J. Anat. (1970), **106**, 2, pp. 213-231
With 33 *figures*
Printed in Great Britain

Electron microscopic observation on the adrenal medulla and extra-adrenal chromaffin tissue of the postnatal rabbit

R. E. COUPLAND AND BRENDA S. WEAKLEY

Department of Human Morphology, University of Nottingham and Department of Anatomy, University of Dundee

(*Received* 24 *February* 1969)

Previous work using both light (Coupland, 1956, 1965*a*) and electron microscopy (Coupland & Weakley, 1968) has demonstrated the presence of primitive sympathetic cells, phaeochromoblasts and typical chromaffin cells during foetal life in the pre-aortic region and in the vicinity of the developing adrenal gland. Although no electron-dense granular inclusions of a type which corresponds to typical chromaffin granules were observed in primitive sympathetic cells they were present in small numbers in phaeochromoblasts and were abundant in typical chromaffin cells. The mean size of adrenal medullary chromaffin granules increased from *c.* 60 nm in 14 d-old foetuses to 148 nm (adrenaline storing) or 86 nm (noradrenaline storing) in 28 d-old foetuses while the extra-adrenal granules remain at a mean diameter of 62 nm.

After initial fixation in glutaraldehyde and post-osmication adrenaline-storing (A) granules exhibit moderate electron-density with evidence of a fine internal granularity while noradrenaline-storing (N) granules are highly and usually homogeneously electron-dense (Coupland & Hopwood, 1966). Applying this tenchnique to foetal chromaffin tissue and comparing the results with amine assay it was possible to show that the extra-adrenal chromaffin cells of the foetus store almost only noradrenaline while both A and N granules can be identified in adrenal chromaffin cells of 20-d foetal and later prenatal specimens, the relative number of A granules increasing with age. In 28-d-old foetuses and early postnatal specimens many chromaffin cells were observed which contained only A granules (Coupland & Weakley, 1968).

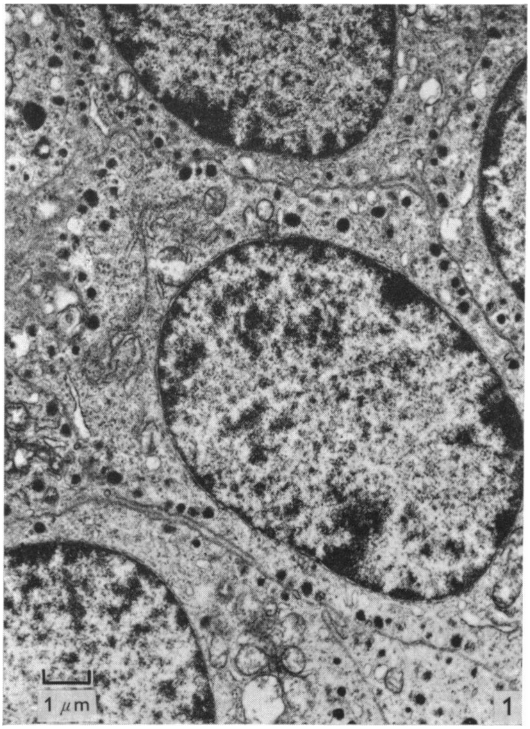
Typical cholinergic-type nerve endings were observed on adrenal chromaffin cells but not on extra-adrenal elements.

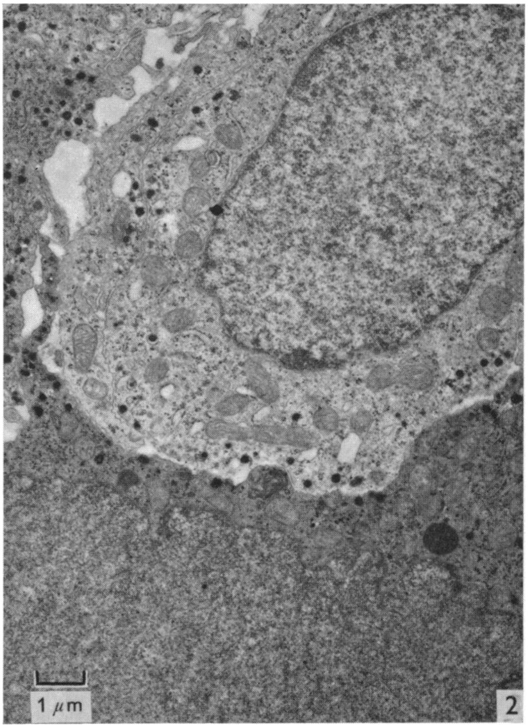
During the last 4 days of foetal life specimens exhibited a similar variety of cytoplasmic organelles to those described in the adrenal medulla of the adult rat (Coupland, 1965*b*).

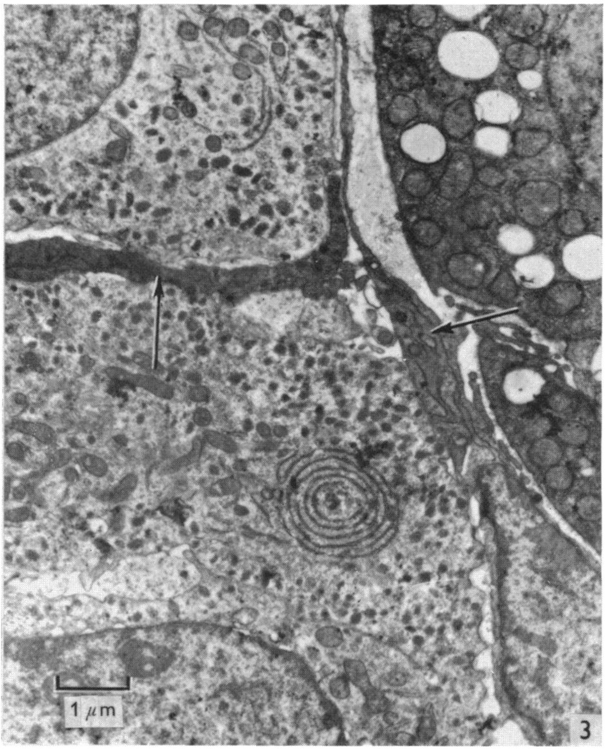
The present work relates to the changes within adrenal and extra-adrenal chromaffin cells during the period between birth and 3 months of age.

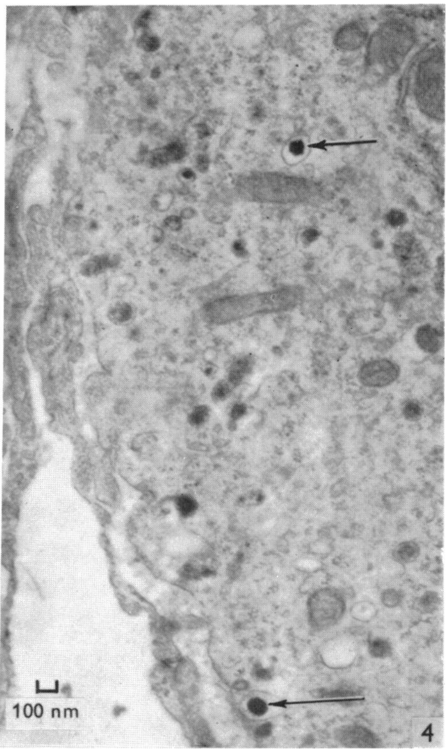
MATERIAL AND METHODS

Three or more specimens aged 1, 9, 15, 23 and 25 d, 6 weeks and 3 months were processed for light and electron microscopy using the methods described previously (Coupland & Weakley, 1968). Light microscopic sections were stained routinely with haemotoxylin and eosin or toluidine blue. Glycogen was identified by the periodic acid-Schiff reaction and by staining with Best's carmine.


1 μm
1


1 μm
2


1 μm
3


100 nm
4

For electron microscopy fixation in glutaraldehyde with postosmication was usually followed by staining on the grid with lead citrate and uranyl acetate. Some sections were stained with potassium permanganate. The results were compared with assay findings (Coupland & MacDougall, 1966) as a further criterion for the differentiation of A and N storing granules.

The size distribution of chromaffin granules in various specimens was determined from photographic prints having a magnification of × 24000.

