Ultrastructure of adrenal medulla of the prenatal rat

By MAX RATZENHOFER & OTFRIED MÜLLER

From the Department of Pathology, University of Graz, Austria

The ultrastructure of the adrenal medulla in adult laboratory animals has been described in a number of reports (Lever, 1955; Sjöstrand & Wetzstein, 1956; Wetzstein, 1957; de Robertis & vaz Ferreira, 1957; Eränkö & Hänninen, 1960; Yates, 1963, 1964a, b, c; Coupland, 1965a, b; Elfvin, 1965a, b). Since the introduction of the glutaraldehyde fixation technique, it has been possible to distinguish clearly between cells storing adrenalin and those storing noradrenalin by the different densities of their granules (Coupland, Pyper & Hopwood, 1964; Coupland, 1965a; Elfvin, 1965a). In order to study the histophysiology of the secretory process of catecholamines, suprarenal glands have been investigated under normal conditions and under the influence of mechanical, physical and chemical-pharmacological agents (the above references and Graf, 1966). The purpose of the present investigation was to study the structure of adrenal medulla of the rat during the prenatal period and to relate the findings to the process of secretion in the medullary cells.

MATERIALS AND METHODS

Four pregnant Wistar rats were sacrificed by a blow on the neck or decapitation, and the suprarenal glands were quickly removed from the foetuses (20 ± 1 days), divided into small pieces and placed in cold 1% OsO₄ solution buffered with veronal acetate (pH 7-4) for 1 h. After rapid dehydration in alcohol the material was embedded in Epon. Thin sections were cut with a Porter–Blum ultramicrotome, stained with lead hydroxide and examined in the Philips EM 100B electron microscope or in the Akashi Tronscope.

RESULTS

Suprarenal glands from foetuses of the same age were prepared for light microscopic studies by fixation in Regaud's fluid and staining with haematoxylin. Medullary cells (so-called 'spheres') were found between the large
and comparatively dark cells of the cortex (cf. Bachmann, 1954; Smitten, 1962). The cells in the ‘spheres’ are partly arranged in a net-like structure, and are small with little cytoplasm. The nuclei contain moderate amounts of chromatin, are oval and much smaller than the cortical cell nuclei. As compared with the mature medullary cells of adult animals, the cytoplasm is low in amount, basophilic and does not display a distinctly positive chromaffin reaction. In some instances we found nerve cell bodies (neuroblasts) with Nissl substance and large, characteristic vesicular nuclei.

With the electron microscope the foetal adrenal medulla of the rat shortly before birth is seen to consist mainly of immature chromaffin cells or pheochromoblasts. In addition, a few neurons, many axons, Schwann cells and vascular connective tissue are observed. The pheochromoblasts are arranged in epithelial units (Plate 1) and are separated by narrow intercellular gaps or wider intercellular spaces which contain axons or, more rarely, branches of Schwann cells. Adjacent to connective tissue spaces with blood vessels (Plate 5) or neighbouring cortical cells, the medullary cell groups are bounded by a basement membrane that is not always complete (Plate 3). The larger spaces between cells adjacent to blood vessels contain a few connective tissue fibrils and numerous microvilli of the cortical cells (Plates 3, 5). The spaces between medullary and cortical cells are mostly narrow, gap-like, and lack collagen fibrils. In such cases the cortical cells either have no or very few microvilli. The basement membrane of the medullary cells is not distinct (Plate 5). The nerve bundles of axons and Schwann cell processes as well as nerve cell bodies and dendrites are separated from the pheochromoblasts by narrow intercellular gaps and spaces which lack a distinct basement membrane (Plates 1, 2, 4, 10). Single axons may be deeply grooved into the surface of pheochromoblasts (Plates 1, 5). The endothelial cells of the capillaries are separated from the pheochromoblasts by a narrow connective tissue space. In Plate 5, the minimum distance between the surface of the medullary cell and the capillary lumen in the area of so-called fenestrae of the endothelium (Elfvin, 1965b) is approximately 420 μm. Pinocytotic vesicles are frequently observed in the cytoplasm of endothelial cells.

