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# Growth and cytodifferentiation of the fetal lamb adrenal cortex prior to parturition

D. P. BOSHIER, HILARY HOLLOWAY AND G. C. LIGGINS*

*Department of Anatomy and * Postgraduate School of Obstetrics and Gynaecology, University of Auckland, New Zealand.*

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## INTRODUCTION

During late pregnancy the pituitary–adrenocortical axis influences developmental activities both within and outside the fetus. Internally maturation of the small intestine (Moog, 1953) and lung (Buckingham, McNary, Sommers & Rothschild, 1968; Liggins, 1969) are strongly influenced by corticosteroid hormones, while externally this axis has a primary role in the initiation of parturition in a number of species (Liggins, 1968; Nathanielsz, 1978). Each of these events is associated with fetal secretion of corticosteroids, a phenomenon which has been most closely studied in sheep.

Rising concentrations of corticosteroids in fetal sheep plasma are apparent several days before birth (Bassett & Thorburn, 1969), and result from a sharply increasing rate of secretion by the fetal adrenals (Alexander *et al.* 1968; Comline, Nathanielsz, Paisey & Silver, 1970; Liggins *et al.* 1973). The rise in fetal plasma cortisol appears to be associated with the gland's rapid growth (Comline & Silver, 1961), greater sensitivity to ACTH (Madill & Bassett, 1973), and an increased synthetic capacity, which is linked with the appearance of a more mature histological state (Alexander *et al.* 1968).

Late in pregnancy there is evidence of a change from a state of adrenocortical refractoriness to ACTH to one of responsiveness (see Liggins *et al.* 1977; Nathanielsz *et al.* 1977). It is unlikely, though, that ACTH is the primary signal for the maturational change in the fetal adrenal responsible for increased corticosteroid production (Jones, Boddy & Robinson, 1977), for the ACTH concentration increases *after* the rise in fetal plasma corticosteroid (Rees, Jack, Thomas & Nathanielsz, 1975). Nevertheless, the fetal adrenal gland needs ACTH for the normal prepartum surge of cortisol (Robinson, Challis, Pooley & Thorburn, 1977).

Such physiological results have rarely been backed up by morphological evidence, strengthening Jost's (1975) contention that the prenatal development of the sheep adrenal cortex needs further elucidation. We have therefore investigated the growth pattern and the ultrastructural maturation of those parts of the fetal sheep adrenal cortex concerned with the production of glucocorticoids (Long, 1975) in late pregnancy, hoping to be able to correlate these features of the cortex with the rise in fetal cortisol secretion in the last week of pregnancy.

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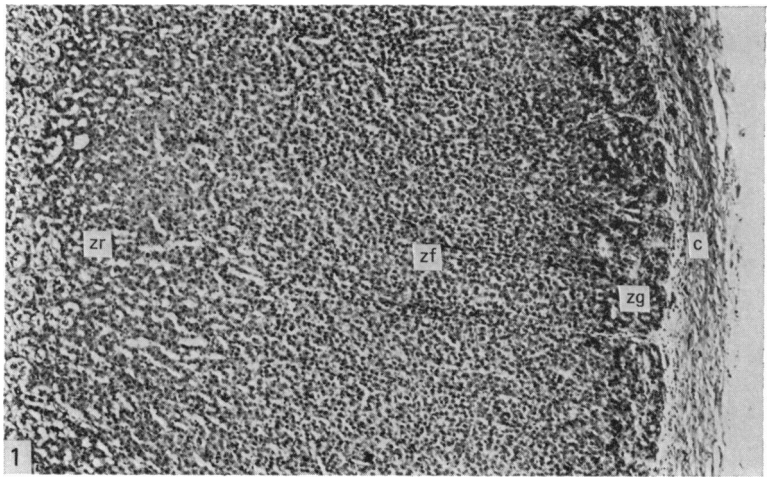



Fig. 1. Transverse section through cortex of fetal lamb adrenal gland at 144 days gestation. The capsule (*c*), zona glomerulosa (*zg*) and zona fasciculata (*zf*) are easily discerned, whereas the zona reticularis (*zr*) is imperfectly defined by the confluence of the sinusoids into the plexus reticularis. H. & E., green filter. × 56.

## MATERIALS AND METHODS

Accurately dated pregnancies were obtained by natural mating of New Zealand Romney ewes after synchronization of oestrus by vaginal tampons containing medroxyprogesterone acetate (MPA). The adrenal glands were obtained from fourteen fetuses delivered by Caesarean section between 136 and 148 days of gestation, and from one newborn lamb delivered vaginally after spontaneous parturition at term.

The adrenal glands, removed from the donor animals immediately after delivery, were bisected longitudinally or left whole, depending on size, and placed in ice-cold, half-strength, Karnovsky's (1965) fluid. About 1 hour later all specimens were cut into slices and placed in fresh fixative for at least 24 hours at 4 °C.

After preparation for paraffin embedding and light microscopy by standard procedures, 8 $\mu$m sections were stained with haematoxylin and eosin and then, using randomly selected slides, the sizes and histological appearances of the cortex and its zones were determined. At least nine measurements from the middle of each adrenal gland were usually made. Standard statistical techniques were employed in the data analyses.

Tissue utilized in the electron microscopical studies were post-fixed in $OsO_4$ for 90 minutes at 4 °C, dehydrated through alcohols, and embedded in Epon 812. Ultrathin sections were stained with aqueous uranyl acetate and lead citrate, then examined in a Philips EM 300.

## RESULTS

### *Adrenocortical histology*

Although all glands examined (Table 1) showed cortical zonation, the zona glomerulosa, zona fasciculata, and zona reticularis were not easily demarcated at their junctions (Fig. 1), the reticularis in particular being very variable in its degree of development.