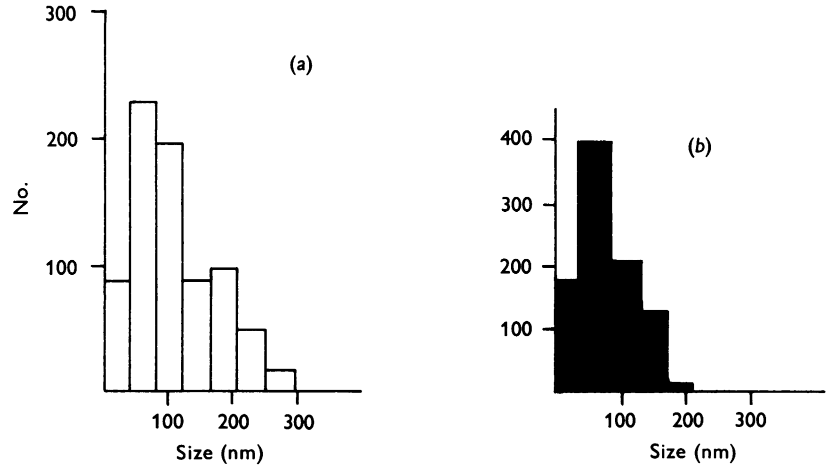


Fig. 5. Histogram showing size-distribution of profiles of (*a*) adrenal and (*b*) extra-adrenal chromaffin granules.

RESULTS

Light microscopy was used mainly for orientation and identification of chromaffin elements and no features other than those detailed previously (Coupland, 1956, 1965*a*) were observed. Glycogen was not observed in chromaffin cells after birth. The following observations relate to the ultrastructure of chromaffin cells in the various specimens.

1-*d post-partum*

Adrenal medulla

Chromaffin cells are similar to those observed in 6 h post-partum specimens (Coupland & Weakley, 1968). Nuclei are mainly oval and contain irregularly dispersed clumps of chromatin. As compared with foetal specimens nucleoli are no longer a prominent feature (Fig. 1).

Variations in electron density of elements allows the differentiation of light and

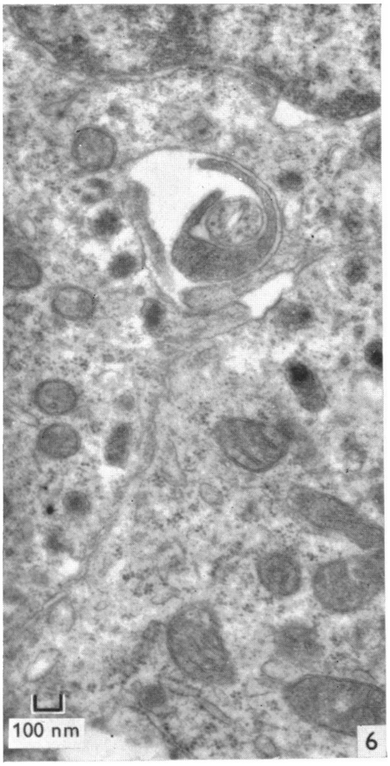
---

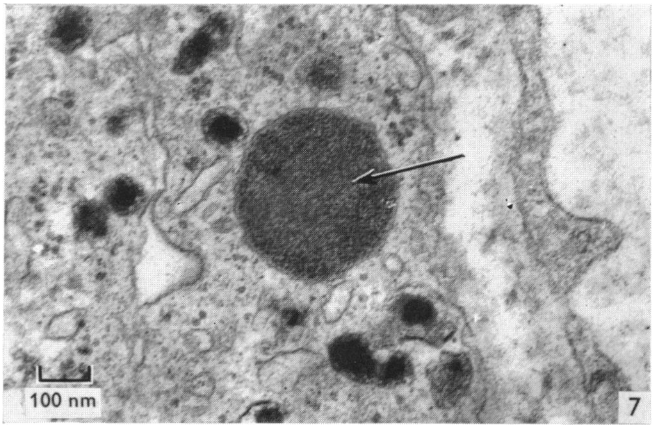
Fig. 1. Noradrenaline-storing adrenal chromaffin cells of l-d-old rabbit.

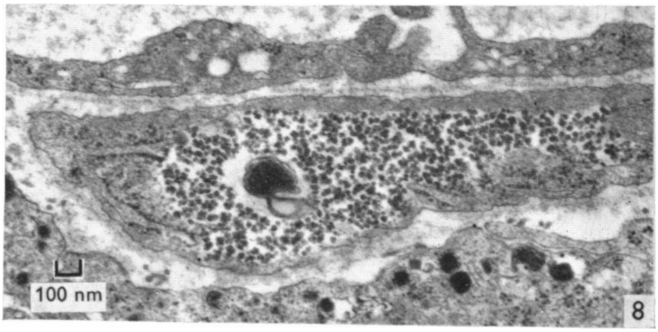
Fig. 2. Dark and light noradrenaline-storing chromaffin cells of 1-d-old rabbit adrenal medulla.

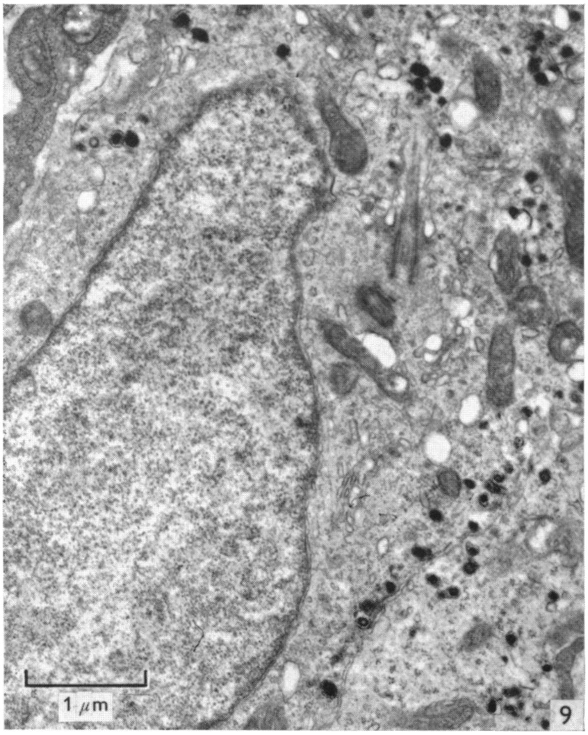
Fig. 3. Cortico-medullary junction of 1-d-old rabbit adrenal. Arrows indicate Schwann cell cytoplasm. Cortical cells lie to the right.

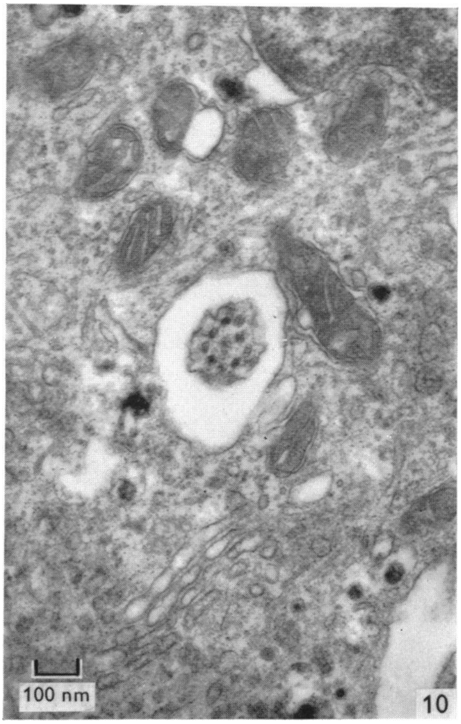
Fig. 4. Adrenal chromaffin cell of a 1-d-old rabbit. Most of the granules are adrenaline-storing but those indicated by arrows store noradrenaline.


100 nm
6


100 nm
7


100 nm
8


1 μm
9


100 nm
10

dark chromaffin (Figs. 2, 3), cortical and Schwann cells. Some electron-dense (dark) cortical cells in the juxta-medullary zone contain large cytoplasmic vacuoles and myelin figures. No primitive sympathetic cells have been observed.

Chromaffin granules have an average profile diameter of 108 nm and in size extend to 294 nm (Fig. 5). They tend to be peripherally distributed (Figs. 1, 2). The majority are moderately electron-dense A granules but some are highly electron-dense N granules. Mixtures of A and N granules may occur in the same cell (Fig. 4) and in such cells A granules are usually the most abundant.

Chromaffin cells are often covered externally by tongues of Schwann cell cytoplasm (Fig. 3) and this may enclose fine nerve fibres. Fine nerve fibres and ensheathing Schwann cell cytoplasm may occasionally be traced into intercellular spaces (Fig. 6). At points of close apposition plasma membranes of Schwann and chromaffin cells are separated by a gap of *c.* 20 nm and no basement membrane intervenes. By contrast basement membrane clothes the surface of both chromaffin cells and Schwann cells where these are exposed to connective tissue spaces.

Free ribosomes are often observed singly or in clusters in chromaffin cells (Fig. 7). Ribosomes are also commonly associated with endoplasmic reticulum. The cisternae of the endoplasmic reticulum may be irregularly scattered, arranged in stacks or in circular arrays (Fig. 3). They may be associated with ribosomes but in some areas the latter are absent or very sparsely distributed along the membranes (Fig. 6).

