# Cell junctions in the adrenal cortex of the postnatal rat

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#### INTRODUCTION

Several kinds of cellular junction in the adrenal gland of mammalian fetuses have been described recently (Black, 1972; Joseph, Slack & Gould, 1973). In the adrenal cortex of adult mammals, including the rat, gap and septate junctions have been described (Friend & Gilula, 1972*a*, *b*). The postnatal period, however, has been neglected in this respect apart from Joseph, Slack & Gould's (1973) description of gap junctions in the rabbit adrenal cortex. Nussdorfer (1970) in his work on the postnatal adrenal gland of the rat did not mention cellular junctions.

It was therefore decided to investigate cellular junctions in the adrenal cortex of the postnatal rat.

#### MATERIALS AND METHODS

The adrenal glands of 44 Wistar rats were studied.

(1) Thirty two rats, divided into groups of four according to their ages (1, 2, 5, 7, 14, 16, 22 and 26 days) were anaesthetised with nembutal and perfused through the aorta with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH, 7.4, for 10 minutes. This was followed by immersion of 1 mm blocks of tissue in the same fixative for 4 hours, post-fixation in 2% osmium tetroxide in the same buffer, dehydration in acetone and embedding in Durcupan (Fluka).

(2) Twelve rats, divided into groups of two according to their ages (1, 2, 7, 14, 16 and 22 days) were anaesthetised with nembutal and perfused through the aorta with 3% glutaraldehyde in 0.2 M s-collidine buffer, pH 7.5 (Goodenough & Revel, 1970). Small blocks of tissue were post-fixed in a mixture (1:1) of 2% OsO<sub>4</sub>-collidine buffer (2:1) and 4% lanthanum nitrate, prepared according to Revel & Karnovsky (1967). The blocks were dehydrated in alcohol and embedded in Epon 812.

Staining with uranile acetate was carried out in the blocks during dehydration, the thin sections being stained with lead citrate and/or uranile acetate; some of them were not stained. Sections were observed and photographed under a Hitachi HU-12A electron microscope.

#### RESULTS

The layered structure of the neonatal adrenal gland of the rat is similar to that of the adult animal: the cells are disposed in cords or groups surrounding fenestrated capillaries. In the glomerular zone the arrangement is least regular, clumps of cells irregularly disposed being joined by rudimentary desmosomes (Fig. 1). These cells are more like the fetal type, being elongated, with big nuclei and not much cytoplasm, the latter containing a few liposomes, some free polyribosomes, a few mitochondria, a small Golgi complex and scanty smooth endoplasmic reticulum (Fig. 1). The cells of the fascicular and reticular zone, however, have all the characteristics of active

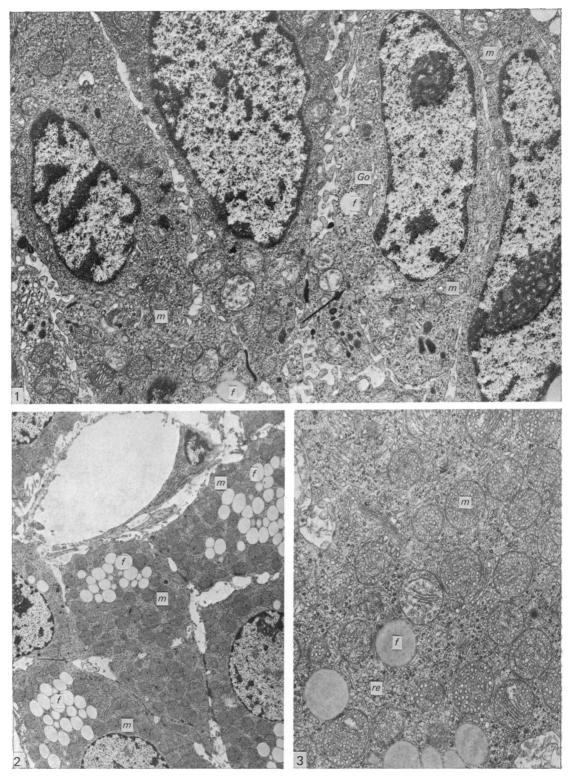


Fig. 1. Two days old rat. Glomerular zone showing very undifferentiated cells with big nuclei and scanty cytoplasm, a few liposomes (f), a few mitochondria (m) and a poorly developed Golgi complex (Go). A few rudimentary desmosomes can be seen between these cells (arrow).  $\times 4000$ .