Table 1. *Dimensions of fetal sheep adrenal in late gestation*

| Ewe number | Gestation length (days) | Combined adrenal weights (g) | Thickness of cortical regions (μm) (mean ± S.D.) Capsule | Zona glomerulosa | Z. fasciculata + z. reticularis |
|---|---|---|---|---|---|
| 181*† | 136 | 0·43 | 113 ± 13 | 162 ± 30 | 501 ± 83 |
| 76* | 136 | 0·43 | 82 ± 15 | 131 ± 20 | 381 ± 60 |
| 858* | 139 | 0·53 | 100 ± 22 | 107 ± 17 | 568 ± 50 |
| 830* | 141 | 0·67 | 104 ± 29 | 85 ± 20 | 787 ± 63 |
| 826* | 141 | 0·70 | 102 ± 24 | 111 ± 18 | 784 ± 75 |
| 832* | 141 | 0·76 | 92 ± 18 | 88 ± 21 | 1012 ± 220 |
| 811† | 142 | 0·70 | — | — | — |
| 9* | 143 | — | 108 ± 33 | 114 ± 16 | 503 ± 66 |
| — | 144 | 0·48 | — | — | — |
| — | 144 | 0·82 | — | — | — |
| 831* | 144 | 0·89 | 99 ± 22 | 89 ± 26 | 1216 ± 121 |
| 45*† | 145 | 0·58 | 104 ± 19 | 129 ± 18 | 529 ± 82 |
| 50*† | 147 | 0·63 | 87 ± 18 | 95 ± 18 | 550 ± 72 |
| — | 147 | 0·82 | — | — | — |
| — | 147 | 0·84 | — | — | — |
| 29*† | 148 | 0·60 | 118 ± 15 | 161 ± 25 | 511 ± 85 |
| 69† | 147 (Newborn) | 0·79 | — | — | — |

* Source of tissues for light microscopy and mensuration.
† Source of tissues for electron microscopy.

A dense connective tissue capsule surrounded the gland, and from it, trabeculae, in which were major blood vessels, passed radially to the medulla. The columnar or cuboidal cells of the outer cortical zone, organized into clustered ovoid groups similar to those of the mature glomerulosa, were enclosed in a connective tissue framework and surrounded by networks of capillaries. Individual cells had round, darkly stained nuclei and vesicular cytoplasm of varying density, some cells containing a few large clear vacuoles.

The cells of the inner zone were arranged in radiating cords one or two cells thick separated from each other by narrow, irregularly spaced, radial sinusoids. As in the glomerulosa, the cells of the fasciculata possessed round nuclei, but they tended to be slightly larger and less densely stained. Their cytoplasm was also variable in appearance, a few cells being densely eosinophilic whereas others were typical adrenocortical spongiocytes. In some preparations the fasciculata extended to the medulla. In others the innermost zone cells, which histologically were similar to those of the fasciculata, were arranged in anastomosing cords separated by large intersecting sinusoids. Consequently, the reticularis varied in the extent to which it surrounded the medulla, and histologically it could not be easily demarcated from the fasciculata. In the full term fetuses the reticularis completely enclosed the medulla.

*Adrenocortical dimensions*

In all specimens examined, the transition from glomerulosa to fasciculata, but not that from fasciculata to reticularis, could be defined with reasonable accuracy. Consequently, the thicknesses of the capsule, glomerulosa, and inner cortex

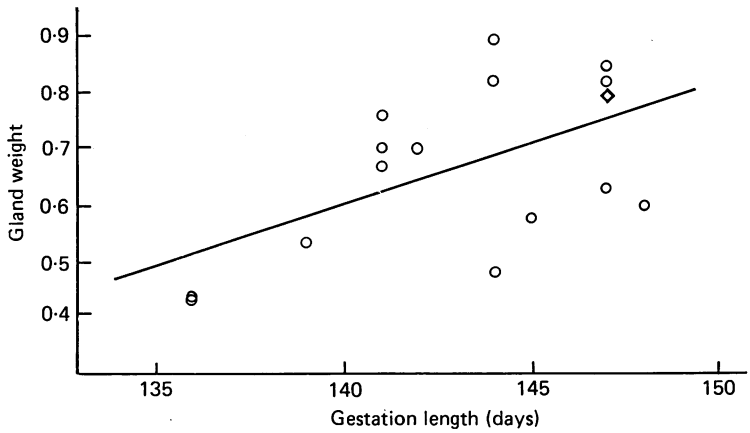



Fig. 2. Relationship between gestation length and weight of combined fetal lamb adrenal glands ($r = 0{\cdot}57$, $P < 0{\cdot}05$). The regression line equation is $\hat{Y} = -2{\cdot}48 + 0{\cdot}022X$. ◇ = newborn lamb.

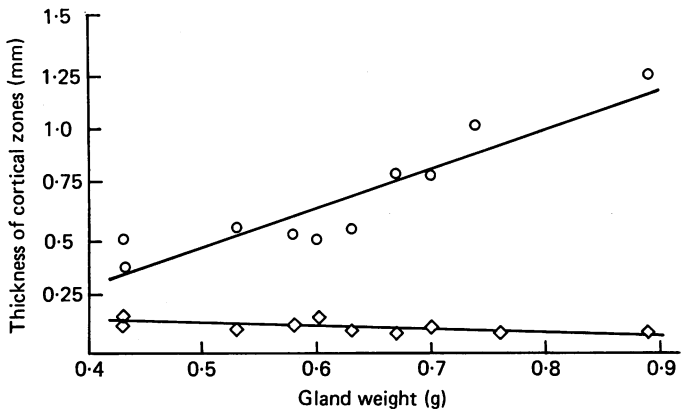



Fig. 3. Relationships between weight of fetal lamb adrenal glands and thickness of zona fasciculata+zona reticularis (○; $r = 0{\cdot}93$, $P < 0{\cdot}01$) and zona glomerulosa (◇; $r = 0{\cdot}69$, $P < 0{\cdot}05$). The regression line equations are respectively $\hat{Y} = -0{\cdot}38 + 1{\cdot}71X$ and $\hat{Y} = 0{\cdot}20 - 0{\cdot}14X$.

(fasciculata plus reticularis) were measured for analyses of adrenocortical growth patterns (Table 1).