Mitochondrial profiles may be rounded or elongated, and have the typical form with a moderately dense matrix and cristae which may run transversely, longitudinally (Fig. 6) or obliquely (Figs. 2–9).

Dense bodies having the typical appearance of lysosomes (Fig. 6) are often observed. The Golgi membranes form a moderately compact group of vesicles and lamellae. Some of the vesicles contain moderately electron-dense granular material but no highly electron-dense contents have been observed in day-old specimens. Cilia are commonly encountered and have a similar structure and attachments to those described and illustrated below.

Although glycogen has not been identified in chromaffin cells by either light or electron microscopy it occurs in pericapillary cells associated with the medial part of the adrenal medulla, i.e. the region of junction between intra- and extra-adrenal chromaffin tissue (Fig. 8).

At the cortico-medullary boundary chromaffin and cortical-cells may be separated

---

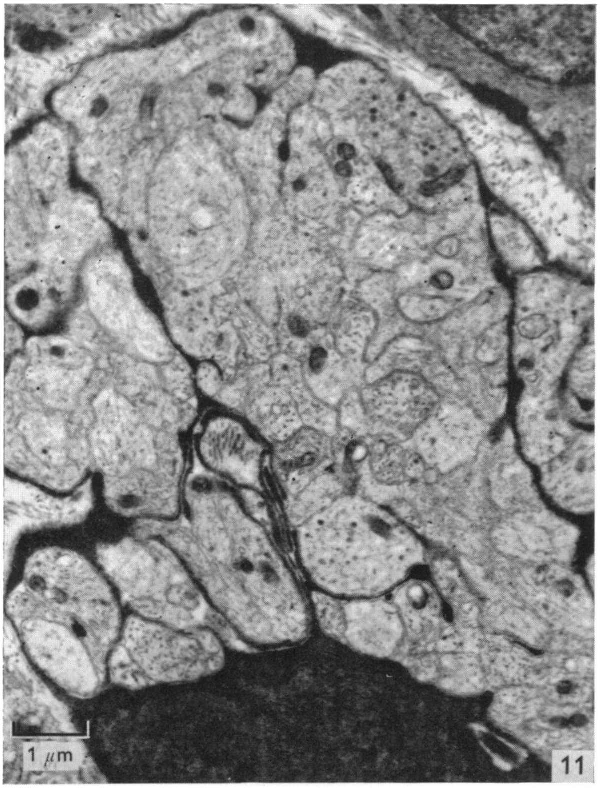
Fig. 6. Adrenal chromaffin cells of 1-d-old rabbit. Granules are adrenaline-storing. Note the fine nerve fibre and surrounding Schwann cell cytoplasm in an intercellular space.

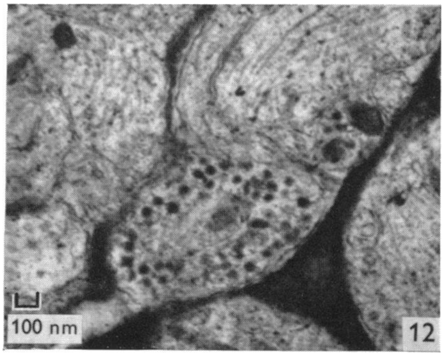
Fig. 7. Adrenaline-storing adrenal chromaffin cell of a 1-d-old rabbit containing a dark body (probably a lysosome) indicated by arrow. Endothelium of a capillary (right) exhibits pores bridged by a single membrane. Note basement membranes adjacent to both the chromaffin and endothelial cells. Ribosomes are usually free and may form chains or clusters.

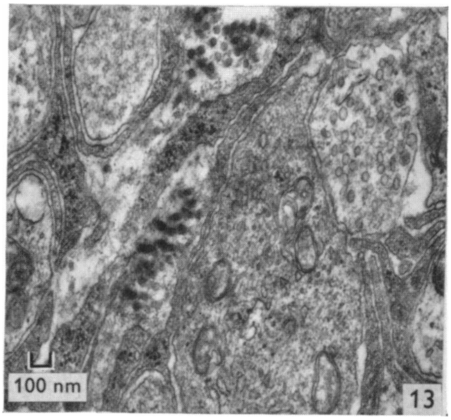
Fig. 8. Capillary (above) from the medial extremity of the adrenal medulla of a 1-d-old rabbit. A pericapillary cell contains glycogen granules.

Fig. 9. Noradrenaline-storing extra-adrenal chromaffin cell. Note perinuclear zone free from inclusion granules; this region contains centrioles with a cilium arising from the distal centriole, some Golgi vesicles and mitochondria.

Fig. 10. Noradrenaline-storing extra adrenal chromaffin cell showing Golgi membranes and cytotubules (or filaments). A cilium with a 9+2 fibril pattern lies in an intracellular tunnel.


1 μm
11


100 nm
12


100 nm
13


100 nm
14


100 nm
15

by blood sinuses or by a thin layer of connective tissue elements—in particular fibroblast processes and scattered collagen fibres.

Typical cholinergic-type nerve endings containing large numbers of small clear-centred (*c.* 40 nm diameter) vesicles and small numbers of larger vesicles (*c.* 80 nm diameter) which possess electron-dense granular central inclusion have been seen. At the point of contiguity plasma membranes of nerve fibre and chromaffin cells approach to *c.* 20 nm but pre- and postsynaptic thickenings of membranes have not been observed in this age group.

Extra-adrenal chromaffin tissue

Chromaffin cells are fusiform and usually separated from each other by gaps of *c.* 20 nm. Intercellular spaces are, however, common and may appear empty or contain occasional collagen fibres. Nuclei of chromaffin cells may be ovoid, circular or irregular (Fig. 9) in profile and background nucleoplasm is usually more uniformly granular than that of adrenal chromaffin cells. Both light and dark chromaffin cells occur.

Cytoplasmic organelles resemble those of the chromaffin cells of the adrenal medulla. Chromaffin granules have a mean diameter of 76 nm and a maximum size of 168 nm (Fig. 5). The majority (90 %) are homogeneous highly electron dense noradrenaline-storing elements and they are mainly distributed around the periphery of the cells. A perinuclear zone containing Golgi membranes and mitochondria is usually free from chromaffin granules (Fig. 9).

Cilia are commonly associated with chromaffin cells and extend from the distal centriole (Fig. 9) which forms the basal body into intracellular tunnels and less commonly into intercellular spaces. They may have the typical 9+2 fibril pattern of motile cilia (Fig. 10) or possess smaller numbers of fibrils with one pair lying in a central or paracentral position. Cytotubules or filaments *c.* 18 nm diameter are often seen in the vicinity of the Golgi zone (Fig. 10).

Schwann cell cytoplasm is moderately electron-dense and encloses unmyelinated nerve fibres (Fig. 11). It also occasionally covers the surface of chromaffin cells having a similar relationship to the chromaffin elements to that observed in the adrenal medulla. Unmyelinated nerve fibres usually contain neurofibrils and neurotubules

---

Fig. 11. Pre-aortic nerve fibres of a 1-d-old rabbit containing neurotubules, neurofilaments, mitochondria and in some cases vesicles with electron-dense centres. Note electron-dense Schwann cell cytoplasm.

Fig. 12. Pre-aortic nerve fibre of a 1-d-old rabbit containing nerve vesicles with highly electron-dense centres, these probably store noradrenaline.

Fig. 13. Axo-dentritic synapse on neuron lying adjacent to extra-adrenal chromaffin body of 9-d-old rabbit. Axon contains many clear centred small vesicles and two larger vesicles which show moderately electron-dense inclusions.

Fig. 14. Nerve fibres containing small clear centred synaptic vesicles and larger vesicles with electron-dense centres surrounded by Schwann cytoplasm in the main extra-adrenal chromaffin body of a 9-d-old rabbit.

Fig. 15. Nerve fibre partially covered by electron-dense Schwann cell cytoplasm and containing neurofibrils, neurotubules and electron-dense granular inclusions lying in contiguity with an extra-adrenal chromaffin cell in a 1-d-old rabbit.

but some possess the dense-cored vesicles 40–80 nm diameter and small mitochondrial profiles typically found in adrenergic nerve endings (Figs. 11, 12).

Nerve endings have not been observed on extra-adrenal chromaffin cells at this stage.

Glycogen has been observed within pericapillary cells but not in chromaffin cells.

Fibroblasts and scattered collagen fibres are occasionally observed in perivascular and intercellular spaces but do not form a prominent feature.