Since the surface of medullary cells is essentially smooth with practically no microvilli, the intercellular gaps between pheochromoblasts are very narrow, about 30–70 μm in width (Plates 6, 7). However the gaps widen in areas where

---

**PLATE 1**

Group of foetal medullary cells in a ‘sphere’ (Markballen); 20th day of development. The cell in the middle with the two nuclei contains no granules (immature cell, spongioblast of Smitten). The other cells contain a few chromaffin granules (pheochromoblasts). Note many axons between the medullary cells, running partly in channels (arrow). Left and below the ‘sphere’, a bundle of many axons and Schwann cell processes is seen, sectioned longitudinally and across. Nuclei of Schwann cells (SC). Below right, an endothelial cell with Golgi zone; capillary lumen (L). Left, large cortical cell with mitochondria, vesicles, lysosomes, and some microvilli at the surface. Note the small connective tissue space between the cortical cell and the nerve bundle. (×9000.)
Four medullary cells surround a bundle of axons which contain many neurotubules and filaments as well as smooth vesicles. Above, a pheochromoblast. In the broad sinus of the nucleus is located the Golgi zone, adjacent to it a group of polymorphous mitochondria. In the cytoplasm numerous rough and smooth vesicles. Some chromaffin granules are noted near the Golgi zone and the plasma membrane. (×9300.)
Adrenal medulla of prenatal rat

cilia or axons are found (Plates 10, 11B), or where three or more cells oppose one another (junctional intercellular spaces of Coupland (1965a)). The intercellular gaps most frequently appear empty but on occasion contain a slightly electron-opaque material.

Ultrastructure of pheochromoblasts

The foetal medullary cells possess little cytoplasm and contain very few opaque granules. The cells are much smaller than in the adult, and are mostly polyhedral with three to five rounded corners or tongue-shaped processes. No distinct microvilli are observed. The nuclei are round to ovoid, and the karyoplasm is finely granular. The nucleolus exhibits a reticular structure. The nucleus occasionally forms small bud-like evaginations which project into an enlarged perinuclear cisterna (Plate 4).

The endoplasmic reticulum is of two varieties, the granular reticulum with ribosomes (ergastoplasm), and the agranular reticulum which lacks ribosomes.

The ribosomes on the outer surface of the ergastoplasmic membranes are arranged in small circles or elliptic or coiled figures (Plate 6), as have been described in growing cells, e.g. in fibroblasts in tissue culture (Goldberg & Green, 1959), in wounds (Ross & Benditt, 1964) and in root cells (Bonnett & Newcomb, 1965). Between these ribosome groups the ergastoplasmic membranes are smooth, and form bulges and evaginations, which may communicate with the smooth reticulum. The flat cisternae of the ergastoplasm are connected with the perinuclear cisternae and are present in all parts of the cell. They are either arranged in groups (Plates 3, 6), or diffusely distributed in the cytoplasm. The ergastoplasm also forms medium-sized spherical vesicles, which are not connected with each other (Plate 4).

The agranular endoplasmic reticulum is abundant in the cell and is represented in part by a three-dimensional network, in part by small to medium-sized vesicles (Plates 4, 9). The small vesicles are often very close to each other or are connected by microtubules or fibrils (Plate 9). It can be imagined that during dispersion of the vesicles the connecting tubes are stretched to fine threads and finally break. In this way the vesicles and fragments of the tubes and threads would come to lie free in the cytoplasm (Text-fig. 1). The membranes of the vesicles are much finer than those of the ergastoplasm. The contents of the vesicles are uniformly dense and, on average, more electron-opaque than the ergastoplasmic sacs. The contents appear to be most dense in the smallest vesicles of the smooth reticulum (Plate 9). On the periphery of the Golgi zone the Golgi vesicles cannot be distinguished from the vesicles of the smooth reticulum (Plates 4, 5).

The Golgi zone is in a paranuclear position or in a sinus of the nucleus (Plate 2) and forms a rather sharply outlined field. It is similar to the Golgi complex described in other epithelial cells.

