells are separated from connective tissue spaces by a distinct basement membrane similar in appearance and arrangement to that associated with chromaffin cells and Schwann cells (Coupland, 1965*c*). With age lipofuchsin granules become increasingly numerous in the neuronal cytoplasm, otherwise the most prominent cytoplasmic features are polyribosomes, small aggregates of rough endoplasmic reticulum, mitochondria and lysosomes.

The nature of these medullary neurons is currently an enigma. In view of the developmental origin of the adrenal medulla it is clearly possible that they arise from thoracolumbar neural crest and are peripheral sympathetic neurons. However, the possibility exists that as with the enteric neurons and some other peripheral neurons associated with abdominal viscera, they are parasympathetic elements that originated from brainstem neural crest. Recently the vagal innervation of the adrenal gland in the guinea-pig and rat has been demonstrated (Coupland, Parker, Kesse & Mohamed, 1989). Furthermore the innervation by neurons whose somata lie in the dorsal nucleus of the vagus is greater in the guinea-pig, which has a larger number of adrenal medullary neurons. Thus a vagal innervation becomes a distinct possibility. A final answer must await the results of experimental procedures including nerve fibre tracing and use of immunohistochemistry.

No evidence of an adrenergic innervation of chromaffin tissue of the adrenal medulla has been obtained during the present work, though the elongated processes of SGC cells could readily be misinterpreted as adrenergic nerve fibres when the only organelles evident are storage granules; this applies particularly if they include an aggregate of the small synaptic-like vesicles (SSV) with electron-dense contents which were first described in the adrenal SGC cells of the mouse (Kobayashi & Coupland, 1977). Small granular chromaffin cells can be identified in all specimens after the age of four days due to the increase in diameter of chromaffin granules (Table 5) in typical A and NA cells after that time and the relative small amount of rough endoplasmic reticulum in A and NA cells (particularly the latter) prior to old age. Small synaptic-like vesicles are not usually observed in younger adult specimens in rats though they are a characteristic feature of SGC cells in aged rats and are occasionally observed in hyperplastic chromaffin cells which contain typical NA or mixed NA and A storage granules. The most useful criteria for distinguishing the elongated profile of an SGC cell from that of an adrenergic axon is the absence of neurotubules, the presence of normal (large) mitochondria typical of chromaffin cells (Fig. 23) and the absence of small mitochondria typical of nerve endings.

Appearances suggest that the contents of SSV as well as amine storage granules are normally discharged through the cell membrane into tissue spaces. However, the occasional aggregates of SSV adjacent to symmetrically thickened cell membranes (Fig. 24) suggests the possibility of synaptic transmission. To date such aggregates and membrane densities have only been observed with respect to SGC and NA cells.

Since the work of Thoenen, Müller & Axelrod (1969) on the trans-synaptic

induction of adrenal tyrosine hydroxylase, much further evidence has accumulated on the importance of neuronal transmission in the regulation of enzymes involved in the synthesis of adrenal catecholamines. It is of interest to note that present findings demonstrate that in the normally innervated gland the relative proportions of adrenaline and noradrenaline in the adrenal glands correlate well with the relative proportions of A and NA cells from 7 days of life to old age (Table 9) even though a small amount of noradrenaline is stored in A cells (Verhofstad, Coupland, Parker & Goldstein, 1985). It is also of interest to note that throughout adult life the amine content of the A granule remains constant (Table 10) even though in old age cellular hypertrophy results in an increase in the amine content of A and NA cells.

Throughout adult life the number of synaptic endings on A and NA cells remains essentially constant at roughly 5 per cell. SGC cells are similarly innervated but since they form a relatively small number (less than 1% of chromaffin cells in the rat) meaningful quantitation is difficult. Since axonal profiles were only counted as synaptic when they possessed synaptic vesicles and exhibited zonal membrane densities, the calculated number per cell is almost certainly an underestimate. Nevertheless, it is of interest to note that the results are very similar to those of Case & Matthews (1985) who reported 3–6 afferent nerve terminals per catecholamine-storing small granule-containing cell in the rat superior sympathetic ganglion.