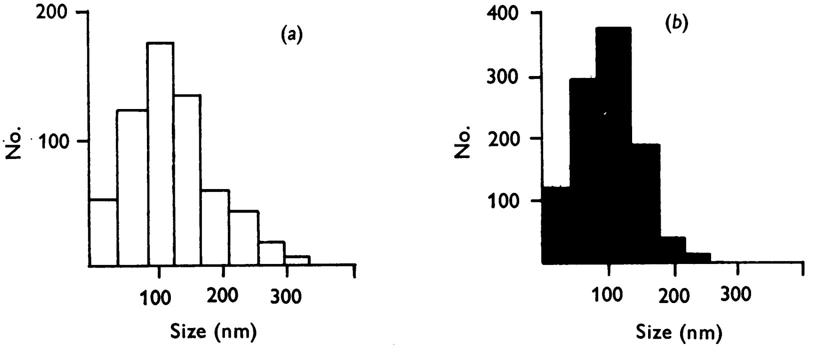


Fig. 16. Histogram showing size-distribution of chromaffin granule profiles of (*a*) adrenal medulla and (*b*) extra-adrenal chromaffin body of a 9-d-old rabbit.

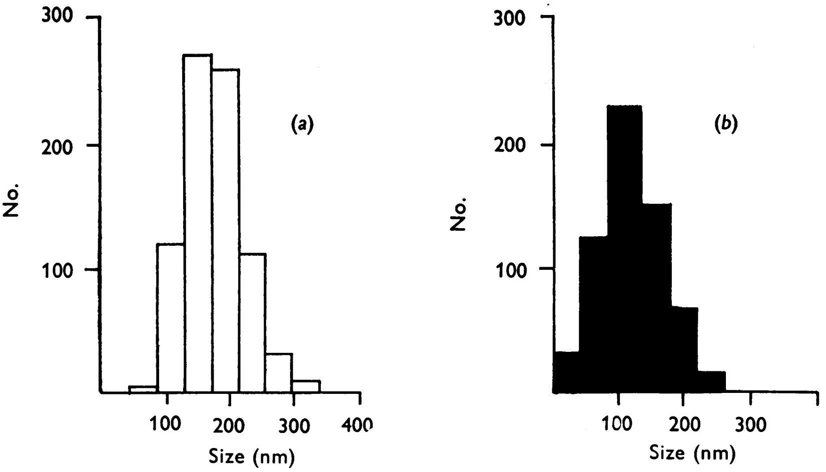


Fig. 17. Histogram showing size-distribution of chromaffin granule profiles of (*a*) adrenal medulla and (*b*) extra-adrenal chromaffin body of a 15-d-old rabbit.

2–15 *d post-partum*

The general appearance and arrangement of both adrenal and extra-adrenal chromaffin cells is similar to that of day-old specimens. Dark cells are less numerous and rarely observed in specimens aged more than 9 d post-partum. Granules of both intra- and extra-adrenal chromaffin cells show an increase in mean profile diameter and range as compared with those of 1-d-old specimens (Table 1 and Figs. 16, 17).

Adrenal medulla

In adrenal chromaffin cells secretion granules usually show moderate electron density and fine internal granularity typical of adrenaline storage. A few cells contain mixtures of moderate and highly electron-dense granules. Small highly electron-dense inclusions have never been observed within the Golgi membranes of adrenaline-storing adrenal chromaffin cells though larger inclusions having moderate or high electron density have been observed in cells which contain mixtures of A and N granules.

Table 1. *Profile diameters of chromaffin granules of post-natal rabbits*

	Adrenal		Extra-adrenal	
Age	Av. diam. (nm)	Range (nm)	Av. diam. (nm)	Range (nm)
1 d	108	0–294	76	0–168
9 d	122	0–336	94	0–252
15 d	176	0–336	114	0–252
6 wks	204	0–378	137	0–462
9 wks	204	0–420	171	0–378

Extra-adrenal chromaffin cells.

In 9-d-old and older specimens plasma membrane thickenings on chromaffin cells typical of desmosomes have occasionally been observed on adjacent chromaffin cells. A single unmyelinated nerve fibre containing synaptic vesicles and mitochondria has been observed lying partly embedded in Schwann cell cytoplasm and near to a chromaffin cell (Fig. 14). This represents the closest approach to what is usually described as a 'typical' synapse on an extra-adrenal chromaffin cell seen during the present work. Contiguity between an extra-adrenal chromaffin cell and a nerve fibre containing neurotubules, mitochondria and large granular vesicles has also been observed on one occasion in the same specimen (Fig. 15). Axo-dendritic synapses have been observed on preaortic sympathetic neurons (Fig. 13).

During this period the number of collagen fibres lying in perivascular and intercellular regions increases slightly as compared with earlier specimens.

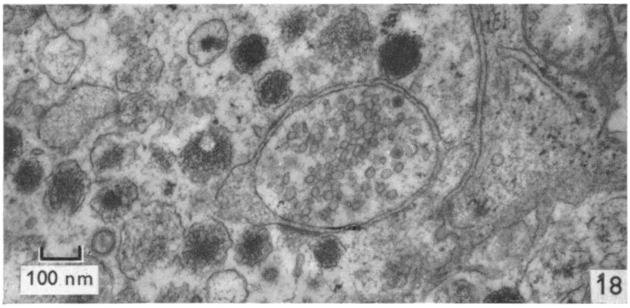
23 *d to* 3 *months post-partum*

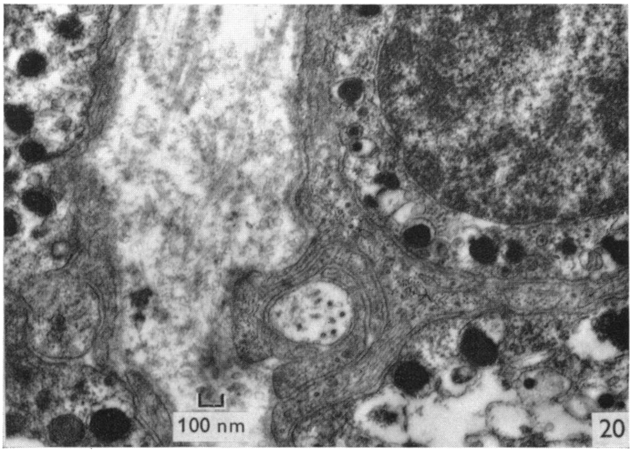
No dark chromaffin cells have been observed during this period. The general form of the cells resembles that of earlier specimens.

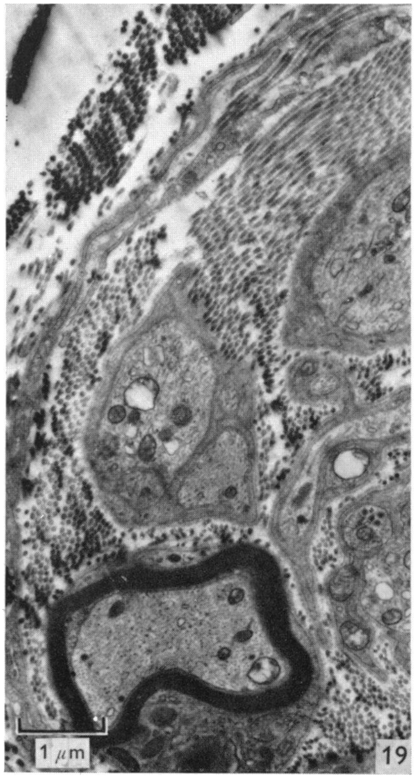
Adrenal medulla

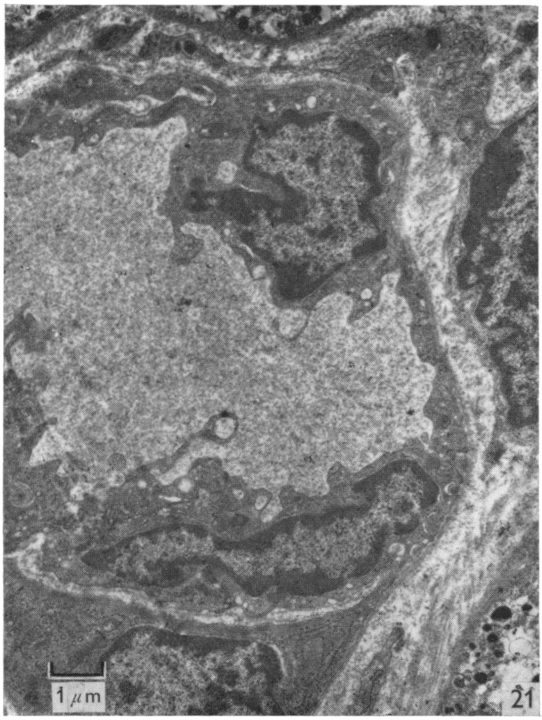
Adrenal chromaffin cells retain the close-packed epithelial arrangement; a slight increase in the number of collagen fibres in perivascular regions and in the cortico-medullary junctional region is observed but does not form a striking feature.

