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Morphometric analyses of adrenal gland growth in fetal and neonatal sheep. II. The adrenal medulla, with some observations on its ultrastructure

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INTRODUCTION

In the previous paper (Boshier & Holloway, 1989) it was demonstrated that although the adrenal gland in fetal and neonatal sheep grows in an essentially logarithmic fashion, the cortex and the medulla contribute to differing degrees, at different times, to the total gland volume during its fetal and neonatal development. While the 53 days fetal adrenal contained 16% of medullary tissue, the 100 days old gland was 37% medulla, a proportion which changed little through the remainder of gestation; but which decreased to 26% during the end of gestation and the perinatal period because of rapid cortical growth at that time.

It is accepted that the adrenal medulla has its origins in migratory neural crest cells but little is known of what attracts the medullary precursor cells to the developing cortical cells. Coupland (1965) described three types of fetal cells which are associated with medullary development in mammals, the primitive sympathetic migratory cells being totipotent and capable of differentiating into neurons or chromaffin cells. The differentiating chromaffin cells are either phaeochromoblasts, which have not yet begun to store catecholamines, or phaeochromocytes, which contain catecholamine-storing granules of either the adrenaline or noradrenaline type. There are now numerous reports of this fetal and neonatal differentiation process in a range of species: man (Hervonen, 1971); primates (Wilburn, Goldsmith, Chang & Jaffe, 1986); rabbit (Weakley & Coupland, 1965); rat (Elfvin, 1967); mouse (Jurecka, Lassmann & Hörander, 1978); sheep (Frydman & Geffen, 1973; McMillen *et al.* 1988); pig (Stadnicka & Van Wynsberghe, 1982), and the American opossum (Carmichael *et al.* 1987). While these accounts demonstrate a common epigenetic pattern, they also confirm Comline & Silver's (1966) observation that there is a wide variation between species in the degree of development of the adrenal medulla at birth.

As is the case with the fetal adrenal cortex, the fetal adrenal medulla is an important component of the homeostatic control systems of the fetus and neonate (Comline & Silver, 1966; Jones & Ritchie, 1978; Boshier, 1983; Phillippe, 1983). The sympatho-adrenal system maintains fetal homeostasis through the physiological and metabolic responses to the vicissitudes of fetal life (Artal, 1980), as well as contributing to the maturation of the fetal lung (Lawson *et al.* 1978; Walters & Olver, 1978). During birth, catecholamines released by the fetal adrenal medulla play a key role in the adaptation of the newborn to extrauterine life by providing both physiological and trophic signals to the fetus and the neonate (Slotkin & Seidler, 1988).

It is clear that the adrenal cortex and medulla must be considered as constituting not

only an anatomical, but also an integrated functional unit in both the prenatal and postnatal mammal (Carballeira & Fishman, 1980; Weinkove & Anderson, 1985). We have therefore attempted to increase the understanding of this relationship by studying the development of the fetal sheep adrenal medulla during fetal and immediate postnatal life, using morphometric and microscopic techniques.

MATERIALS AND METHODS

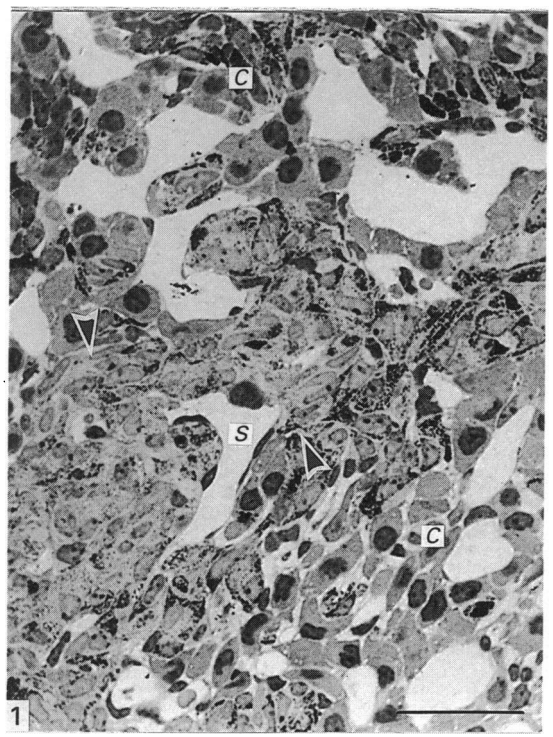
Details of experimental animals, tissue preparation, microscopic study, morphometric and statistical analyses have been reported in the companion paper (Boshier & Holloway, 1989).

In summary, adrenal glands were obtained, following perfusion fixation with cold (4 °C) Karnovsky's electron microscopy (EM) fixative diluted 1:1 with 0·1 M phosphate buffer at a pressure of 70 cm water for 10 minutes, from New Zealand Romney sheep fetuses of 53 days (5), 100 days (5), 130 days (5), 144 days (5) gestation and 2 days *postpartum* (3). The adrenals were weighed and measured, then embedded for microscopy in Epox 812 resin (Ernest F. Fulham) following standard EM procedures.

For light microscope studies, 30 one μm equally spaced sections were collected from one randomly selected gland of each pair, thereby giving an accurate representation of the histological organisation of the gland. The 53 days sections were stained for argentaffin cells (Stevens, 1977), and then, as for all other sections, with 1:1 toluidine blue (1 % aq.) and Azure II (1 % aq.). In some of the 2 days *postpartum* sections which had been prepared as segments of the entire gland because of the size of the organ, neither the central medullary cells nor the blood vascular volume density data may be as accurate as those from the fetal glands, for in some instances it was not possible to determine with full confidence the extent to which the central and other main veins contributed to the full medullary area. Data obtained for individual juxtacortical and central medullary cells, however, are valid.

Standard point-count methods for biological stereology as described by Weibel (1979) were used in all morphometric analyses. Point-count data were collected using a BBC B^+ microcomputer-controlled, stepping-motor driven stage on a Leitz Orthoplan microscope. Test line units (d) from 100 μm to 1 mm in length arranged in a coherent multipurpose system were selected to allow the collection and storage in the computer of the appropriate number of points per animal and experimental group. To allow calculation of nuclear and secretory cell volumes, the longer and shorter nuclear axes were measured in approximately 1250 nuclei in the juxtacortical and central regions of the combined medullae of each age group. For later calculations of nuclear volumes as volume equivalent spheres using the Abercrombie correction, the mean tangent diameters where the axial ratio exceeded 1·5:1 were corrected using Figure 5.3 in Weibel (1979). For statistical analyses of the four age groups, *t* tests for determining differences between two proportions were used.

Ultrastructural studies of juxtacortical and central medullary cells were undertaken using ultrathin sections stained with saturated aqueous uranyl acetate and Sato's (1967) lead. The randomly selected sections from each age group were then viewed and photographed in a Hitachi H-7000 transmission electron microscope.