The mitochondria frequently lie near the Golgi zone (Plate 2) and exhibit a characteristic polymorphous form. The following forms are found: ovoid,
elongated, bent and buckled, 'V' or 'Y' shaped. They often exhibit bulges and processes (Plate 1). The lamellae of the inner structure are, as a rule, short and bent, and only rarely run through the entire diameter of the mitochondrion. Free ribosomes are abundant, arranged in small groups, and can be found in all parts of the cell except in the Golgi zone. The centriole has the usual appearance (Plate 5). The cilia (Plate 9) arise from the basal body and pass obliquely in a tunnel of the peripheral cytoplasm to the cell surface, ending in the intercellular space (Plate 10).

Each cell contains numerous multi-vesicular bodies (Plates 4, 5) which are usually round and enclosed by a single membrane. The interior of the bodies is moderately dense with a varying number of very small, almost empty vesicles. Plate 4 shows an accumulation of cytoplasm vesicles and granules around two multi-vesicular bodies. Cytosomes (lysosomes, dark bodies) (Plate 9) are rare in the cells. The limiting membrane of the cytosome seems single, and the interior dense with very fine granules. In almost all cells we have found the previously mentioned fine fibrils or tubules (Plates 4, 6, 9), often running for long distances and frequently in a straight course. The diameter of the tubules is 25–30 mμ. The finest fibrils are only ca. 5 mμ in diameter, and do not seem to be homogeneous although a distinct periodicity could not be distinguished. The pheochromoblasts also contain glycogen (Plates 7, 8) as reported by Graumann & Neumann, 1964.

Text-fig. 1. Hypothetical scheme of the transition of the smooth reticulum (1) into vesicles of increasing size with connecting microtubules and fibrils (2, 3).

Paranuclear section through a pheochromoblast. The basement membrane separates the cell from the surrounding connective tissue space (CS). In this space are located some precollagen fibrils and microvilli of the adjacent cortical cells. The cytoplasm of the pheochromoblast contains specific granules of different densities, ribosomes, and vesicles. In the upper region of the cell many ergastoplasm sacs (E) are seen. The arrows (PV) indicate two special pinocytotic vesicles; the arrow (G) indicates a small, probably newly-formed granule. In the right upper corner of the cell many mature osmiophilic granules with large intensely dense contents are shown. In the middle are large osmiophobic granules with or without dense material. (x 12900.)
Part of a pheochromoblast. The nucleus (right) shows an evagination into the perinuclear cisterna. Few polymorphous mitochondria are seen. Many medium sized vesicles of the rough reticulum ($E$) with electron-light contents and small vesicles of the smooth reticulum with more opaque contents are seen. Multivesicular body ($M$), and in the neighbourhood an aggregation of many small vesicles and chromaffin granules. To the left of the Golgi zone some smooth filaments are seen ($F$). Near the surface membrane are granules of different size and contents, processes of pheochromoblasts ($P$), and some axons. ($\times$ 15 500.)
Adrenal medulla of prenatal rat

Special pinocytotic vesicles (Roth & Porter, 1962; David-Ferreira & Manaka, 1965) exist in great numbers in each cell, and are particularly localized at the cell surface below the plasma membrane (Plates 3, 8). They are characterized by an opaque inner structure and particularly by dense regular and radially arranged material around the surrounding membrane (‘coated vesicles’ of Bunge, Bunge & Peterson 1965). Their origin and relationship to the plasma membranes can be seen in Plate 3. Ordinary pinocytosis seems to occur occasionally.

The specific granules

The granules vary greatly in appearance; they are either distributed throughout the cytoplasm (Plates, 3, 4), or arranged in groups. Accumulations near the Golgi zone are observed only occasionally (Plate 2). On the other hand, the granules are sometimes present in relatively large numbers near the plasma membrane (Plates 1, 6–8). The form of the granules is round to oval and they are always surrounded by a limiting membrane which is triple layered (unit membrane structure) (Plate 8). The interior of the granules varies somewhat in electron density, and this is why they can usually be divided into osmiophilic or osmiophobic. In the small and medium-sized osmiophilic granules, the secretory grain (core) is very electron-opaque (Plates 4, 6, 7). We regard the large-sized granules with intensely dense cores as mature ones. They show only a narrow light zone (halo) between the black centre and the limiting membrane. Most of the granules are osmiophobic and contain a moderately dense material, sometimes with a fine granular (paracrystalline) ultrastructure (Plate 8). These osmiophobic granules often contain small particles of intensely dense material which we consider to be remains of the osmiophilic contents of the mature granule. Finally, there are transitions to limiting membranes with or without residual osmiophobic contents. Such forms are found both in the cell interior and in accumulations below the cell surface (Plates 4–8) and some of the largest may enter into contact with the plasma membrane (Plate 8), but we have never observed with certainty an expulsion of the granule contents through the membrane.