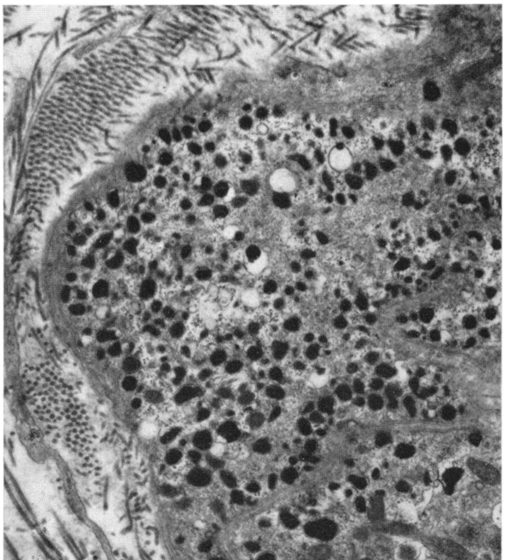
Chromaffin granules of the adrenal medulla conform to the moderately electron-dense adrenaline-storing variety (Figs. 18, 25) and these increase in mean profile size and range throughout the period (Table, 1; Figs. 23, 24).


100 nm
18


100 nm
20


1 μm
19


1 μm
21


1 μm
22

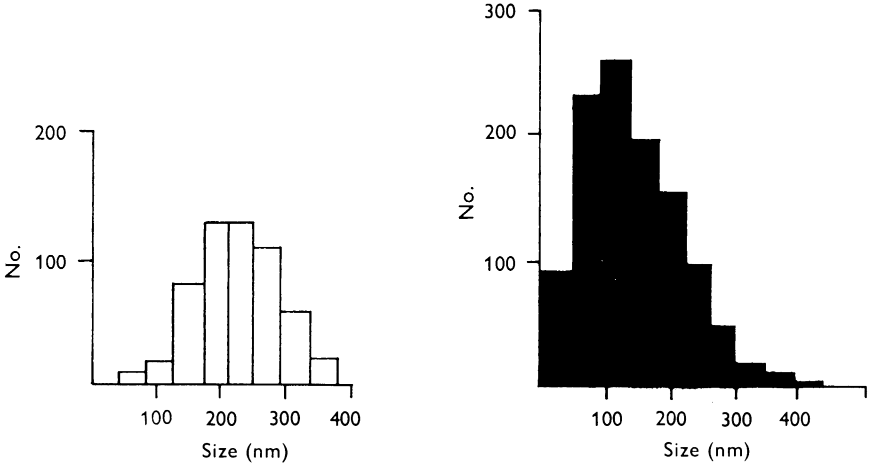


Fig. 23. Histogram showing size distribution of chromaffin granule profiles of (*a*) adrenal medulla and (*b*) extra-adrenal chromaffin body of a 6-week-old rabbit.

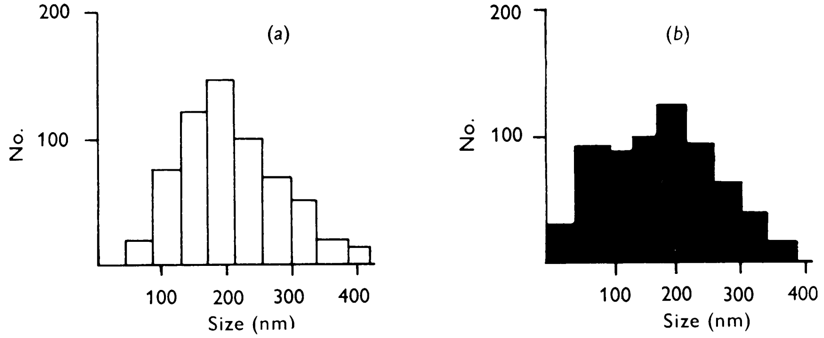


Fig. 24. Histogram showing size distribution of chromaffin granule profiles of (*a*) adrenal medulla and (*b*) extra-adrenal chromaffin body of a 3-month-old rabbit.

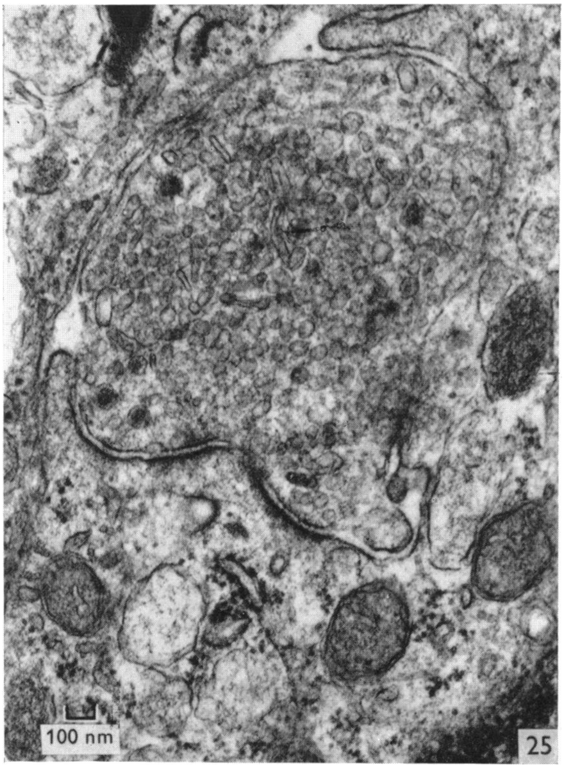
Fig. 18. Adrenaline-storing adrenal chromaffin cell of 25-d-old rabbit with invaginated cholinergic nerve fibre with synaptic vesicles. Pre- and postsynaptic membrane thickenings are evident.

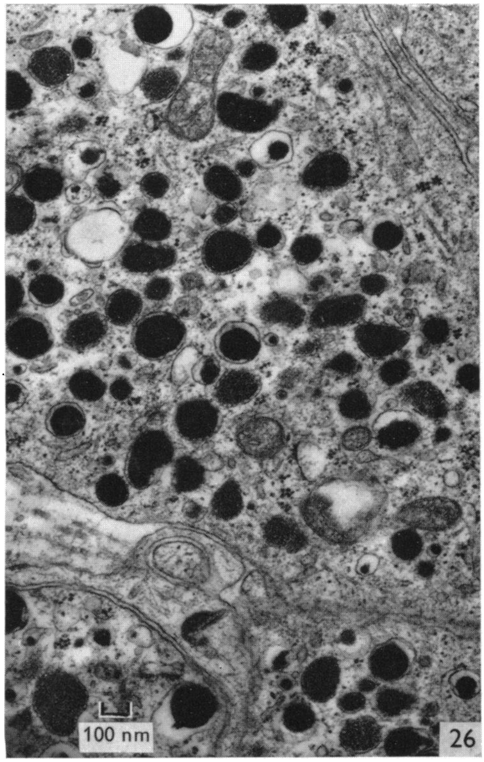
Fig. 19. Preaortic nerve fibres of a 3-month-old rabbit. Only one fibre is myelinated. Note large numbers of collagen fibres lying between nerve fibres which are covered by Schwann cell cytoplasm.

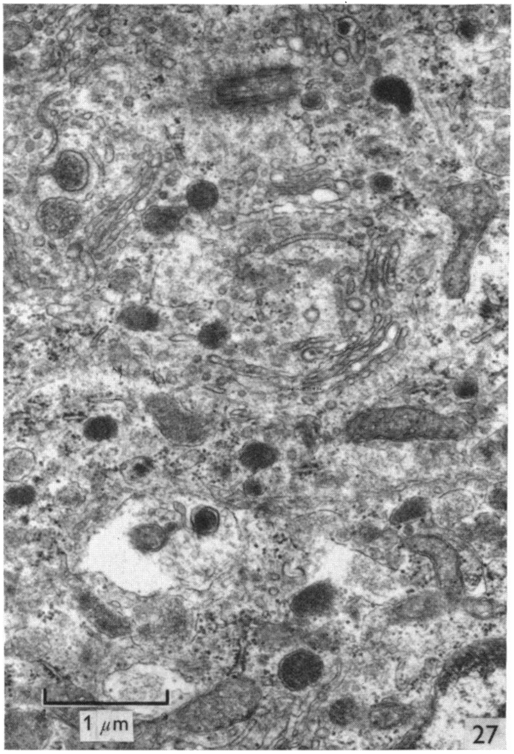
Fig. 20. Schwann cell cytoplasm covering the surface of an extra-adrenal chromaffin cell in a 6-week-old rabbit. The Schwann cell envelopes a fine nerve fibre. A basement membrane separates Schwann cell cytoplasm from adjacent connective tissue spaces and these contain collagen fibres.