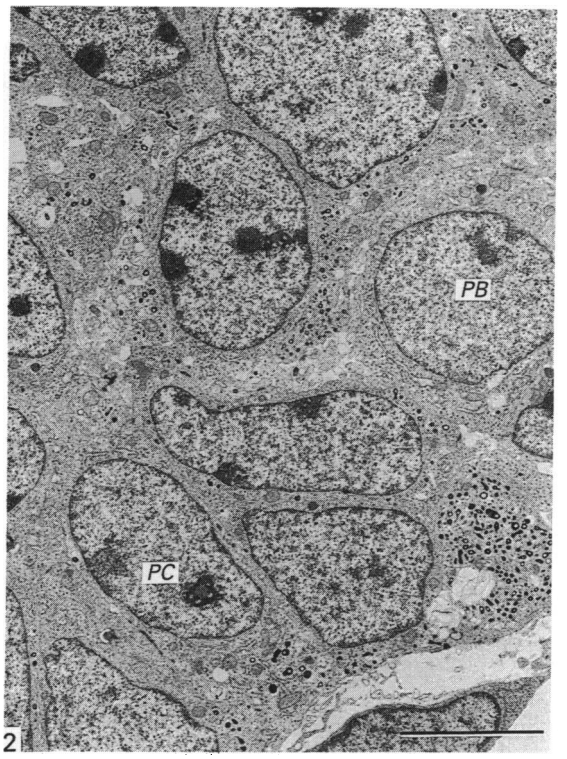


Fig. 1. Light micrograph of 53 days adrenal gland in which migratory medullary cells, present as whorls and columns (arrowheads), are located among the cortical cells (*C*): both phaeochromoblasts and phaeochromocytes are present. The sinusoids (*S*) are lined by endothelial cells. Bar: 40 μm.

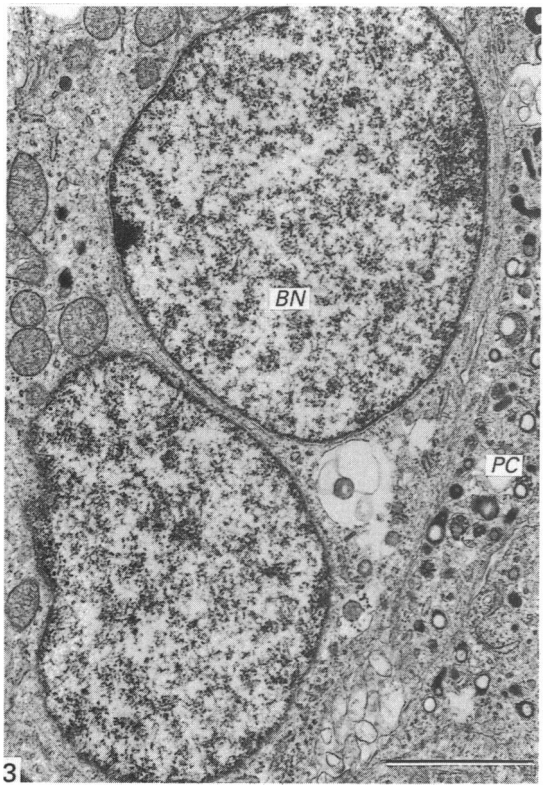
Fig. 2. Electron micrograph of 53 days medullary cell whorl showing phaeochromoblasts (*PB*) and phaeochromocytes (*PC*). Note the endothelial cell nucleus in bottom right corner. Bar: 5 μm.

RESULTS

Cellular differentiation

By 53 days, whorls and columns of migratory sympathochromaffin cells were located among the cortical cells and in some sections were continuous with phaeochromoblasts present in the capsular region (Fig. 1). The whorls contained both phaeochromoblasts and phaeochromocytes (Fig. 2). The former cells had large rounded nuclei containing finely dispersed chromatin and one, or sometimes two, nucleoli. A small number of cells were binucleate (Fig. 3). The cytoplasm, a thin rim around the nucleus, was devoid of secretory granules; but contained large rounded mitochondria, sparse profiles of rough endoplasmic reticulum and numerous polysomes (Figs. 3, 4). As shown in Figures 3 and 4, the phaeochromocytes contained varying numbers of granules, the majority of which were darkly stained, asymmetric in form and possessed clear halos of different sizes within the granule limiting membrane – characteristics typical of noradrenaline (NA)-containing vesicles (Coupland, 1965, 1971). These cells were elongated, had oval nuclei with up to three nucleoli and finely dispersed chromatin, and had cell organelles that were little different from those of the phaeochromoblasts. None of the Golgi body profiles seen contained condensing granules. There was no evidence of sympathetic innervation.

In the 100 days specimens, separation of the medulla from the cortex was complete.



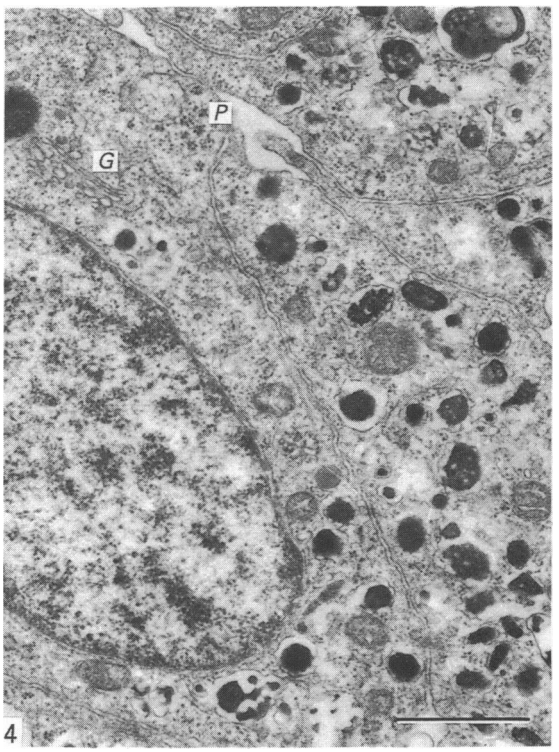


Fig. 3. Electron micrograph of binucleate cell (*BN*) with sparse granules. Compare phaeochromocyte NA granules in cell to right. Bar: 2 μm.

Fig. 4. Higher power electron micrograph of 53 days phaeochromocytes showing Golgi body cisternae (*G*), polysomes (*P*) and pleiomorphic NA granules. Bar: 1 μm.