The size of the granules in the micrographs depends upon the plane of section, individual size, development, fixation, and upon the secretory phase of the granules (see discussion and Text-fig. 2). Osmiophilic mature granules including their terminal membranes have a diameter from 140 to 250 mμ. It seems evident that in these largest granules the central core is in lysis (Plates 3, 7, 8).

Nerve tissue

Very often we observed bundles of non-myelinated nerve fibres associated with Schwann cells and their processes on the surface (Plate 1) or in the interior of the spheres (Plate 2). Non-myelinated axons are found either singly or in rows in the intercellular spaces between the pheochromoblasts (Plates 1, 11B). The Schwann cell processes are plump and short and as a rule they do not envelop the axons as in the adult adrenal medulla. On the surface of the pheo-
chromoblasts, thin axons are invaginated in the cells in deep grooves. In the axons there are numerous bundled neurofilaments, neurotubules and fine vesicles of the smooth-surfaced endoplasmic reticulum that are sometimes arranged beside the flat ergastoplasmic sacs (Plate 10). In spite of the numerous contacts between axons and chromoblasts we only saw one nerve ending that could be positively identified as such (synapsis, bouton) (Plate 6). There appears to be an indentation into the chromaffin cell at the point of contact (Coupland, 1965b). The portions of the plasma membranes facing each other are thickened and in the clear cytoplasm of the axon ending there are many fine smooth vesicles and an absence of large ones with electron-opaque centres.

Nerve cell bodies are rare (Plate 11). They contain large mitochondria with dense matrix and lamelliform cristae, ergastoplasmic sacs, smooth vesicles and bundles of neurofilaments and neurotubules that apparently have their origin near the Golgi zone.

**DISCUSSION**

In the late development of adrenal medulla the so-called spheres consist mainly of differentiating parenchymal cells, and contain connective tissue with blood vessels and much nervous tissue. The latter includes axons, differentiating nerve bodies, and Schwann cells. The parenchymal cells include ungranulated ones not yet identified as chromaffin elements (spongioblasts of Smitten, 1962) as well as immature chromaffin cells with initial granule development. These small and more rounded cells with few chromaffin granules are referred to as pheochromoblasts in contrast to the larger polyhedral mature chromaffin cells of the adult rat which contain numerous chromaffin granules and give a positive chromaffin reaction. For this latter cell type we propose the name pheochromocyte.

The terms 'pheochromoblast' and 'pheochromocyte' were introduced by Poll (1906). The 'pheochromoblasts' were considered by Poll, and later by Garafolini (1924), Hett (1925), and Dietrich & Siegmund (1926) as intermediate elements in the differentiation of adrenal medulla, ranging between the immigrated sympathicus cells and the granulated chromaffin cells.

There are many other differences between immature and mature chromaffin cells. It is noteworthy that the ultrastructure of the pheochromoblast seems to
Pheochromoblast with abundant ergastoplasm. On the outside of the sacs the ribosomes are arranged in small rosettes, in an elliptical and spiral manner. Small vesicles with moderately dense contents in contact (arrow) with the ergastoplasmic sacs or free in the cytoplasm. Many groups of free ribosomes. Near the plasma membrane chromaffin granules, some with lytic centre. In the right upper corner a nerve ending on the pheochromoblast. Circumscribed thickenings of the corresponding plasma membranes (synapsis). In the knob-like nerve ending only smooth vesicles of different sizes, no neurotubules. (×22000.)
Three pheochromoblasts. Accumulation of granules in the peripheral cytoplasm. Very different appearances of the granules: mature osmiophilic granules (m) with electron-dense contents and small halo (black granules); in the osmiophobic granules (o) fine granular and less dense material or small areas of the intensely stained black material (r); large lytic granules (l). Smooth vesicles (v), free ribosomes, ergastoplasm are also seen. (x 40400.)