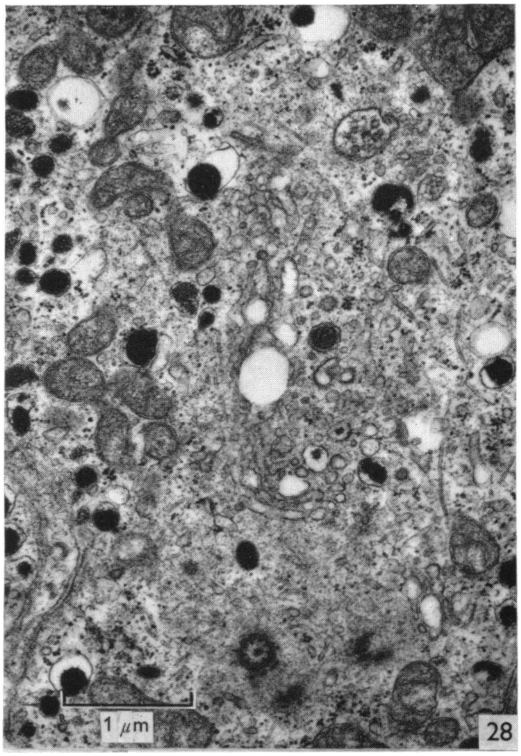
Fig. 21. Capillary blood vessel with pericapillary cell lying in the main preaortic extra-adrenal chromaffin body of a 6-week-old rabbit. Note wide space containing collagen fibres between the endothelial and chromaffin cells.

Fig. 22. Extra-adrenal chromaffin cells of a 6-week-old rabbit. Schwann cell cytoplasm covers the external surface of the cells and extends between two adjacent cells from below. Note numerous collagen fibres; some of these (below) are invaginated into Schwann cell cytoplasm.


100 nm
25


100 nm
26


1 μm
27


1 μm
28

The Golgi membranes of A cells of the adrenal medulla show no evidence of highly electron-dense noradrenaline-containing inclusions (Fig. 27).

Typical cholinergic-type nerve endings or synapses which exhibit both pre- and postsynaptic thickenings (Fig. 25) have been observed on adrenal chromaffin cells. Some synaptic regions are invaginated into chromaffin cells cytoplasm (Fig. 18) although the plasma membranes remain intact.

Extra-adrenal chromaffin cells

There is an increase in fibrous connective tissue in association with extra-adrenal chromaffin cells and in 3-month-old specimens large numbers of collagen fibres may be identified around the periphery of groups of nerve fibres (Fig. 19), around blood vessels (Fig. 21) and around collections of chromaffin cells (Fig. 22).

Although fine nerve fibres have been identified ensheathed by the cytoplasm of Schwann cells which cover the external surface of some extra-adrenal chromaffin cells (Fig. 20) synaptic contacts between nerve fibres and extra-adrenal chromaffin cells have not been observed. Groups of collagen fibres are occasionally ensheathed by Schwann cell cytoplasm (Fig. 22).

More than 90 % of the secretion granules present in extra-adrenal chromaffin cells are N storing (Figs. 20, 22, 26 and 28)—the remainder, which contain only moderate electron dense inclusions and show internal granularity, may be A or N granules which have lost their catecholamine (Figs. 26, 33). Extra-adrenal chromaffin granules increase in mean profile diameter and range during this period (Figs. 23, 24) and in 3-month-old specimens the size distribution histograms of intra- and extra-adrenal chromaffin granules are more alike (Fig. 24) than histograms of the two groups in younger specimens.

Some golgi membranes in N-storing extra-adrenal cells contain small highly dense inclusions which probably contain noradrenaline (Fig. 28); in addition moderately electron dense inclusions which exhibit fine internal granularity are also seen in some sections through this region. Multivesicular bodies and lysosomes are occasionally observed in both intra- and extra-adrenal cells.

Ribosomes may be observed in small clusters while others are associated with the rather scanty endoplasmic reticulum which may form irregular strands, lamellae or concentric arrays. In 25-d-old and older specimens, in addition to typical ribosomes electron-dense granules of *c.* 30 nm diameter (Figs. 29, 33) are observed in both intra- and extra-chromaffin cells but more commonly in the latter. These granules may

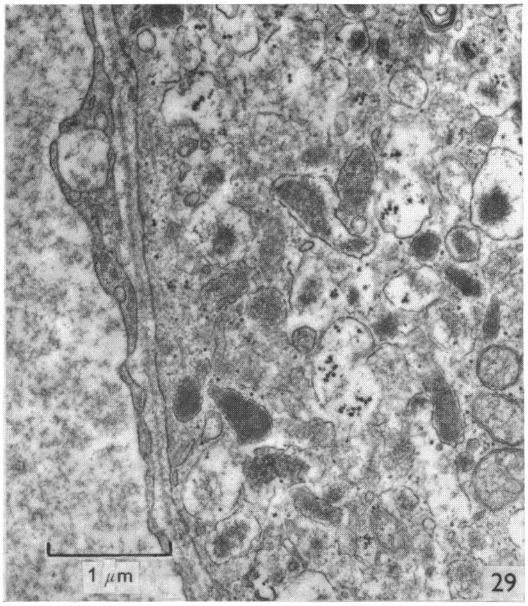
---

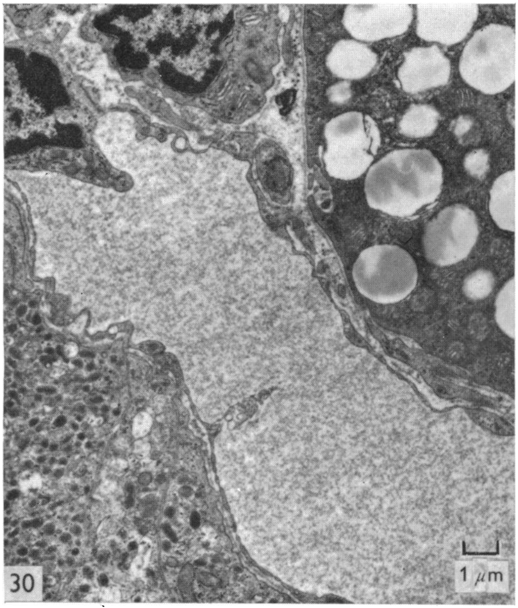
Fig. 25. Cholinergic nerve ending containing small clear centred vesicles and larger vesicles with moderately electron-dense centres on an adrenaline-storing adrenal chromaffin cell from a 25-d-old rabbit.

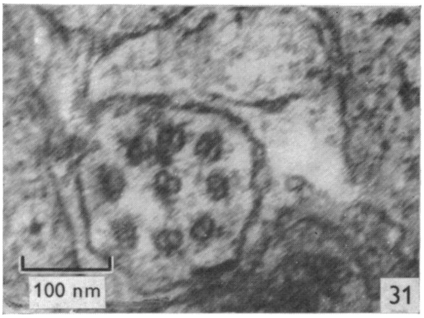
Fig. 26. Extra-adrenal chromaffin cells of a 23-d-old rabbit. Note Schwann cell cytoplasm containing a nerve fibre separates lower chromaffin cells from connective tissue space. The upper cell is covered by a basement membrane only.

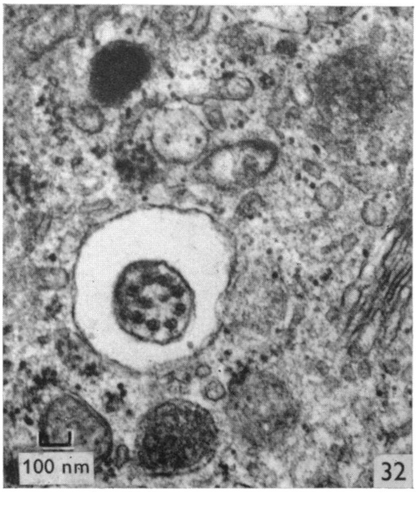
Fig. 27. Golgi zone of an adrenal chromaffin cell of a 25-d-old rabbit. Note lamellae and vesicles. Some contain moderately electron-dense inclusions but no highly electron-dense noradrenaline-storing material is visible.