Figure 2, which illustrates the growth pattern of the fetal adrenal gland during the last 2 weeks of gestation, shows that, during this period, the gland almost doubled in weight, increasing on average from 0·45 to 0·80 g. A major component of this growth was in the cortex, particularly the inner zone (Fig. 3). Over the period considered, this inner zone grew from some 0·33 mm to approximately 1·25 mm in thickness, whereas the outer glomerulosa appeared to decrease very slightly in size. The growth curves of these three parameters were essentially linear until birth. Medullary growth was negligible: in the youngest glands its mean radial width was 1·43 mm, while in the heaviest glands it was 1·48 mm.

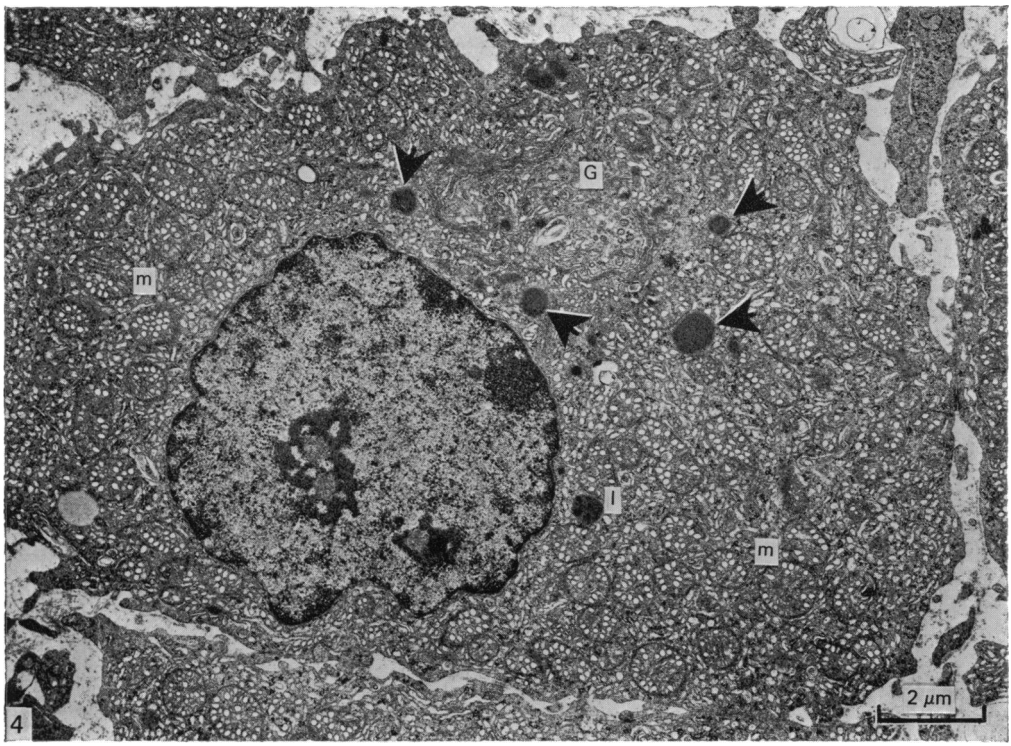



Fig. 4. Electron micrograph of dark adrenocortical cell, illustrating location and format of Golgi complex (G) with perinuclear dark granules (arrowed), lysosome (*l*), and mitochondria (*m*) with vesicular cristae. The dark cell may be the cell concerned principally with hormone synthesis and storage.

*Ultrastructural features*

The inner cortical cells in general possessed the ultrastructural characteristics of steroid-secreting cells (Fawcett, Long & Jones, 1969), but they were present in three different forms, namely dark cells, light cells and intermediates.

The dark cells were cuboidal, had dense cytoplasm, and an irregularly shaped nucleus with clumped chromatin and one or two distinct nucleoli (Fig. 4). They possessed short cisternae, and infrequently, small stacks of rough endoplasmic reticulum (RER) but abundant saccules of smooth endoplasmic reticulum (SER) (Figs. 4, 5). Cisternae of the SER were occasionally continuous with those of the RER, and they were frequently in intimate association with the mitochondria (Fig. 5). Numerous clumped or free ribosomes were scattered through the cytoplasm (Figs 5, 6).

The Golgi complex was well developed with a number of circumferentially placed Golgi stacks composed of four or five closely apposed smooth surfaced cisternae (Fig. 4). Vacuoles of varying sizes and coated vesicles were present internal (trans) to the Golgi stacks. These cells also possessed numerous ovoid or spherical mitochondria, usually of orthodox configuration, and with a dense matrix (Figs 4–6). Internally the mitochondria contained tubules which were closely associated with the inner mitochondrial membrane, yet not derived from it (Fig. 6); numerous vesicular cristae which occasionally filled the inner mitochondrial compartment; and

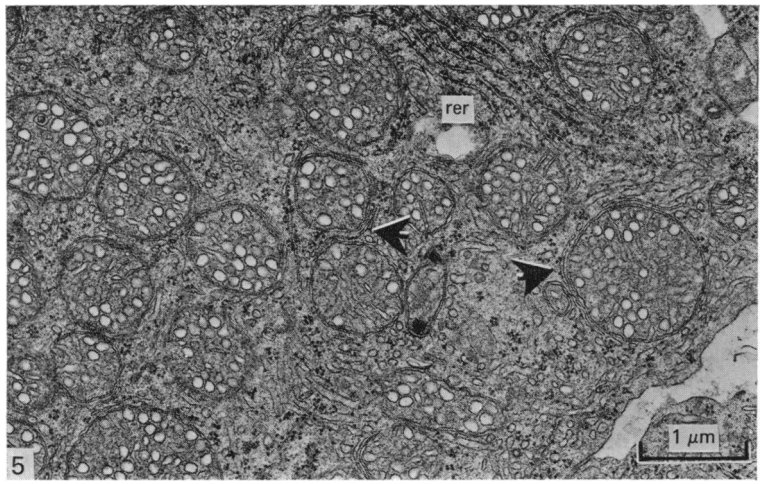



Fig. 5. Section of dark cell in which numerous mitochondria are associated with whorls of smooth endoplasmic reticulum (arrowed). The rough endoplasmic reticulum (*rer*) is arranged in parallel stacks and as single cisterna.