M. RATZENHOFER & O. MÜLLER
Three pheochromoblasts. Near the plasma membranes (PM) osmiophobic granules with fine granular opaque contents (paracrystalline pattern of the matrix). Note the three layers of the granule membranes (arrows) and of the plasma membrane. Empty granulum (I), membrane fused with the plasma membrane, but no discharging of material at the cell surface; in the intercellular gap very little opaque material. Coated vesicle (cv) with corona near the obliquely cut plasma membrane. Many free ribosomes and glycogen particles. (x 72000.)
Pheochromoblast with very few granules. Dense network of smooth reticulum (arrows) with early vesiculation. Below left many vesicles connected by microtubules. Many free microtubules and microfilaments. Multivesicular body. Cilium, arising from the basal body and passing through a channel of the cytoplasm. In the cilium longitudinally cut pairs of tubules and some very fine vesicles. In the inset an obliquely cut cilium with a central pair and 9 peripheral pairs of tubules and a small vesicle. Cytosome with finely granulated dense contents and single dark outer membrane. (x 26300.)
Left, two pheochromoblasts. In the upper cell large Golgi zone, basal body and cilium, protruding into a widened intercellular space, adjacent to a bare axon (a). In the lower cell numerous ribosomes and granules, partly in contact with the plasma membrane. No distinct discharging of granule contents. Right, bundle of thick axons, partly in direct contact with the two pheochromoblasts. Attenuated Schwann cell process (SP). In the axons bundles of fine neurofilaments and neurotubules, smooth reticulum (SR) and larger vesicles without electron-dense centre. Special pinocytotic vesicle (cv). Mitochondrion, very few ribosomes. (x 16600.)

M. RATZENHOFER & O. MÜLLER
Adrenal medulla of prenatal rat

be represented more easily and distinctly than that of the mature pheochromocyte. With the same fixing and embedding technique, the endoplasmic reticulum and fine cytoplasmic vesicles as well as the hyaloplasm of the pheochromoblast are better preserved than in the pheochromocyte. Two factors are to be taken into account in explaining this more favourable preservation of the foetal medullary cell: its lower contents of specific granules and catecholamines, and the fact that the innervation of the medullary cell is not yet fully developed. These two features result in a lower secretory activity of the foetal cell after irritation. Consequently when the pregnant female animal is killed, and hormones and other substances are released from the adrenal medulla (Cannon's alarm reaction, Wetzstein, 1957) the foetal cells cannot undergo such a massive physico-chemical response as the mother animal and it is perhaps for this reason that the fixation is better by comparison.

Another difference from the adult rat concerns the nature of the cell surface and thus the topographical relationship of the medullary cells to one another. In the adult rat, the free surface and the intercellular surface of the chromaffin cells show small localized invaginations and evaginations, and some microvilli in interlocking zones of adjacent cells with 15–20 mμ intercellular gaps after fixation (Coupland, 1965a). On the contrary in the foetus the plasma membrane is as a rule smooth on all sides, and the intercellular gaps are simple and narrow (30–70 mμ). The intercellular spaces formed by three or more approaching cells are also narrow and contain nerve fibres, cilia, and only occasionally microvilli. Some larger invaginations of the cell surface contain closely impressed axons and axon endings as described by Coupland 1965b. We have observed small grooves, so-called 'caveoles', regarded by Coupland (1965a) as points of previous discharge of chromaffin granules, but we relate them to the development of the special pinocytotic vesicles of David-Ferreira & Manaka (1965) (the coated vesicles of Bunge et al. 1965) and not to the discharge of granules. Distinct thickenings of the plasma membrane and adjacent cytoplasm (attachment zones, Elfvin, 1965a) or distinct desmosomes (Coupland, 1965a) have not been observed in the foetal cell.

Endoplasmic reticulum and granulopoiesis

It is probably due to the proliferative activity of the pheochromoblast that it contains much more rough-surfaced endoplasmic reticulum (ergastoplasm) and free ribosomes than the fully differentiated pheochromocyte. The profiles of the ergastoplasm are tubulosaccular or vesicular. Very often we saw smooth parts of the ergastoplasmic sacs with evaginations which seem to be places of connexion of the smooth and the rough reticulum. Similar impressions have been obtained in other cell types by other authors (Goldberg & Green, 1964).