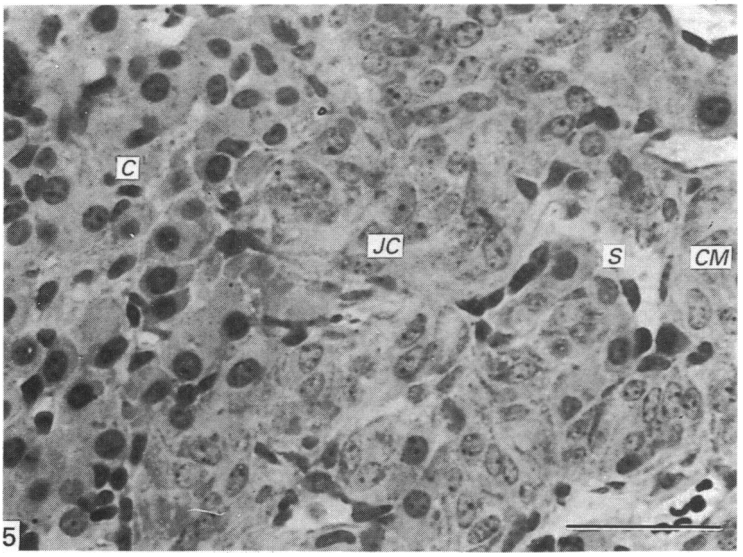
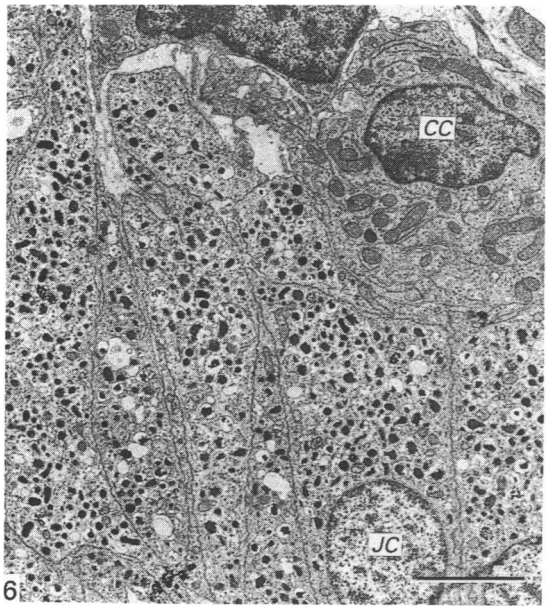


Fig. 5. Light micrograph of 100 days adrenal gland illustrating relationship of juxtacortical cells (*JC*) to cortical cells (*C*) and central medullary cells (*CM*). The juxtacortical cells maintain a close association with the sinusoids (*S*). Bar: 40 μm.



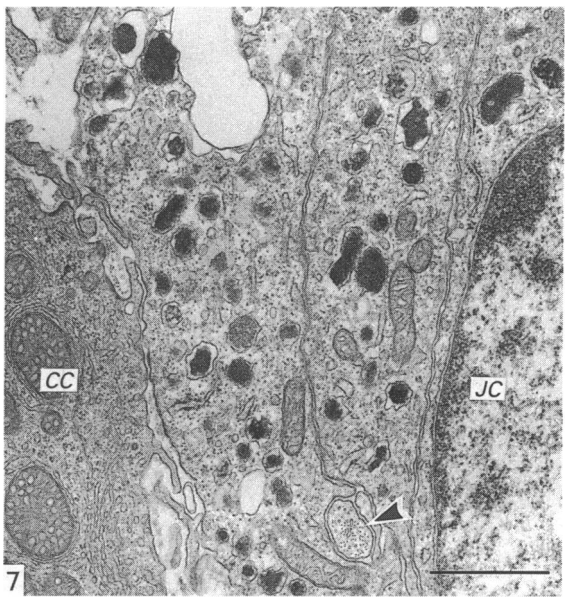


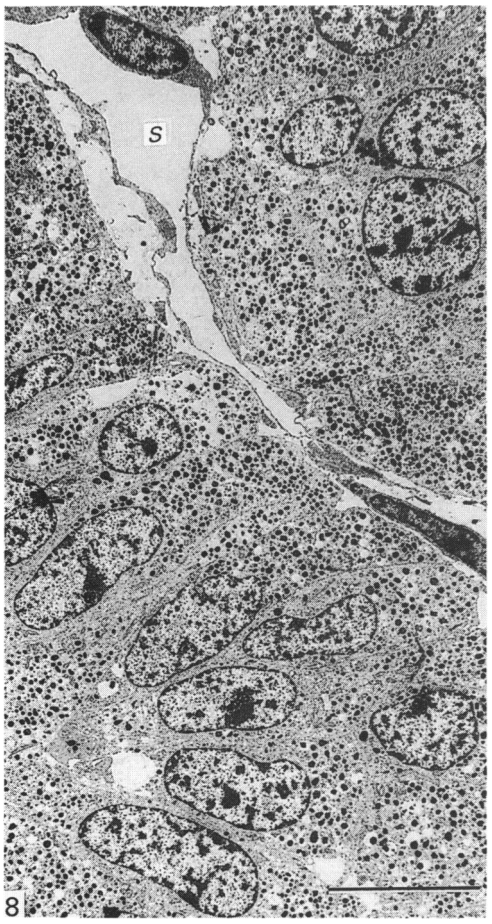
Fig. 6. Electron micrograph of 100 days juxtacortical medullary cells illustrating their columnar form and increased granularity. A cortical cell (*CC*) is in close association. Bar: 3 μm.

Fig. 7. Higher power electron micrograph of juxtacortical cells adjacent to cortical cells at 100 days gestation. The catecholamine-containing granules are predominantly of the NA type. An axon of the sympathetic innervation is shown (arrowhead). Bar: 1 μm.

However, the medulla was composed of two populations of cells. The juxtacortical cells were typically arranged in palisades adjacent to the blood sinusoids draining the cortex, and enclosed the central medullary cells (Fig. 5). Some whorls of cells near the cortex were like those of the 53 days specimens in that the constituent cells were both phaeochromoblasts and phaeochromocytes (Fig. 5). The juxtacortical cells had elongated, sometimes rounded, nuclei similar to the phaeochromocyte nuclei at 53 days. The cells were elongated and contained numerous pleiomorphic granules of the NA type (Figs. 6, 7). Some axonal profiles, but no synapses were seen (Fig. 7).

During the next 30 days of development, the juxtacortical cells retained their relationship with the corticomedullary blood vessels and underwent further functional differentiation, for at 130 days the granule population also contained greyish, powdery granules located more centrally within the enclosing membrane and showing little or no halo (Figs. 8, 9). These granules satisfy Coupland's (1965, 1971) criteria as containing adrenaline (A). The A- and NA-containing granules were in mixed populations in some cells whereas other cells contained NA granules predominantly (Fig. 9). The central medullary cells contained NA granules almost entirely (Fig. 10). Axonal profiles of the preganglionic sympathetic nervous system were frequently seen between the secretory cells and contained neurotubules, neurofilaments, mitochondria and two populations of vesicles (Figs. 9, 10, 12). Synapses between the axons and the catecholamine granule-containing cells were present at 130 days of gestation (Figs. 11, 12).