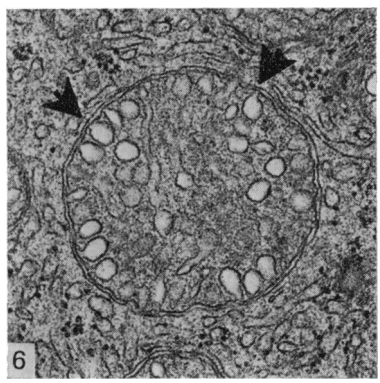



Fig. 6. The majority of mitochondria contain tubular vesicles (arrowed) which are linked with, but are not a continuation of, the inner mitochondrial membrane.

infrequently, small numbers of lamellar cristae. Lipid droplets were rarely seen. A few, dark, membrane-bound granules were present.

Light cells increased in number proportionately more during the period of observation than did the dark cells. The earliest light cells examined (Fig. 7) had a round or irregularly shaped nucleus with densely staining, clumped chromatin and distinct nuclear pores. A ribosome-studded outer membrane surrounded the nucleus, and the perinuclear cisterna was dilated in many areas (Figs. 7, 8). Scattered through the pale cytoplasm of the cell were numerous profiles of a vesicular endoplasmic reticulum, some possessing a few external ribosomes, polysomes and dark granules (Figs. 8, 9). The Golgi complex (Fig. 8), of more flattened form, was composed of stacks of three or four flattened cisternae. Its trans region contained numerous bristle coated vesicles and clear vesicles of similar form to those found through the cytoplasm. Small coated vesicles were also present outside (cis) the Golgi stacks. Light cell mitochondria were numerous, of orthodox configuration, and spherical or ovoid in shape (Figs. 7–9). Internally the mitochondria had an expanded, clear

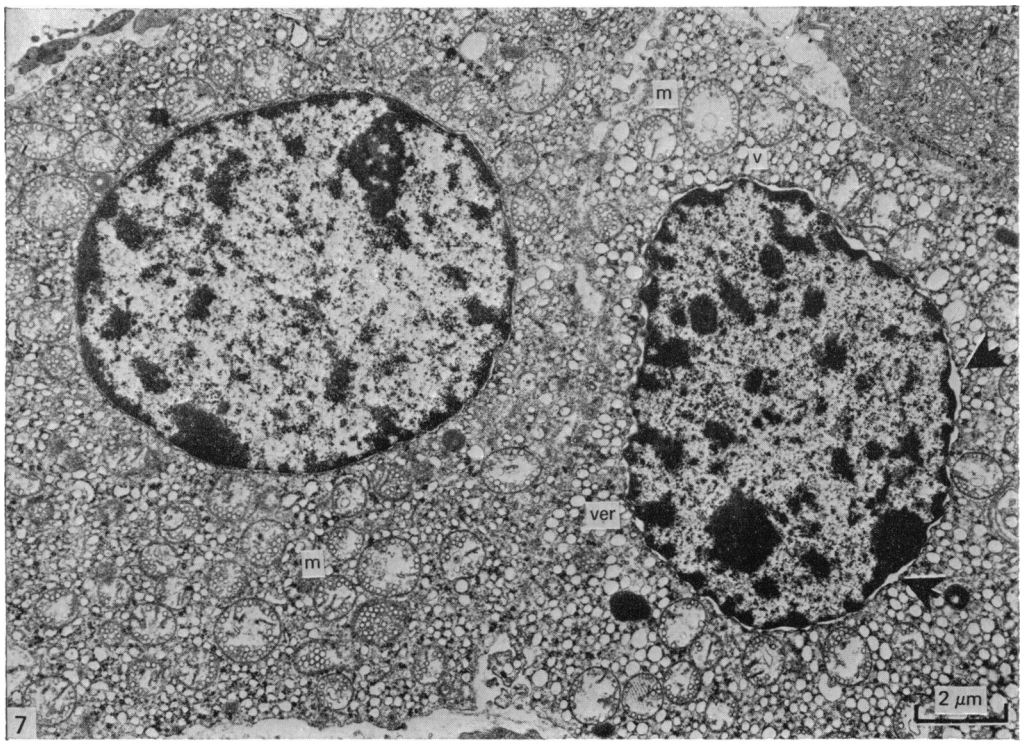



Fig. 7. Electron micrograph of two adjacent light cells illustrating degree of development of vesicular endoplasmic reticulum (*ver*). The mitochondria (*m*) contain numerous vesicular cristae which are frequently dispersed to the boundaries of the organelle; the majority of mitochondria possess a relatively clear matrix. Note the degree to which the perinuclear cisterna may be dilated (arrows). The light cell may be the primary secretory cell.

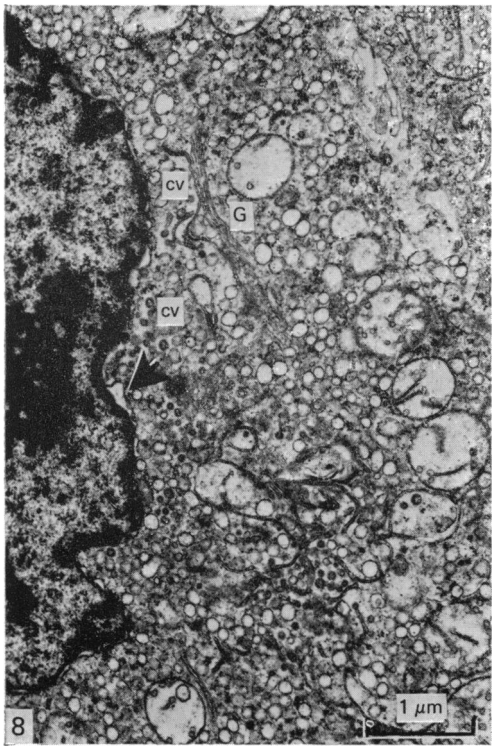
matrix space which contained vesicular cristae which were usually closely apposed to the inner mitochondrial membrane, and a few lamellar cristae, some of which possessed terminal dilatations. A few dark, membrane-bound granules were scattered through the light cell cytoplasm (Figs. 7, 9).