A remarkable property of the foetal chromaffin cell not previously described in the normal adult cell is the abundance of thin-walled smooth vesicles with electron-opaque contents and of different sizes, in all parts of the cell. The zones with chromaffin granules contain fewer vesicles than granule-free areas. The
origin of these vesicles cannot be determined with certainty. A continuous formation of vesicles (vesiculation) may result from "pinching off" of the smooth reticulum (see Text-fig. 1) or from peripheral parts of the Golgi zone (Plates 4, 5). This abundance of small vesicles was also observed in the mature chromaffin cell, but only under experimental conditions after intense treatment with reserpine (Clementi & Zocche, 1963; Yates, 1963, 1964; Elfvin, 1965a; Graf, 1966) or insulin (Fujita, Kano, Kunishima, & Kido, 1959; Yates, 1964a). Under such conditions the number of granules was strongly reduced or the density of the central core decreased. Two or more days following cessation of the reserpine treatment Yates (1963) observed a decrease in the number of smooth vesicles and a reappearance of electron-opaque granules. Thus the number of vesicles and granules are in an inverse ratio following reserpine or insulin treatment. We have obtained similar findings in the foetal adrenomedullary cell, with an abundance of vesicles and relatively few granules.

The question arises as to the site of granule formation in the cell. According to Yates (1963) and Coupland (1965a) the formation of the specific granules probably occurs in the Golgi area. In our investigations fine vesicles with small dense centres could be observed in all parts of the pheochromoblasts (Plate 3); we regard them as newly formed granules. The fact that smooth cytoplasmic vesicles are related both to the Golgi zone and to the endoplasmic reticulum makes it impossible to say whether the granule formation occurs only in the Golgi zone or elsewhere in the cytoplasm from the smooth reticulum. Since we have not observed any accumulation of such small newly formed granules in the periphery of the Golgi zone, we suggest the possibility of an ubiquitous formation of granules in the cell.

The varying densities of the granules and the process of secretion

In agreement with the results hitherto obtained on medullary cells of adult animals we believe that granules with a very dense core and narrow halo are the mature chromaffin granules containing large amounts of catechol amines (pre-secretory phase, Plate 7). Since our material was fixed only with osmium tetroxide we cannot say which catecholamines (noradrenaline and/or adrenalin) are stored in the cells. If, however, the core stains intensely black in only a few places and the remaining contents are less densely granular, we consider this indicates a smaller amount of osmium-reducing amine, or none at all (Plates 7, 8). Countings have shown that such osmiophobic granules with reduced opacity of the central core are, on an average, larger than the intense dark ones. As
a result, we believe that the osmiophobic granules are older than the small and medium-sized mature osmiophilic granules. We suppose the less dense granules to be in the process of secretion, and the largest ones with least fine grained or diffuse contents to be exhausted granules. Many of them consist only of a limiting membrane and cannot be clearly distinguished from smooth vesicles (Plates 7, 8).

Regarding the question of the mechanism of secretion from the pheochromoblasts we must emphasize again that in our prenatal rat material we have not observed a discharge of granule contents on the cell surface, and the cell membranes do not show form variations indicating such expulsion of material (cf. de Robertis & vaz Ferreira, 1957; de Robertis & Sabatini, 1960; Coupland, 1965a; and Elfvin, 1965a, on adult animals). To interpret the process of secretion in the foetal adrenal medulla we think it is important to note that osmiophobic granules can be seen in all parts of the cell, in the interior (Plates 3, 7) as well as on the cell periphery, sometimes touching the plasma membrane (Plate 8). We conclude therefore that the liberation of catecholamines may take place anywhere in the cell and we term this process \textit{intracellular granulolysis}.

This theory of release of catecholamines by intracellular lysis of granules as a secretion mechanism has already been suggested by Lever (1955), Wetzstein (1957), and Yates (1963, 1964c) and it seems to be in general agreement with some experimental results obtained from reserpine- or insulin-treated adult animals, where numerous empty vesicles are to be seen within the chromaffin cell cytoplasm. These may correspond to granules after disintegration of the central core. de Robertis & vaz Ferreira have found granules in the adult rabbit medulla 'that seem to undergo a loss of catecholamine content while in the interior of the cytoplasm.'