During the later stages of gestation and in the immediate *postpartum* period, differentiation within the juxtacortical cells was largely a continuation of the changes present at 130 days. The central medullary cells also increased in number but showed little change ultrastructurally. The medullary cells during this period became more



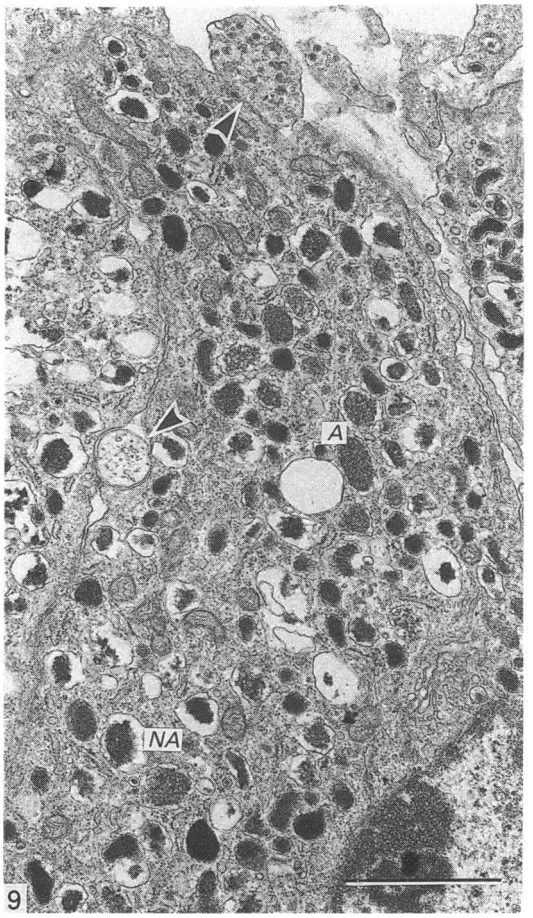


Fig. 8. Low power electron micrograph of 130 days juxtacortical medullary cells illustrating their columnar form, catecholamine-containing granules and relationship with sinusoids (*S*). Bar: 10 μm.

Fig. 9. Higher power of 130 days juxtacortical cells with both adrenaline (*A*) and noradrenaline (*NA*)-containing granules. Associated axons are shown by arrowheads. Bar: 2 μm.

discretely organised, taking on a locular form with several cells enclosed by a connective tissue sheath in which ran the blood vessels and the innervating axons (Figs. 13, 14). Some juxtacortical cells contained A granules almost entirely, some had a mixed population of A and NA granules, while others contained a predominance of NA granules (Figs. 15, 16, 17). There were numerous sympathetic axons between the secretory cells, frequent synapses, and numbers of puncta adherentia (Peters, Palay & Webster, 1976) along the interface between the axons and the catecholamine-containing cells (Fig. 17). The central medullary cells contained only NA granules.

Morphometry

Volume densities within the developing adrenal medulla

The volume densities calculated for the juxtacortical cells, central medullary cells, blood vessels and the intercellular, connective and neural tissue space are presented in Table 1. The volume densities of the juxtacortical and central regions are further divided into nuclear and cytoplasmic components. During the study period, the medulla increased markedly as a fraction of the entire gland between 53 and 100 days

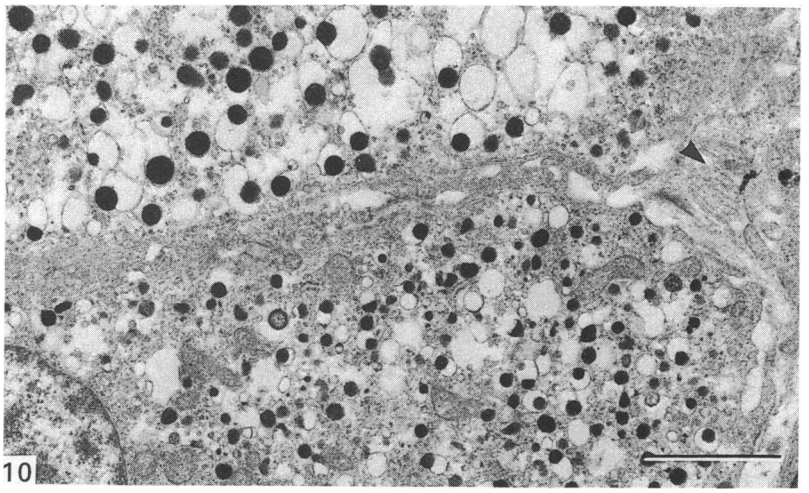
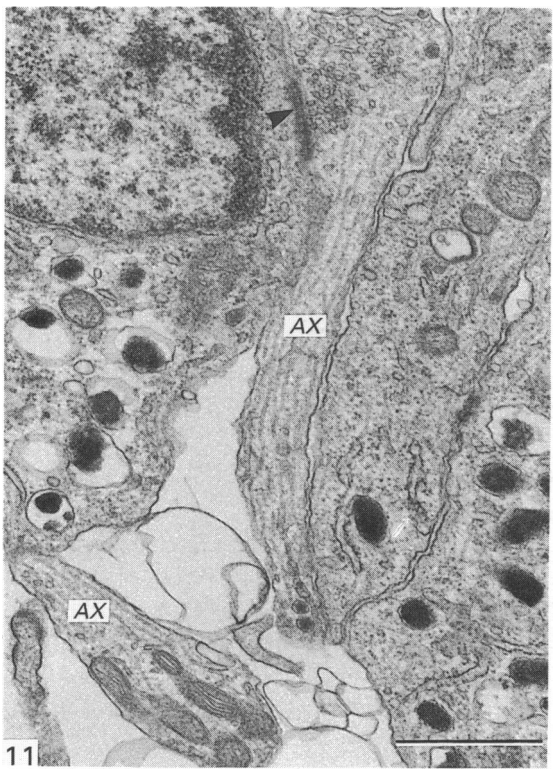


Fig. 10. Central medullary cells of 130 days adrenal gland containing NA granules. These cells show little ultrastructural change after their separation at 100 days. Axons (arrowhead) are located between the cells. Bar: 2 μm.