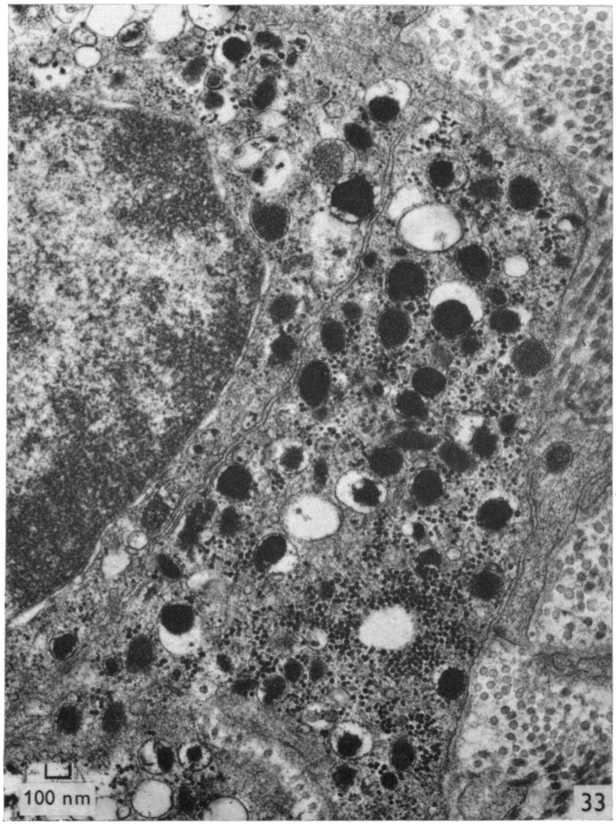
Fig. 28. Golgi zone of an extra-adrenal chromaffin cell from a 23-d-old rabbit. Most of the granular inclusions are highly electron-dense and probably contain noradrenaline. The diameter of the inclusion varies greatly. A multivesicular body and a centriole are visible.


1 μm
29


30
1 μm


100 nm
31


100 nm
32


100 nm
33

apparently lie free in the cytoplasmic sap, within typical chromaffin granules between the peripheral membrane and the aggregated granular contents, or within membrane-bound areas which may represent dilated portions of smooth endoplasmic reticulum or the membrane of chromaffin granules without their typical inclusions. These particles are larger than typical ribosomes and do not conform in structure to typical glycogen storage particles; histochemical reactions for glycogen are negative.

Cilia are commonly observed within intracellular tunnels or in intercellular spaces free from connective tissue elements in both the adrenal medulla and extra-adrenal chromaffin tissue. The fibril pattern is frequently 7+2 or 8+2 (Fig. 31) but irregular arrangements of fibrils (Fig. 32) are common even though the total number of groups may add up to 10.

Close proximity of chromaffin cells to blood capillaries and sinuses is normally observed in the adrenal medulla and only a single basement membrane may separate endothelial and chromaffin cells (Fig. 29). At the cortico-medullary junction cortical and chromaffin cells often abut the same venous sinus (Fig. 30). The association between blood vessels and chromaffin cells is less close in extra-adrenal sites than in the adrenal medulla and, in addition, to the basement membranes associated with both cell types a connective tissue space containing collagen fibres is commonly interposed (Fig. 21).

DISCUSSION

During the period birth to 3 months of age there is a gradual increase in the mean diameter and size range of chromaffin granules. In the adrenal medulla this change is accompanied by the disappearance of the highly electron dense N granule and in specimens of more than 9-d-old no granules which could be unequivocably identified as N types were observed.

Although extra-adrenal chromaffin granules show the same tendency to enlarge they tend to lag behind those of the adrenal medulla in this change and at all stages show a lower mean size. More than 90 % of these granules conform to the N form, and the few which show contents with only moderate electron density and internal granularity represent either N granules which have lost amine immediately prior to or during sacrifice of the animal and fixation procedures, or A granules. The occur-

Fig. 29. Adrenaline-storing adrenal chromaffin of a 25-d-old rabbit lying adjacent to a blood capillary (left). Note single basement membrane and adjoining spaces separating the endothelial and chromaffin cells and pores bridged by a single unit membrane in the endothelial cell. Some membrane-bound spaces contain small (*c.* 30 nm) electron-dense granules and others lie in the cytosol.

Fig. 30. Adrenal gland of a 25-d-old rabbit showing a cortical cell (top right) and chromaffin cells (bottom left) lying adjacent to a blood sinus. Fibroblast processes intervene between the cortical cell and the endothelium. The chromaffin cells are separated from endothelium by a single basement membrane and adjoining spaces.

Fig. 31. Transverse section of a cilium with 8+2 fibril pattern lying in an intercellular space in the adrenal medulla of a 15-d-old rabbit.

Fig. 32. Transverse section of a cilium with 8 peripheral pairs of fibrils and two para-central groups lying within an intracellular tunnel of an adrenal chromaffin cell from a 25-d-old rabbit.

Fig. 33. Chromaffin cell of extra-adrenal body of a 3-month-old rabbit. Most of the chromaffin granules exhibit highly electron-dense centres indicative of noradrenaline storage. A few contain inclusions of only moderate electron-density and fine internal granularity and may store adrenaline. Note large numbers of small (*c.* 30 nm) particles lying usually within the cytosol and less commonly in membrane-bound areas. Collagen fibres are numerous in extracellular spaces.

rence of a small number of A granules in extra-adrenal bodies would be in keeping with the assay findings (Coupland & MacDougall, 1966) and there is no electron histochemical evidence to indicate that a significant change occurs in the type of amine stored in extra-adrenal chromaffin bodies during the first 3 months of life.

The phenomena of storage of predominantly the primary amine noradrenaline by extra-adrenal cells and its methylated derivative adrenaline by adrenal medullary cells has been discussed previously (Coupland, 1965*a*), and experimental works (Coupland & MacDougall, 1966; Coupland, 1968*a*) have demonstrated that extra-adrenal cells may be induced to modify their activity and synthesize and store adrenaline instead of noradrenaline by exposing organ cultures of rabbit extra-adrenal chromaffin tissue to high but physiological concentrations of corticosterone; deoxycorticosterone was without effect. These *in vitro* results represented the first direct demonstration that adrenocortical hormones affect the ability of chromaffin cells to synthesize and store the methylated amine adrenaline. They are in accord with the observations of Eränkö, Lempinen & Räisänen (1966), who showed that the extra-adrenal chromaffin body of the newborn rat, which normally degenerates within about 1 week of birth (Lempinen, 1964), can be induced to persist and to store adrenaline instead of noradrenaline by subjecting the animals to daily injections of 0·1 mg. hydrocortisone. Further evidence of the effects of corticoids on the methylation of noradrenaline was provided by Wurtman & Axelrod (1966), who showed that daily injections of dexamethazone partially restores the activity of the adrenal medullary enzyme phenylethanolamine *N*-methyltransferase, which falls following hypophysectomy.

In the rabbit, unlike the rat, the para-aortic extra-adrenal chromaffin body persists throughout life (Coupland 1956, 1965*a*), even though it becomes elongated and physically difficult to identify and isolate due to surrounding adipose tissue. During postnatal life the extra-adrenal body shows a marked increase in fibrous stroma, and collagen fibres increase in number in all perivascular and perineural regions; the latter occasionally form bundles which are ensheathed by Schwann cell cytoplasm. Although the fibrous stroma of the adrenal medulla shows a slight increase during the same period the change is much less marked. As in the rat adrenal medulla (Coupland, 1965*a*, *b*) the plasma membranes of chromaffin cells and Schwann cells are separated from adjacent connective tissue elements by a basement membrane which becomes more distinct and complete in specimens more than two weeks old.

Apart from the differences referred to above in the size and types of amine-storage granules present in adrenal medullary and extra-adrenal chromaffin cells, the two groups of cells show differences in form and arrangement: medullary cells tend to be polyhedral and are arranged in close-packed epithelial form, while extra-adrenal cells are fusiform and although often separated by spaces of only 20 nm are not infrequently more loosely associated, especially in older specimens. Furthermore, the arrangement differs in the two situations in so far as the extra-adrenal chromaffin cells commonly show a perinuclear zone which contains Golgi membranes and vesicles, mitochondria, centrioles and a variable amount of cytosol which is largely free from chromaffin inclusion granules. A similar arrangement also occurs in the N cells of the rat adrenal medulla, but not in the A cells of either rat or rabbit. In A cells a more random distribution of cytoplasmic organelles and inclusions is usually observed. It is, therefore, possible that this arrangement reflects the type of amine stored and the metabolic activities associated with the elaboration of either adrenaline or noradrenaline rather than the position of the cell *vis à vis* an adrenal or extra-adrenal situation. Since adrenaline synthesis and storage is associated in these species with locally high concentrations of adrenal corticosteriods it is possible that the latter influence both metabolic activity and intracellular disposition of organelles and that the two phenomena are related.