Cells intermediate between the two major (light and dark cell) types were found. Such cells sometimes possessed mitochondria characteristics of light cells, but within cytoplasm characteristic of dark cells, and sometimes mitochondria of dark cell type but associated with a profusion of endoplasmic reticular vesicles like light cells.

Although their proportions changed, the same cell types remained to term (Fig. 10); but from 142 days onwards increasing numbers of small bristle coated vesicles were present in the Golgi complex and scattered through the cytoplasm in both the dark and light cells (Fig. 11). Over the same period, increased numbers of lipid droplets appeared in the light cells, extracellular dark granules became more apparent, and coated caveolae were more numerous (Figs. 10–13).

The inner cortex of the newborn adrenal was composed predominantly of light cells, typically with round nuclei containing finely dispersed chromatin, and with variably dilated perinuclear cisternae which were usually ribosome-free.

The Golgi complex at this stage was well developed and located in the perinuclear cytoplasm (Fig. 11). It was formed of several discrete stacks of curved cisternae, typically with terminal dilatations, and with the trans faces oriented towards the



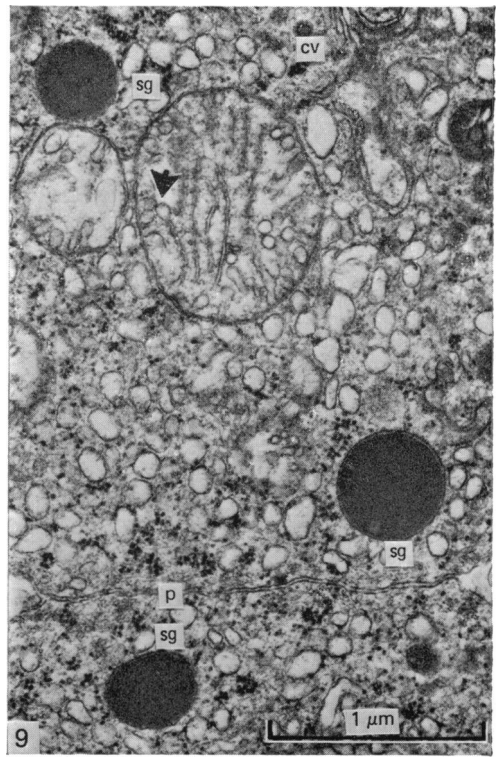




Fig. 8. Higher magnification of perinuclear region of light cell. The perinuclear cisterna shows some dilatation (arrowed), a single Golgi stack (*G*) is present, and trans to it are numerous small coated vesicles (*cv*).

Fig. 9. High power electron micrograph illustrating membrane-bound secretory granules (*sg*) present in two adjacent cells (*p*, plasmalemma). Free ribosomes (*r*) are present and the mitochondria possess vesicles (*cv*) and lamellar cristae, some of which have terminal dilatations (arrowed).

central region of the complex. At the cis regions of the Golgi stacks, clear ribosome-free vesicles of the transitional ER were present. Each Golgi stack consisted of three or four cisternae of varying length and irregular width. Just inside each stack there were one or more narrow cisternae of relatively uniform width, considered to be components of GERL (Novikoff, 1976). The terminal regions of these GERL cisternae were covered to varying degrees along their lengths with small, bristle-like projections. The numerous small, round, bristle coated vesicles present in the interior of the Golgi complex appeared to be formed from the GERL cisternae. Other membrane-bound clear vesicles of differing sizes were also located within the Golgi complex.

The mitochondria (Figs. 12, 14) were similar to those of the light cells, but tended to contain more of the internal tubulovesicular and lamellar components and to have a denser matrix. Dark membrane-bound granules were seen both inside and outside the cell, and numerous clear vesicles of ER and lipid droplets were present within the cytoplasm (Figs. 12–14).