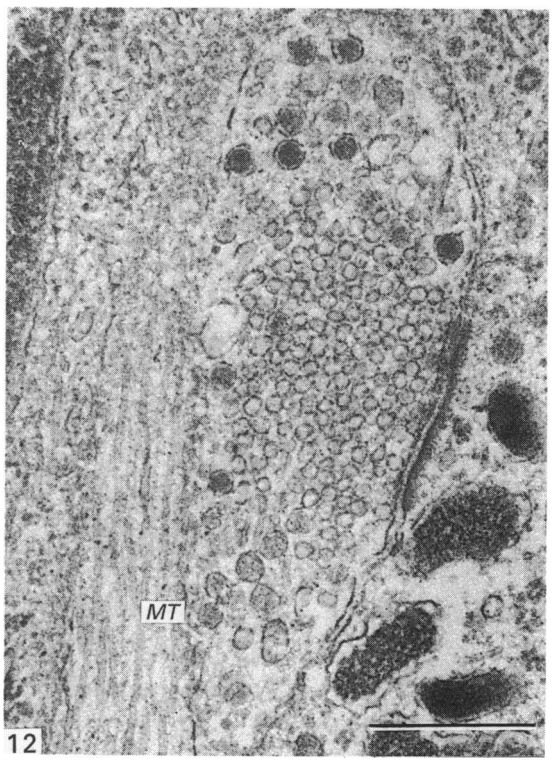
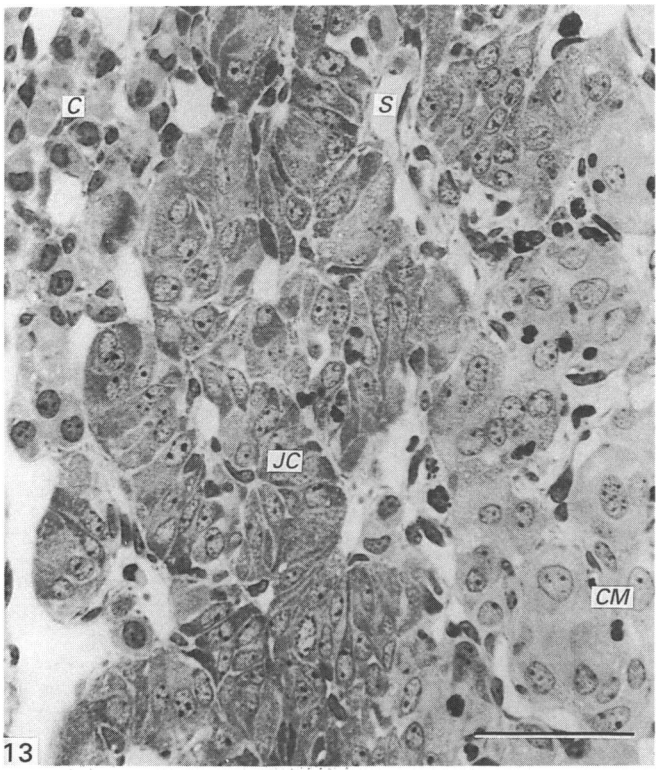


Fig. 11. Electron micrograph illustrating synaptic connection between sympathetic postganglionic axon (arrowhead) and catecholamine-secreting cell. The synaptic region contains presynaptic vesicles adjacent to the synaptic complex. Microtubules, mitochondria, and dense-cored vesicles are present within the axonal processes (*AX*). Bar: 1 μm.

Fig. 12. Higher power electron micrograph of 144 days synaptic region showing that while the presynaptic vesicles are closely associated with the synaptic complex, the dense-cored vesicles are not. Microtubules (*MT*) are clearly seen in an adjacent axon. Bar: 500 nm.



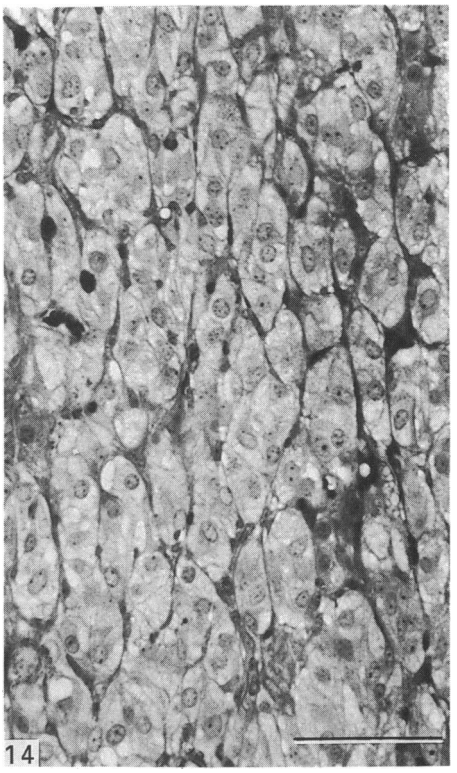


Fig. 13. At 144 days gestation, both the juxtacortical and the central medullary cells have become organised into more discrete cellular associations. Bar: 40 μm.

Fig. 14. Light micrograph of 2 days old adrenal gland illustrating extent to which medullary cells have become organised into connective tissue-enclosed complexes. Bar: 40 μm.

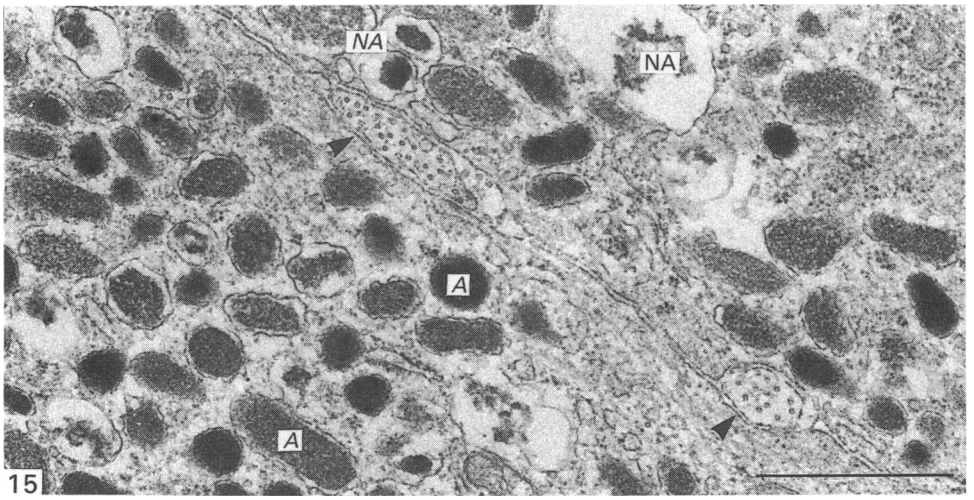


Fig. 15. By 144 days of gestation, many of the juxtacortical cells contain a predominance of *A* granules. Two axonal profiles are shown (arrowheads). Bar: 1 μm.