Cilia have been observed in association with chromaffin cells in all specimens and conform to those described previously in the rat adrenal medulla (Coupland, 1965*b*). They arise from the distal centriole, which functions as a basal body. The number of fibrils and their arrangement in transverse sections shows some variation. Since, however, cilia with a typical 9+2 motile pattern of fibrils have occasionally been seen it seems likely that as suggested previously (Coupland, 1965*b*) many or all represent the motile form of cilia, albeit not uncommonly incomplete in fibril content, and that if functional they are more likely to be concerned with fluid movement than sensory activity.

Golgi membranes of rabbit chromaffin cells have the same form and contents as those of the rat. Although granular inclusions of various dimensions may be associated with either membranes or vesicles, no evidence has been obtained which suggests that noradrenaline is stored within the Golgi zone of A cells, even though highly electron-dense deposits typical of noradrenaline storage have been observed in the Golgi membranes and vesicles of N cells. Hence, the present work has thrown no light on the problem of the intracellular site of methylation. The present findings would be in keeping with the view that in the older postnatal specimens, assuming that the normal sequence of amine synthesis involves the methylation of noradrenaline, the primary amine is only transiently present and is almost immediately methylated.

The association between blood vessels and chromaffin cells is much closer in the adrenal medulla than in the extra-adrenal chromaffin bodies. In the former the basement membranes of chromaffin and endothelial cells may fuse and the two cells be separated by a gap of only *c.* 80 nm, which includes a layer of basement membrane some 30 nm thick. Endothelial pores bridged by a single unit membrane have been observed, but true discontinuities have not been seen in well-fixed specimens. In extra-adrenal chromaffin bodies chromaffin cells and blood vessels are separated by a variable space which contains connective tissue elements and these increase in amount with age. As in the foetus (Coupland & Weakley, 1968) pericapillary cells of the extra-adrenal chromaffin tissue and medial extremity of the adrenal medulla contain glycogen in 1-d-old specimens but not in older animals.

The significance and nature of the *c.* 30 nm granules in the cytosol, membranes and chromaffin granules of older chromaffin cells is a matter for conjecture. Small numbers of particles of similar size and staining characteristics may be observed in some nuclei and it is possible that they represent large ribosomes. If so this may account for the report by Philippu & Schümann (1964) that ribonucleic acid occurs in the large granular fractions of the adrenal medulla—a finding which is at variance with that of many other workers who have prepared relatively pure fractions of chromaffin granules.

The majority of nerve fibres in the preaortic region and adrenal medulla are non-myelinated though occasional myelinated fibres are observed. Judging by the types of granules present in fibres of the pre-aortic region many of these are adrenergic. The existence in the rabbit of fine unmyelinated nerve fibres ensheathed by Schwann cell cytoplasm, which is closely applied to the surface of chromaffin cells, is reminiscent of the situation in the rat (Coupland, 1965*c*). Both intra- and extra-adrenal cells show this particular neurocellular association, but differ in other respects in so far as a typical cholinergic synapse has never been seen on an extra-adrenal cell during either foetal or postnatal life, though synaptic contacts or endings of this type have been seen on both adrenal chromaffin cells and sympathetic neurons of all stages. This negative finding suggests that the extra-adrenal chromaffin cells may not receive a typical preganglionic sympathetic motor innervation. The contiguity of extra-adrenal cells with nerve fibres which are free from typical synaptic vesicles, but which contain neurofibrils and tubules, suggests that some of these nerves may subserve a sensory function. Such contacts are, however, very rarely encountered and the majority of extra-adrenal chromaffin cells of the preaortic chromaffin body appear to be without nerve synapses or endings even though they are often partially covered by tongues of Schwann cell cytoplasm which may contain fine nerve fibres. The adrenal chromaffin cells of the rabbit exhibit similar nerve synapses and associations to those described in the rat (Coupland, 1965*c*). At the point of contiguity between a nerve fibre and an adrenal chromaffin cell the surface of the cell may or may not show an indentation, and occasionally a definite invagination occurs which allows the nerve fibre to approach the more central part of the cell, even though the individual plasma membranes remain intact.

As in the rat (Coupland, 1965*c*) the synaptic-type endings on adrenal medullary chromaffin cells are associated with two distinct populations of nerve vesicles. The smaller vesicles, which have a mean profile diameter of *c*. 35 nm, possess a homogeneous and only slightly electron dense centre. The larger vesicles, which are fewer in number, possess granular moderately electron-dense centres, which are separated by a narrow halo from the external limiting membrane. Similar large vesicles exist in the adrenergic nerve fibre of the rat vas deferens (Coupland, 1968*b*); they do not, however, store noradrenaline since after gluteraldehyde-osmium tetroxide fixation the inclusions are only moderately electron-dense and non-homogeneous. The significance of this type of vesicle is not currently understood, though from their appearance and reactions it is likely that the contents are proteinaceous, and hence may be enzymic in nature; they may represent small lysosomes.

SUMMARY

In the rabbit at birth both adrenaline- and noradrenaline-storing chromaffin cells may be identified following initial fixation in glutaraldehyde and post-osmication. Some adrenal cells contain mixtures of both types of amine-storing granules. In 9-d-old and older specimens noradrenaline-storing granules are rarely observed in the adrenal medulla and when present are limited to cells of the most medial part of the gland adjoining the main extra-adrenal chromaffin body.

Extra-adrenal chromaffin cells contain mainly (> 90 %) noradrenaline-storing

granules throughout the period. Throughout the developmental period typical cholinergic-type nerve endings have been observed on chromaffin cells of the adrenal medulla but not on extra-adrenal cells. Adrenal chromaffin cells are more closely associated with blood capillaries than are the extra-adrenal elements.

Although noradrenaline-storing granules have been observed within the Golgi complex of noradrenaline-storing extra-adrenal chromaffin cells they have not been observed within the Golgi membranes of adrenaline-storing cells.

The mean profile diameter of chromaffin granules increases throughout the period studied. At birth and up to 6 weeks of age the adrenaline-storing granules of the adrenal cells have a larger mean diameter than have the extra-adrenal noradrenaline-storing granules. In 3-month-old rabbits the mean diameter and size distribution patterns of both types of granules is more similar than at earlier stages but a larger proportion of smaller granules still exists in the extra-adrenal cells.

The authors wish to thank Mrs Elspeth Lloyd Davis and Mr A. S. Pyper for technical assistance. This work was partly supported by an M.R.C. grant to R. E. Coupland for which the authors are most grateful.

REFERENCES

COUPLAND, R. E. (1956). The development and fate of the abdominal chromaffin tissue in the rabbit. *J. Anat.* **90**, 527–537.

COUPLAND, R. E. (1965*a*). *The Natural History of the Chromaffin Cell*, pp. 47–88 and 143–147. London: Longmans.

COUPLAND, R. E. (1965*b*). Electron microscopic observations on the structure of the rat adrenal medulla. I. The ultrastructure and organization of chromaffin cells in the normal adrenal medulla. *J. Anat.* **99**, 231–254.

COUPLAND, R. E. (1965*c*). Electron microscopic observations on the structure of the rat adrenal medulla. II. Normal innervation. *J. Anat.* **99**, 255–272.

COUPLAND, R. E. (1968*a*). Corticosterone and methylation of noradrenaline by extradrenal chromaffin tissue. *J. Endocr.* **41**, 487–490.

COUPLAND, R. E. (1968*b*). Localization of noradrenaline in chromaffin cells and nerve fibres. In *Roy. Micr. Soc. European Symposium 'Cytochemistry in Electron Microscopy'*, p. 30.

COUPLAND, R. E. & HOPWOOD, D. (1966). The mechanism of the differential staining reaction for adrenaline and noradrenaline-storing granules in tissues fixed in glutaraldehyde. *J. Anat.* **100**, 227–243.

COUPLAND, R. E. & MACDOUGALL, J. D. B. (1966). Adrenaline formation in noradenaline-storing chromaffin cells *in vitro* induced by corticosterone. *J. Endocr.* **36**, 317–324.

COUPLAND, R. E. & WEAKLEY, B. S. (1968). Developing chromaffin tissue in the rabbit: an electron microscopic study. *J. Anat.* **102**, 425–455.

ERÄNKÖ, O., LEMPINEN, M. & RÄISÄNEN, L. (1966). Adrenaline and noradrenaline in the organs of Zuckerkandl and adrenals of newborn rats treated with hydrocortisone. *Acta physiol. scand.* **66**, 253–254.

LEMPINEN, M. (1964). Extra-adrenal chromaffin tissue of the rat and the effect of cortical hormones on it. *Acta physiol. scand.* **62**, 1–91.

PHILIPPU, A. & SCHÜMANN, H. J. (1964). Ribonuclaseaktivität isolierter Nebennierenmarkgranula. *Experientia* **20**, 547–548.

WURTMAN, R. J. & AXELROD, J. (1966). Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. *J. biol. Chem.* **241**, 2301–2305.