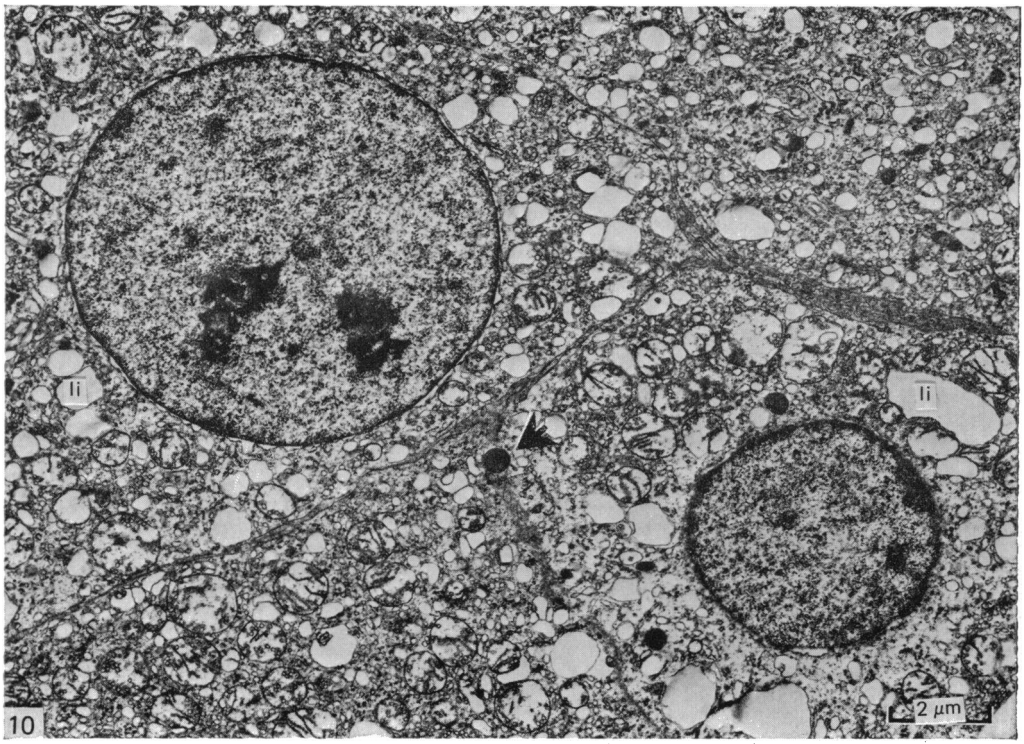



Fig. 10. Electron micrograph of light cells as they are seen in the newborn lamb. Numerous ER vesicles are present and lipid droplets (*li*) are characteristic of some cells. Note the extracellular secretory granule (arrowed).

## DISCUSSION

The pronounced changes known to occur in the activity of the fetal sheep adrenal cortex were not reflected in its histological appearance, for over the last ten days of gestation tissue changes were quantitative rather than qualitative. By the beginning of the transition period (Nathanielsz, 1976) the gland had attained the tissue relationships characteristic of the mature gland. However, it was only close to term, principally because of increasing sinusoidal confluence and the formation of the plexus reticularis adjacent to the medulla, that the reticularis came completely to surround the medulla and to be distinguishable histologically from the fasciculata with assurance.

Growth of the gland, as measured by increases in weight and organ dimensions, was essentially linear over the period of examination. The rapid growth phase characteristic of late gestation (Comline & Silver, 1961) had already begun by 136 days, the glands almost doubling in weight over the following 12 days. Our figures were like those of Comline & Silver (1961) in showing considerable variability and an approximate doubling of gland weight over the same period. Careful consideration of these authors' data does not support Nathanielsz's (1976) contention that the increase in weight over the period is fourfold.

There was a very close correlation between the weight of the gland and cortical thickness, indicating that the increased size of the gland resulted from differential growth of the inner cortical zone, for neither the glomerulosa nor the medulla grew much during the period under examination. Radial measurements of the cortical

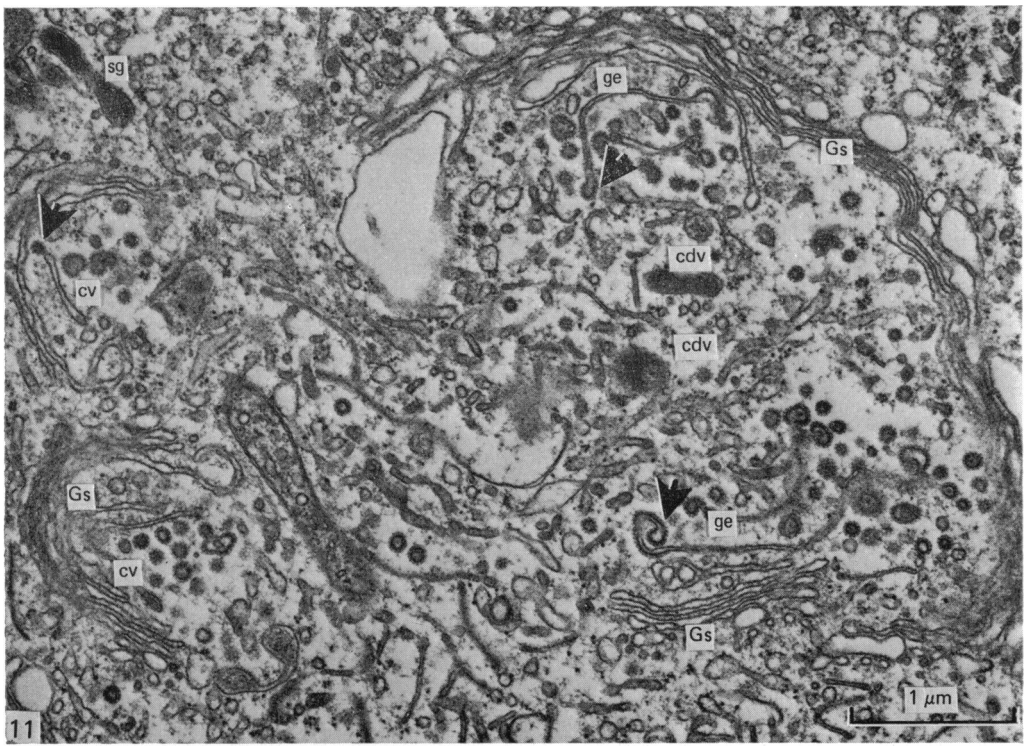



Fig. 11. High power view of Golgi complex in newborn animal. Numerous cisternae of the Golgi stacks (*Gs*) enclose GERL (*ge*) cisternae, some of which contain bristle coated regions or have budded small coated vesicles (arrowed). Within the Golgi complex are several condensing vacuoles (*cdv*) and vacuoles of the endoplasmic reticulum. *cv*, coated vesicles.

zones showed that growth of the inner zone was also essentially linear during its fourfold increase in thickness from some 0·33 mm to approximately 1·25 mm. This degree of growth resulted in a sixfold increase in the volume of the fasciculata + reticularis. Thurley (1972) presented comparable data and observed that no adrenal from a live newborn lamb had a central cortical area of less than 15 $mm^2$. Consequently, there must be a lower limit to the mass of cortical tissue capable of producing sufficient cortisol to initiate parturition.

The control of growth in the adrenal cortex during its period of enhanced secretion is not yet understood. Fetal hypophysectomy leads to decreased adrenal growth (Jost, 1977), yet the adrenal cortex will continue to grow without the hypothalamo-hypophyseal system. Maintenance of normal cellular structure, however, does depend on the presence of this central endocrine axis (Daikoki, Kinutani & Sako, 1976).