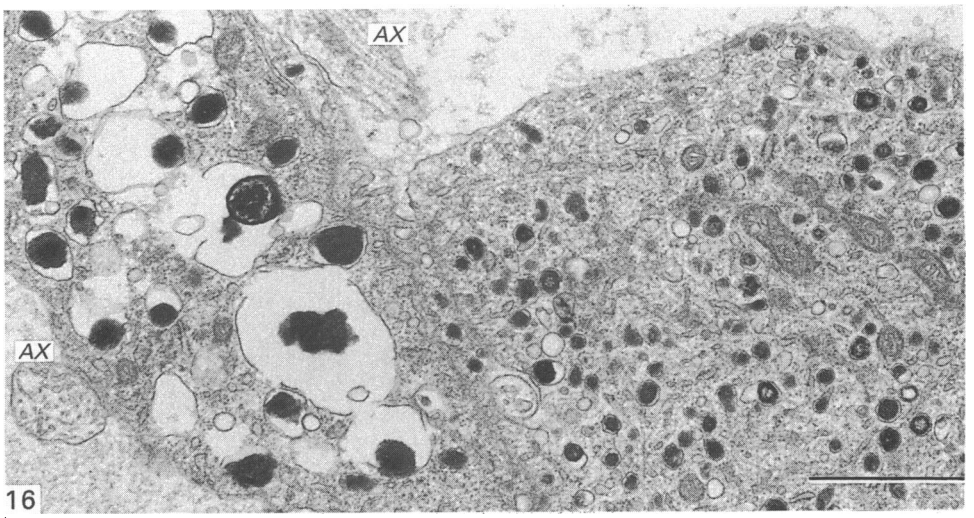


Fig. 16. Electron micrograph of 2 days *postpartum* juxtacortical cells illustrating range of granule forms present. Those on the left are predominantly *NA* granules, while those on the right are predominantly *A* granules. Bar: 1 μm.

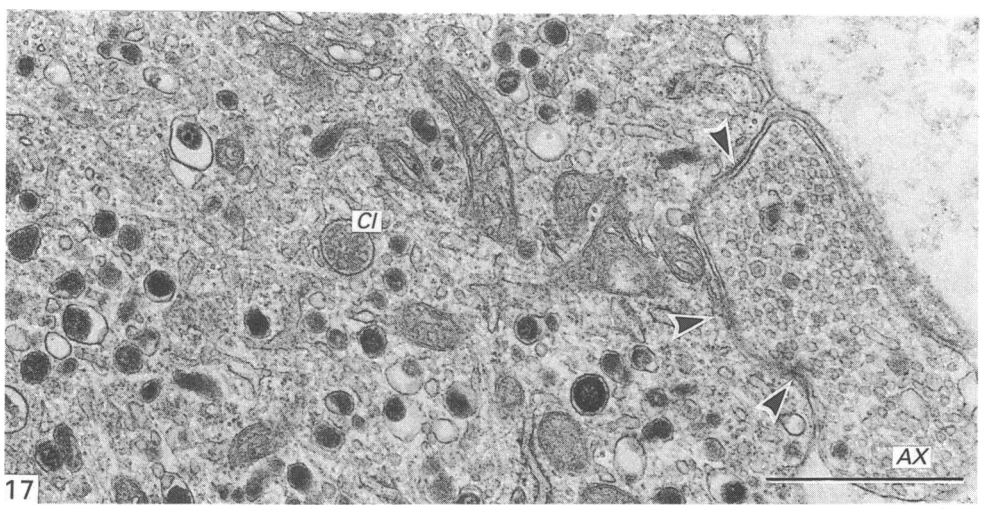


Fig. 17. In this 2 days *postpartum* electron micrograph, a number of puncta adherentia (arrowheads) are present between the axon and the adjacent catecholamine-secreting cell. Note the circular profile of a sectioned cilium (*CI*) within the cell. Bar: 1 μm.

of development, by which time it had become a discrete central element. Over the next 44 days, the medullary volume density remained constant; then, as a result of the second phase of accelerated cortical growth described earlier (Boshier & Holloway, 1989), it fell by one third over the birth and perinatal period.

Within the medulla, the juxtacortical cells occupied 0·33 of its volume at 100 days, a fraction which decreased to 0·22 by 144 days and then remained constant until 2 days *postpartum*. The volume density of the central medullary cells, however, increased proportionately, rising from 0·19 at 100 days to 0·30 at 144 days. As discussed above, the volume density of 0·25 at 2 days postnatally may not represent the true volume of

Table 1. *Volume density of major regions within adrenal medulla of fetal and neonatal sheep**

	Gestational age (days)				Postnatal age (days)
	53	100	130	144	2
Number of glands studied	5	5	5	5	3
Volume density					
Juxtacortical (j/c) cells	—	0·33±0·08	0·27±0·03	0·22±0·05	0·22±0·02
Nucleus j/c cells	0·28±0·03†	0·34±0·08	0·37±0·08	0·26±0·04	0·21±0·05
Cytoplasm j/c cells	0·72±0·03†	0·66±0·06	0·63±0·08	0·74±0·05	0·79±0·05
Central medullary (c/m) cells	—	0·19±0·06	0·26±0·08	0·30±0·04	0·25±0·10
Nucleus c/m cells	—	0·26±0·07	0·30±0·07	0·21±0·02	0·18±0·05
Cytoplasm c/m cells	—	0·74±0·07	0·70±0·07	0·79±0·02	0·82±0·05
Blood vessels	—	0·24±0·05	0·28±0·04	0·33±0·04	0·32±0·11
Connective tissue and intercellular space	—	0·24±0·05	0·19±0·05	0·15±0·03	0·21±0·06

* Mean±95% C.I.
† Values for all cells of migratory whorls.

Table 2. *Proportionate comparisons of secretory cells with other medullary components at different developmental stages of fetal and neonatal sheep**

	Age groups comparisons (days)		
	100/130	130/144	144/+2
Juxtacortical:central medullary cells	$P < 0·05$	$P < 0·05$	
Proportional value	1·74 1·04	1·04 0·73	
Nucleus:cytoplasm/juxtacortical cells	N.S.	$P < 0·05$	N.S.
Proportional value	0·52 0·59	0·59 0·35	0·35 0·28
Nucleus:cytoplasm/central medullary cells	N.S.	$P < 0·05$	N.S.
Proportional value	0·35 0·43	0·43 0·27	0·27 0·22
Blood vessels:secretory cells	N.S.	$P < 0·05$	
Proportional value	0·46 0·36	0·36 0·64	
Connective tissue, neural tissue and intercellular space:secretory cells	$P < 0·05$	$P < 0·05$	
Proportional value	0·46 0·36	0·36 0·29	

* The proportional values are the volume ratios of the first component of the contrasted pair to the second at the development age specified, using the volume data presented in Table 3.

the central medullary cells. In both groups of medullary cells, the nucleus progressively took up a lesser, and the cytoplasm a greater, proportion of the glandular cells. Between 100 days and 144 days of fetal development, while the blood vascular compartment occupied an increasing fraction of the medulla, the connective tissue, neural tissue and intercellular space occupied progressively less. However, for the reasons given above, the volume density of the blood vessels in the newborn may be underestimated.