Figs. 2-3. Five days old rat. Fascicular zone: its pericapillary cells are similar to those of the adult animal. Their cytoplasm shows multiple lipid droplets (f), an abundance of mitochondria with tubular cristae (m) and membranes of smooth endoplasmic reticulum (re). × 2500; × 10000.

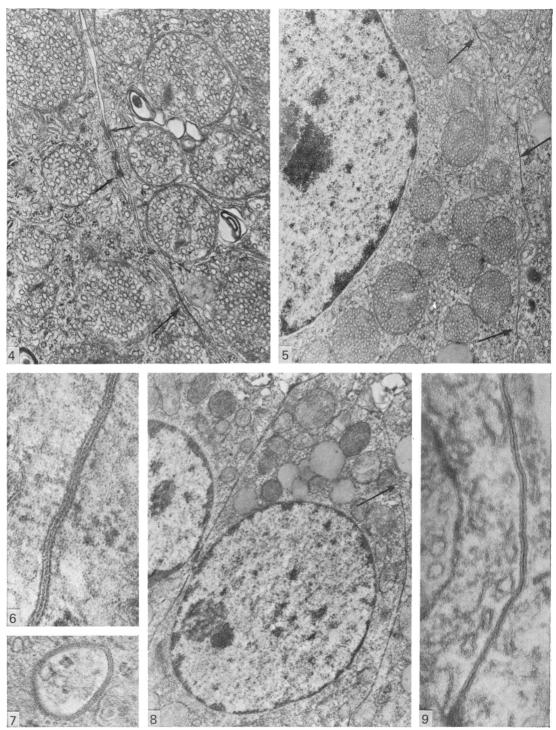


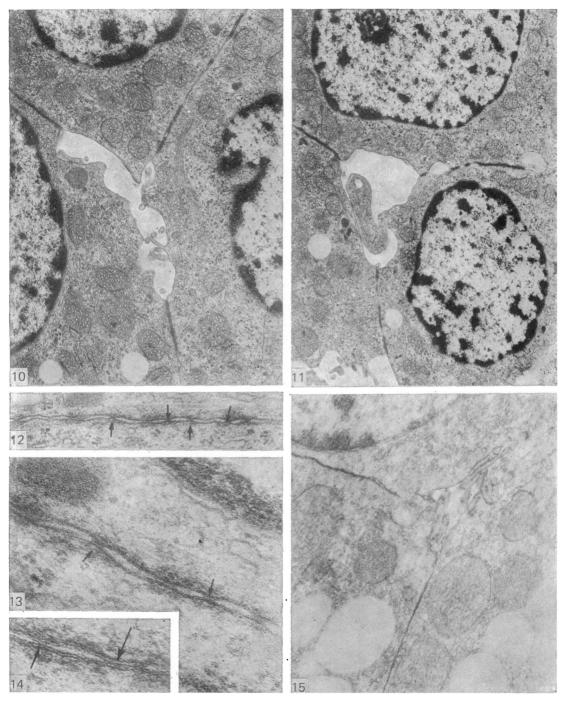
Fig. 4. Five days old rat. Reticular zone. Rudimentary desmosomes (short arrows) and a gap junction (long arrow) are shown.  $\times 15000.$ 

Fig. 5. Seven days old rat. Fascicular zone: gap junctions are seen between cells (arrows).  $\times\,10000.$ 

Figs. 6–7. Fascicular zone. Longitudinal and circular gap junctions. × 80000; × 50000.

Fig. 8. Fourteen days old rat. Lanthanum nitrate. Fascicular zone: the cell outlines are stained with lanthanum, which is penetrating through a gap junction (long arrow).  $\times 3500$ .

Fig. 9. The same gap junction as that shown in Fig. 8 at higher magnification.  $\times$  70000.



Figs. 10–11. Fourteen and sixteen days old rats. Glomerular zone: note uniting plaques between the cells enclosing narrow intercellular channels.  $\times$  8000;  $\times$  6000.

Figs. 12–13. Uniting plaques at higher magnification: Some tight junctions are seen (arrow-heads).  $\times$  20000;  $\times$  50000.

Fig. 14. Tight junction (short arrow) and a more extensive union (long arrow) in the uniting plaques.  $\times$  70000.

Fig. 15. Fourteen days old rat. Lanthanum nitrate. Glomerular zone: the lanthanum has passed through the intercellular uniting plaques.  $\times 12000$ .