Ramachandran, Rao & Liles (1977) concluded that adrenocortical cell proliferation must be induced by factors other than ACTH, possibly one or a combination of the growth factors–growth hormone, somatomedin and insulin. Adrenocortical cell hypertrophy may follow exposure to ACTH (Kahri, 1966), but adrenocortical hyperplasia does not. For an adequate explanation of the rapid increase in growth occurring at about 130 days of gestation, more information on the fetal plasma levels and the growth-promoting effects of the above growth factors is needed. ACTH is

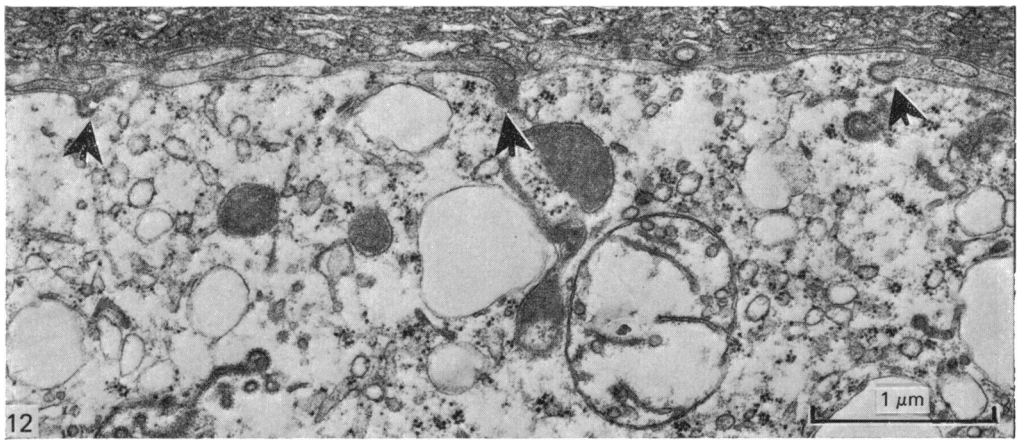



Fig. 12. The caveolae possess bristle coated surfaces (arrowed). Note also the peripheral secretory granules.

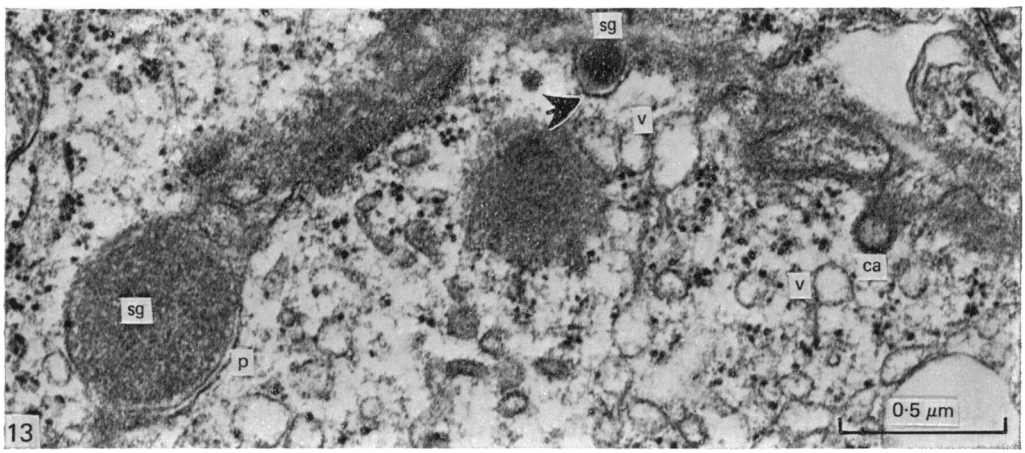



Fig. 13. While the plasmalemma of the caveolae (*ca*) are coated, those of the exocytotic region (arrowed) are not. *sg*, secretory granules.

more involved in the acute stimulation of steroidogenesis, and long term growth and maintenance, than in short term growth stimulation (Ramachandran *et al.* 1977).

Numerous studies have examined the relationship between cell ultrastructure and steroidogenesis in both the fetal (Idelman, 1970; Jost, 1975; Coffigny & Dupouy, 1978) and adult glands (Malamed, 1975). There has been general agreement on the significance of the relationship between a well developed SER, mitochondria with tubular or vesicular cristae, cholesterol ester-containing lipid droplets, and corticosteroid secretion by adrenocortical cells. In our study, all adrenocortical cells examined demonstrated these features, yet there were subtle differences in the degree of cellular differentiation as gestation proceeded.

Although light, dark and intermediate cells (Idelman, 1970) were present in all cortical specimens examined, the light cells were increasingly the most numerous as gestation proceeded. Similar light and dark cells have been described in the rat fetus by Coffigny & Dupouy (1978), and our findings support these authors' conclusions that increased corticosteroid production is linked with decreased numbers of dark

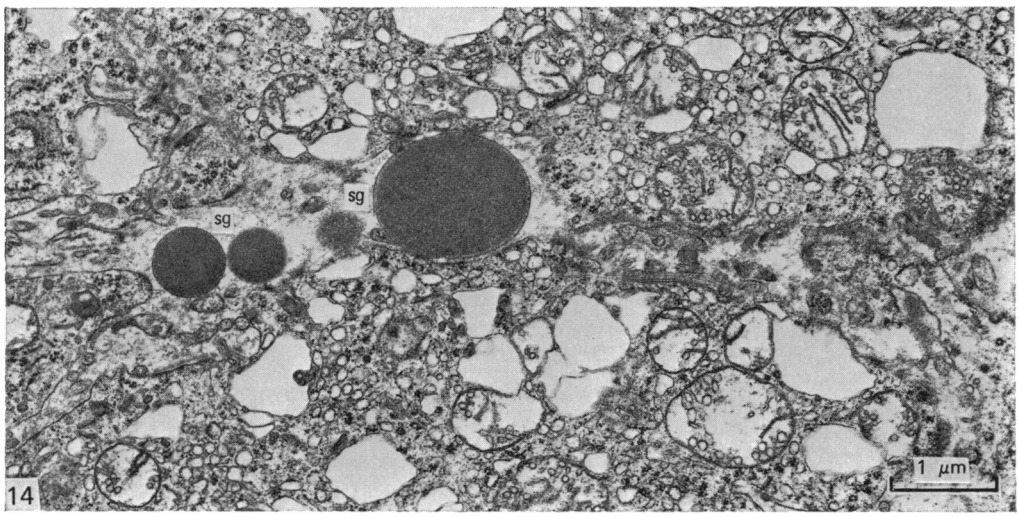



Fig. 14. After they have been released, the secretory granules (*sg*) are located in the intercellular spaces.