When the interrelationships of the medullary components are considered (Table 2), it is apparent that the changes in the intraglandular proportions of the juxtacortical and central medullary cells through later gestation were significant, and confirm that the latter cells became progressively the greater proportion of the total gland. In both

Table 3. *Morphometric characteristics of the adrenal medulla in fetal and neonatal sheep*

	Gestational age (days)				Postnatal age (days)
	53	100	130	144	2
Mean total adrenal wt. (mg)*	22·10 ± 1·60	121·50 ± 7·80	290·00 ± 13·80	560·01 ± 54·50	914·10 ± 71·60
Mean total adrenal vol. (mm^3)*†	22·26 ± 1·60	122·64 ± 7·90	293·01 ± 13·90	566·06 ± 55·10	923·79 ± 72·40
Vv_a adrenal medulla‡	0·16 ± 0·06	0·37 ± 0·06	0·36 ± 0·07	0·37 ± 0·05	0·26 ± 0·06
Volume of medulla (mm^3)	—	45·38	105·48	209·44	240·19
Vol. of connective tissue and intercellular space (mm^3)§	—	10·89	20·04	31·42	50·44
Vol. of blood vessels (mm^3)§	—	10·89	29·53	69·12	76·86
Vol. of medullary cells (mm^3)§	3·56‖	23·61	55·91	108·90	112·89
Vol. of juxtacortical cells (j/c) (mm^3)§	—	14·98	28·48	46·08	52·84
Vv nucleus of j/c cells	0·28	0·34	0·37	0·26	0·21
Mean nuclear diameter (μm)	4·64	5·11	6·12	6·30	6·46
Mean nuclear volume (μm^3)	52·31	69·87	120·02	130·92	141·16
Mean j/c cell volume (μm^3)	186·82	205·50	324·40	503·54	643·64
Number of j/c cells ($\times 10^6$)	—	72·90	87·79	91·15	
Volume of central medullary (c/m) cells (mm^3)§	—	8·62	27·43	62·82	60·05
Vv nucleus c/m cells	—	0·26	0·30	0·21	0·18
Mean nuclear diameter (μm)	—	7·25	8·09	7·49	7·92
Mean nuclear volume (μm^3)	—	199·53	277·23	220·01	260·12
Mean c/m cell volume (μm^3)	—	767·42	924·10	1047·67	1445·12
Number of c/m cells ($\times 10^6$)	—	11·23	29·68	63·97	

* Mean ± S.E., per fetus, post-fixation weight.

† Corrected for shrinkage (+5%; Hayat, 1981) and specific gravity (÷1·039; Malendowicz, 1986). Volume therefore approximates prefixation volume.

‡ Vv_a, Volume density of medulla within whole adrenal gland, mean ±95% C.I.

§ Within adrenal medulla, calculated by using Vv in Table 1.

‖ Data at 53 days are mean values for all cells of migratory whorls.

secretory cell populations, the nucleus:cytoplasm rate decreased significantly between 130 and 144 days of development. Over the same period, the volume of the blood vascular compartment increased significantly when compared with the secretory cell volume. The proportion of intercellular space, however, decreased through the period from 100 days to 144 days.

Growth velocities within the adrenal medulla

From data obtained for the entire adrenal glands, and using the volume densities of the medullary components, we have calculated the real volumes of the compartments making up the medulla (Table 3). Over the period from 100 days to 2 days postnatally, the total medulla increased in volume from 45 mm^3 to 240 mm^3, a more than five-fold increase.

Within the medulla, the secretory cells also increased five-fold in volume, growing from 24 mm^3 of secretory cells at 100 days to 113 mm^3 two days after birth. This increase in secretory cell volume resulted from a three-fold increase in the juxtacortical cell volume and a seven-fold increase in the central medullary compartment between 100 days of gestation and birth. The morphometric characteristics of the 53 days

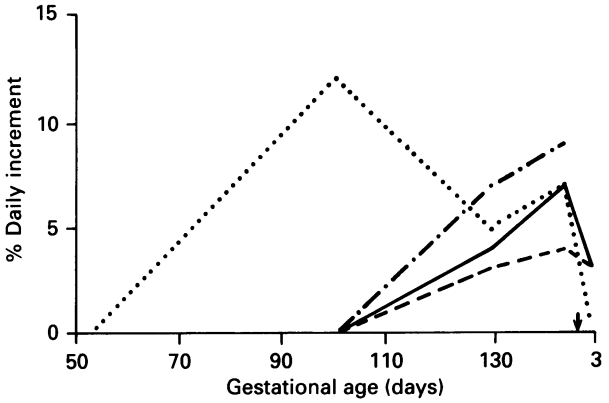


Fig. 18. Mean velocity diagrams of relative changes in medullary volume (——), volume of catecholamine-secreting cells (· · ·), volume of central medullary cells (–·–·–·), and volume of juxtacortical cells (– – –) during growth of the ovine adrenal medulla from 53 days of gestation to two days *postpartum*, where:

$$\text{Daily increment } \% = \frac{\dfrac{\text{Datum}_n - \text{Datum}_{n-1}}{\text{Growth period (days)}}}{\text{Datum}_{n-1}} \times 100.$$

Lines joining the gestational age data points are indicative of the changes occurring, for our data do not allow determination of the true inflections. Term, indicated by the arrow on the age axis, is 147 days.

migratory cells were the average values of the mixed population of phaeochromoblasts and phaeochromocytes, so they do not correspond to the anatomically juxtacortical cells of the 100 days and older glands. In both the juxtacortical and central medullary cells, the nuclei showed their greatest increase in volume between 100 and 130 days of development. Total cell volume in both cell populations, however, increased throughout the period of the study. At all stages the central medullary cells were larger than those of the juxtacortical population.

The rates of change in the volumes of the secretory cell compartments, expressed as an averaged percentage daily increase, are shown in Figure 18. They show that the greatest daily increase in medullary cell volume occurred between 53 and 100 days of gestation then, after a decrease from 100 days, a second growth phase had begun by 130 days. Total glandular growth then slowed markedly at the time of birth. Throughout the period studied, the total volume of the central medullary cells increased at a greater rate than did that of the juxtacortical cells.

The rates of change in the cell numbers of the medullary cell populations can only reasonably be calculated from 100 days to 144 days of fetal life. They show that the rate of increase in juxtacortical cell numbers between 100 days and 130 days was 0·7% per day, and that between 130 and 140 days of gestation was 0·3%. In contrast to this slowing of cell multiplication in the juxtacortical cells, cell division occurred at an increasing rate in the central medullary cells. In the central cells between 100 days and 130 days, there was a 5% per day increase, while between 130 and 144 days, the increase in cell numbers had risen to 8% per day.