The functional nature of the five or more nerve endings (afferent) on each chromaffin cell is still to be determined. In addition to the vagal innervation referred to above, a significant spinal sensory innervation of the adrenal gland has recently been demonstrated in the guinea-pig (Mohamed, Parker & Coupland, 1988) and rat (Afework, 1988) in which a mean of 164 dorsal root ganglion cells were labelled by an *in vivo* Fast Blue injection tracing procedure that results in the labelling of a mean of 698 preganglionic sympathetic neurons (Kesse *et al.* 1988). Hence it is possible, indeed likely, that some of the endings observed are those of neurons whose cell bodies lie in the dorsal root ganglion or in the vagal ganglia.

The latter view is strengthened by the physiological and pharmacological observations of Khalil, Marley & Livett (1984) and Khalil, Livett & Marley (1986, 1987) on the role of sensory fibres in adrenal medullary secretion. Although many synaptic endings on chromaffin cells appear typical of preganglionic cholinergic endings many profiles are less typical. This may be the consequence of chance section through pre-synaptic as compared with synaptic parts of axons. Are the profiles in which aggregates of small mitochondria occur, sometimes together with synaptic membrane thickenings, a different functional type of ending, possibly sensory? The answer to this question will await the simultaneous ultrastructural localisation of different neuropeptides characteristic of preganglionic efferent and sensory neurons, together with nerve fibre tracing.

Previous observations on the sympathetic preganglionic innervation of the adrenal gland using retrograde nerve fibre tracing following the injection of Fast Blue into the adrenal medulla (Parker *et al.* 1988) have shown that in the Wistar rat the number of labelled neurons decreases from about 890 in two days old animals to about 700 in young adults. This decrease is not unexpected in the light of cell death reported in many neuronal groups during development. However, since it is likely that the majority of synaptic endings on chromaffin cells are provided by the telodendria of the axons of some 700 neurons, the implication is that each preganglionic neuron innervates some thousand chromaffin cells. A single typical cholinergic-type ending is occasionally seen innervating adjacent A or NA cells and, as indicated above, up to 5 separate endings have been observed on a single chromaffin cell. During this work

a single ending has not been observed to innervate simultaneously adjacent A and NA cells. The presence of trail-like endings on some A and NA cells adds support to the suggestion based on light microscopic findings using cholinesterase histochemistry (Coupland 1965*a*) that some telodendria may pass from cell to cell proving *boutons de passage*, while others end in terminal synapses.

It is of interest to note that no nerve fibres containing synaptic vesicles have been observed to be specifically related to the blood vessels – medullary arteries, capillaries or venous sinuses – in the specimens examined. In the same specimens an association of nerve fibres containing neuro-peptidergic and/or adrenergic storage vesicles is not infrequently observed (Coupland, unpublished), particularly in the outer zones of the cortex and the capsule.

SUMMARY

The innervation of the adrenal medulla has been investigated in normal Wistar rats from birth to old age and ultrastructural findings compared with biochemical markers of the cholinergic innervation of the adrenal gland and catecholamine storage.

Morphological evidence of the immaturity of the innervation during the first postnatal week is provided and using quantitative morphometry the innervation of chromaffin cells is shown to reach a mean total of 5·4 synapses per chromaffin cell during the period 26 days to 12 weeks of age.

The variation in contents of synaptic profiles is discussed in the light of recent work that demonstrates a major sensory as well as visceral efferent innervation of the gland.

Adrenal medullary neurons usually occur in closely packed groups, intimately associated with Schwann cells. Axodendritic and axosomatic synapses on these neurons are described and the likely origin of axonal processes innervating the neurons discussed.

In old age the density of innervation remains the same as in young adult animals even though the medulla shows evidence of hyperplasia and hypertrophy of individual chromaffin cells.

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