cells. The light cells were characterized by a profuse vesicular SER, contrasted with the tubulovacuolar SER of the dark cells, mitochondria of the orthodox configuration with vesicular rather than tubulolamellar cristae, numerous lipid-containing vacuoles, a well-developed Golgi complex, and an increased number of dark granules, some of which were extracellular.

The majority of the above ultrastructural features are indicative of an increasing response to ACTH. Comparable ACTH-induced transformation of the SER and mitochondria have been described in the fetal human adrenal (Johannison, 1968) and the fetal rat adrenal (Suyama, Long & Ramachandran, 1977). The development of rounded vesicles of SER, and of mitochondria containing microvesicular cristae, universally precedes increased secretion of corticosteroids (Kahri, 1966; Idelman, 1970; Milner, 1971).

At 136 days the Golgi complex was well developed in both dark and light cells; but at term, it was best developed in the light cells. In the mature gland, numerous small bristle coated vesicles and larger clear vacuoles were trans to the Golgi stacks. These small coated vesicles developed from the ends of narrow parallel profiles of GERL, and some appeared occasionally to be incorporated into the vacuoles. These small coated vesicles have either been regarded as primary lysosomes (Friend & Farquhar, 1967; Novikoff, 1976) or as being involved in intracellular transport from the transitional elements of the ER to the condensing vacuoles (Palade, 1975). Coated vesicles in the peripheral cytoplasm were also most numerous near term. They formed after the invagination of coated regions of the plasmalemma. After losing their bristle coat these vesicles have been shown to transport either low density lipoprotein–cholesterol complexes (Anderson, Goldstein & Brown, 1976) or proteins (Hillman, Seliger & Burk, 1975) to the interior of the cells where, after the incorporation of primary lysosomes, the ingested material is degraded and utilised in relevant metabolic activities.

The secretion of corticosteroids may (Gemmell, Laychoch & Rubin, 1977) or may not (Idelman, 1970) be associated with convincing ultrastructural evidence of its

occurrence. Our studies confirm the findings of Gemmell *et al.* (1977), for there was good evidence of condensation and of the formation of dark, membrane-bound granules in the Golgi complex. These granules were in greatest concentration near the Golgi complex, and in late gestation were frequently found near the plasmalemma or else external to it. It is highly likely that these granules, which may be lysosomes, function as translocators of the corticosteroids (Nussdorfer, Mazzocchi, Neri & Robba, 1978).

The two most significant events noted during the last 12 days of gestation were (1) a sixfold increase in the volume of that part of the cortex which synthesises glucocorticoids, and (2) the differentiation in the inner zone cells of the apparatus for maximum steroidogenic capacity. During this period, fetal plasma ACTH concentrations more than doubled (Jones, Boddy & Robinson, 1977) and, by the time of parturition, corticosteroid production had increased ninefold (Liggins *et al.* 1973). Therefore, at term, either enhanced sensitivity to ACTH or sufficient inner zone cell differentiation must have occurred to increase corticosteroid production per unit mass by some 50%.

The stimulus to growth of the inner zone of the cortex is not yet known, but the differentiation of its cells during the 130–140 days period, is, in all probability, a response to the phasic release of ACTH, which reaches levels considerably above the average plasma concentration during episodes of nocturnal low amplitude EEG activity over this period (Nathanielsz *et al.* 1977). This exposure to surges of ACTH should stimulate cytodifferentiation, particularly of the SER and the mitochondria.

As we have seen, increased numbers of mitochondrial vesicular cristae (as demonstrated here) follow ACTH stimulation, and Milner (1971) has demonstrated their close association with increased 11$\beta$-hydroxylation activity. Further, it has been suggested that maturation of the 11$\beta$-hydroxylation system is an important factor contributing to the increased fetal plasma cortisol present at this time (Anderson, Pierrepoint, Griffiths & Turnbull, 1972). This effect of ACTH during long term infusions (Liggins, 1968), and in normal pregnancy, indicates a mechanism by which adrenocortical cells enhance their sensitivity to ACTH and secrete the higher levels of cortisol which precede experimentally induced premature, or normal, parturition.

Our growth data, and the ultrastructural evidence of cellular differentiation during the last 10–12 days of gestation, appear adequate to explain the increased levels of fetal plasma cortisol characteristic of this period of pregnancy.

## SUMMARY

Growth patterns and cytodifferentiation of the fetal lamb adrenal cortex, from 136 days of gestation to birth at normal term, have been examined.

The gland almost doubles in weight over this period (0·45–0·80 g) and the inner cortical zone (zona fasciculata + zona reticularis) nearly quadruples in thickness (0·33–1·25 mm). The phase of rapid growth has begun by 136 days, and the growth patterns are essentially linear.

The inner cortical zone consists of light and dark cells, the former increasing in number proportionately more than the dark cells. This change in the cell ratio is linked with increased fetal production of cortisol. As growth and differentiation proceed the light cells increasingly possess a more vesicular SER; mitochondria of the orthodox configuration, with predominantly vesicular cristae; a well developed Golgi complex with numerous small, bristle coated vesicles; and dark granules near the Golgi complex, in the peripheral cytoplasm, and external to the cells.

The sixfold increase in the volume of the inner cortical zone and the ultrastructural evidence of cytodifferentiation appear adequate to account for the known increase in the fetal synthesis of cortisol during this stage of pregnancy.

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