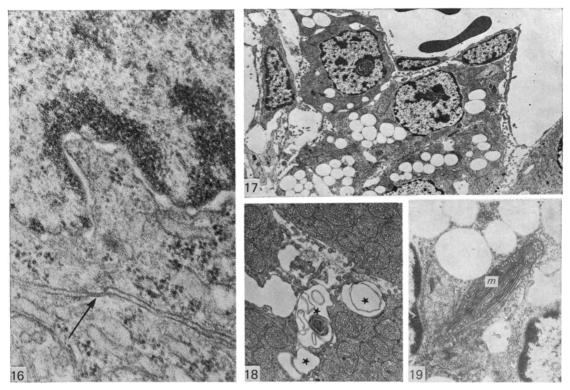


Fig. 16. Twenty two days old rat. A small gap junction between two cells of the glomerular zone (long arrow).  $\times$  25000.

Fig. 17. Sixteen days old rat. Liposomes increased in the cells of the glomerular zone.  $\times$  2500. Fig. 18. Twenty two days old rat. Signs of endoplasmocrine secretion in the glomerular zone (\*).  $\times$  8000.

Fig. 19. Twenty six days old rat. Mitochondria with pseudocrystalline cristae (m).  $\times 10000$ .

steroid-secreting elements, including an abundance of liposomes, smooth reticular membranes and mitochondria with tubular cristae (Figs. 2, 3). The cells in these layers are joined by rudimentary desmosomes and gap junctions (Figs. 4, 5), which generally are longitudinal or circular. At higher magnification, gap junctions are typically pentalaminar (Figs. 6, 7). Lanthanum nitrate showed up a well defined intercellular space, gap junctions being permeable to this marker (Figs. 8, 9). In animals from 2 weeks of age onwards, one could observe extensive, relatively electro-dense plaques of union between the cells of the glomerular layer (Figs. 10, 11) enclosing small intercellular channels. At higher magnification these plaques showed tight junctions and wider ones similar to those found in gap junctions (Figs. 12-14). These plaques were also permeable to lanthanum (Fig. 15). In older animals it was possible to see small gap junctions between the cells of the glomerular zone (Fig. 16). As this type of junction became more frequent in the glomerular layer, the cells gradually acquired the cytological characteristics of the adult animal, with an increase in liposomes, the starting of endoplasmocrine secretion and an increase of mitochondria with tubular cristae as well as some pseudocrystalline forms (Figs. 17-19). No septate junctions like those of the adult rat were seen in any of the animals studied here.

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#### DISCUSSION

Recent investigations have shown that the differentiation of gap junctions could well be under hormonal control (Decker, 1976), especially in steroid-secreting tissues such as ovarian follicles and corpora lutea (Albertini & Anderson, 1974, 1975). It is known that the hypothalamo-hypophyseal system is involved in the regulation of the deeper layers of the adrenal cortex and that the system is functional prenatally (Eguchi, Hirai, Morikawa & Hashimoto, 1973; Daikoku, Kinutani & Sako, 1976). The glomerular zone, however, although it is exposed to some hypothalamic control, is mainly regulated via the renin-angiotensin-aldosterone system which depends on the juxtaglomerular apparatus. The latter does not differentiate until some time after birth (Kazimierczak, 1971). All this is in accordance with the observation that gap junctions are already differentiated in the deep zones of the cortex at birth, but not in the glomerular zone. Nussdorfer (1970) in fact describes the glomerular zone as non-functional in the postnatal rat. The author would agree that this is likely during the first 2 weeks, but from then onwards the zone shows the morphological features of the functioning adult organ (Wassermann & Wassermann, 1974). It is likely that gap junctions accelerate cellular differentiation by establishing metabolic and other functional connexions between cells, especially the exchange of ions and regulatory molecules (Staehelin, 1974; Lawrence, Beers & Gilula, 1978). Such junctions should also be important in the establishment of electric coupling between cells, highly desirable in a well innervated gland like the adrenal cortex (Unsicker, 1971).

#### SUMMARY

The differentiation of cellular junctions in the adrenal cortex of the postnatal rat has been studied. Desmosome and gap junctions were well differentiated at birth in the deeper zones of the cortex, but in the glomerular zone tight and gap junctions were not observed until 2 weeks after birth. The possibility that the development of gap junctions in the different zones of the adrenal cortex is hormonally controlled is discussed.

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