DISCUSSION

Although there is one report providing baseline morphometric data on the mature rat adrenal medulla at the tissue and cellular level (Tomlinson, Durbin & Coupland,

1987), the analyses reported in this paper are the first morphometric studies of the developmental pattern exhibited by one mammalian species. They complement the companion studies (Boshier & Holloway, 1989) and show that the growth of the fetal sheep adrenal cortex and medulla occur in parallel. In both components of the gland, the tissue framework is established by 100 days (0·68 gestation) as the result of rapid growth; then, some four weeks later, mature function, associated with the development of the appropriate enzyme systems and stimulatory mechanisms, begins, and is itself associated with an increase in growth rate. At this stage, it is apparent that there are profound interactions between the adrenal cortex and medulla (Weinkove & Anderson, 1985).

The early migratory pattern of the primitive sympathetic cells and their differentiation pattern from phaeochromoblast to phaeochromocyte follows Coupland's (1965) descriptions. In those species in which the newborn are reasonably mature, it seems that the migration of the medullary cells has begun by the end of the first third of gestation, for we have shown migration and the beginnings of cellular differentiation to be well established by 0·36 gestation, a stage similar to that described in primates and in man (Ikeda, Lister, Bouton & Buyukpamukcu, 1981; Wilburn *et al.* 1986), and in the pig (Stadnicka & Van Wynsberghe, 1982).

While the stimulus to the onset and completion of primitive sympathetic cell migration into the cortex is as yet unknown, there is some evidence that the differentiation into the early phaeochromocytes is influenced by nerve growth factor (NGF) and the adrenal glucocorticoids (Grothe, Hofmann, Verhofstad & Unsicker, 1985; Anderson & Axel, 1986). In the 100 days old fetal sheep, this differentiation process has resulted in the development of two primary populations of medullary cells, the juxtacortical cells and the central medullary cells. The two populations are morphologically distinct, but at this time both have NA-containing granules almost exclusively. By 130 days, however, the juxtamedullary cells contained large numbers of A-containing granules.

That the production of NA within the adrenal medulla precedes that of A in all mammals so far studied is well established (Hökfelt, 1951), as is the close association between the A-synthesising cells and the adrenal cortex (Frydman & Geffen, 1973). The adrenal cortex is the factor which determines the degree of methylation of NA to A via the action of phenylethanolamine N-methyltransferase (PNMT), an enzyme whose synthesis is stimulated by glucocorticoids originating in the adrenal cortex (Coupland, 1953; Wurtman & Axelrod, 1966). The presence of A in the fetal rat adrenal is preceded by one day by increased corticosteroid synthesis (Axelrod, 1977) and the increased number of NA cells in the fetal pig at 100 days gestation correlates with the increased adrenocortical activity observed several days before parturition (Dvorak, 1972; Stadnicka & Van Wynsberghe, 1982). Cortisol has been shown to stimulate catecholamine release from 130–132 days old fetal sheep adrenal medullary cells by Graham, Longo & Cheung (1986); McMillen *et al.* (1988) demonstrated immunocytochemically the presence of PNMT in ovine medullary cells of 80 days gestation and in a peripheral rim of medullary cells at 105 days. We found a significant increase in the numbers of A granules in the juxtacortical cells of 130 days fetuses, a differentiation state which may be linked with the increased plasma levels of cortisol at this time (Alexander *et al.* 1968); it is similar to the pattern of localisation of the catecholamines in the mature bovine adrenal medulla (Livett, Day, Elde & Howe, 1982).

Comline & Silver (1961) and Jones & Robinson (1975) have reported the presence of both NA and A in the mid- and late gestational sheep fetuses, a major rise in A

content occurring towards the end of pregnancy. The former authors found that stimulation of the splanchnic nerves had little effect before 125 days. From about 125 days to term, the rate of release of NA and A rose sharply and after 130 days the output of A was significantly higher than that of NA, a change probably due to the development of the innervation of the adrenal medulla during this period. This hypothesis based on physiological grounds is now confirmed, for our studies have demonstrated a proliferation of the preganglionic sympathetic fibres among the juxtacortical medullary cells between 100 and 130 days of gestation and the presence of well-developed synapses between the sympathetic axons and the catecholamine-secreting cells at 130 days. The number of synapses appears to be more numerous in the 144 day specimens.

Heightened catecholamine secretion from 130 days of gestation in the fetal sheep (Padbury, Polk, Newnham & Lam, 1985) follows the combined effects of increased adrenocortical secretion of cortisol (Alexander *et al.* 1968; Wintour *et al.* 1975) which enhances PNMT activity (Graham *et al.* 1986) and the development of functional synapses between the preganglionic sympathetic axons and the catecholamine-secreting cells which we have demonstrated. In this manner, the catecholamine-secreting system influencing lung maturation and ensuring the viability of the fetus during the later stages of gestation and at birth is established (Abdellatif & Hollingsworth, 1980; Cheng, Goldfien, Ballard & Roberts, 1980; Padbury, Agata, Ludlow & Humme, 1987; Slotkin & Seidler, 1988).

The development of controlled catecholamine synthesis and secretion has been associated with growth in both the juxtacortical and the central medullary zones but the growth rate in the A-producing area, the juxtacortical compartment, was not as rapid as in the central compartment, which was composed, apparently entirely, of NA granule-containing cells. Between 100 and 140 days of development the juxtacortical zone fell from 33% of the medulla to 22%, whereas the central zone increased from 19% of the medulla to 30%. While both zones increased in volume, in the juxtacortical region growth was primarily hypertrophic as the rate of cellular multiplication fell. By contrast, growth within the central zone was both hypertrophic and hyperplastic, for both cell size and cell division rates continued to increase during the later stages of gestation and the immediately postnatal period.

SUMMARY

This account of fetal and neonatal sheep adrenomedullary development is the first such study in mammals using both morphometric and microscopic techniques. At 53 days gestation some cells in the migratory whorls and columns contained noradrenaline (NA) granules whereas by 100 days the medulla, now enclosed by the cortex, was composed of elongated juxtacortical cells and rounded central medullary cells, both populations of cells containing NA granules. In the 130 days glands, many of the juxtacortical cells contained adrenaline granules and had synaptic connection with axons of the preganglionic sympathetic nerve fibres. Later development was essentially growth-related. While the juxtacortical cells decreased from 33% of the medulla at 100 days to 22% at 144 days, the central medullary cells increased from 19% to 30% over the same period. Both cell populations exnibited hypertrophic growth over the study period; but the central cells multiplied at a faster rate.

We conclude that the development of the cortical and medullary compartments of the adrenal gland are closely linked, for both showed rapid mid-gestational growth which slowed with the attainment of definitive tissue organisation. Then a second

phase of growth, associated with increased and controlled catecholamine secretion in the medulla and cortisol secretion in the cortex, occurred in late gestation.

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