A summary schematic representation of our views on the secretory cycle of the pheochromoblast (granulopoesis and granulolysis) is given in Text-fig. 2. According to this hypothesis the disintegration of the granule core may occur both in the interior and in the peripheral part of the cytoplasm. The presence of fully developed chromaffin granules in foetal pheochromoblasts is in agreement with the reports of pressor activity in adrenal glands of human (Coupland, 1953) and rabbit foetuses (Brundin, 1965).

The pattern of the nerves in the foetus is different from that of the medulla of the adult rat. Although many nerve fibres are found between the cells in the foetus, we have consistently noted an absence of envelopment of axons by Schwann cell cytoplasm such as occurs in the adult animal (Coupland, 1965b). Thus the nerves are bare in the intercellular spaces and appear in bundles (Plates 1, 2, 10, 11) or as single axons (Plate 5). This finding corresponds with the observations of Cravioto (1965), who described in human foetal sciatic nerves axon bundles which were separated during subsequent development by dividing Schwann cells into smaller and smaller bundles until isolation of single axons occurred. These were then enveloped by Schwann cell processes. The number of
Schwann cell bodies seem to be lower in the foetus than in the adult rat and the number of nerve endings is much smaller in the foetus. In all our sections observed till now we have noted only one single nerve ending which was a typical ‘bouton’ with synapse as described by Coupland (1965b). Thus, the envelopment of axons by Schwann cell cytoplasm and the formation of numerous nerve endings takes place only after birth when the pheochromoblasts differentiate into pheochromocytes.

Our results seem to indicate that in its function the foetal organ differs considerably from that of the adult animal: in the fully developed adrenal medulla the secretory process is effected through nerve stimulation and may lead to an instantaneous depletion of catecholamines, while during the prenatal period the smaller amounts of specific granules and the undeveloped innervation may permit only a small secretion of hormones, probably by another mechanism.

Since this paper was submitted for publication an article has appeared reporting the embryonic and post-natal development of the secretory granules of the rat adrenal medulla (Elfvin, 1967).

**SUMMARY**

1. Electron microscope investigations of 20-day-old rat foetus show that the medullary cell groups of the adrenal glands are composed of immature chromaffin cells (pheochromoblasts), many non-myelinated nerve fibres, nerve cell bodies, blood vessels, and connective tissue elements.
Adrenal medulla of prenatal rat

2. The pheochromoblasts are distinguished from the pheochromocytes of adult rats by their smaller size and decreased number of secretory granules. The other cytoplasmic organelles of the chromaffin cells are fully developed. An abundance of ergastoplasm with ribosomes and of vesiculated smooth-surfaced endoplasmic reticulum were found. Additional elements observed in the pheochromoblast include filaments, multivesicular bodies, centrioles, cilia, and glycogen.

3. Granulopoiesis occurs in small vesicles in the Golgi zone as well as in the peripheral cytoplasm.

4. In the rat foetus a discharge of granule contents at the cell surface was not observed with certainty and consequently it is assumed that the hormones are released by a process of intracellular granulolysis.

5. Schwann cell processes which consistently envelop the axons of the adult rat medulla are only beginning to appear in the late foetus and thus the axons are for the most part devoid of Schwann cell cytoplasm.

6. Synaptic nerve endings on pheochromoblasts were very rarely observed.

7. The morphological differences account for the low function of adrenal medulla before birth.

ZUSAMMENFASSUNG

Über die Feinstruktur des Nebennierenmarkes der Ratte vor der Geburt

1. Elektronenmikroskopische Untersuchungen an 20 Tage alten Rattenfoeten ergeben, dass die Markballen der Nebennieren zusammengesetzt sind aus unreifen chromaffinen Zellen (Phäochromoblasten), reichlich marklosen Nervenfasern, Ganglienzellen, Blutgefässen und Bindegewebszellen.


3. Die Granulopoese vollzieht sich sowohl in kleinen Bläschen der Golgizone als auch im übrigen Cytoplasma.


5. Während bei der erwachsenen Ratte Fortsätze der Schwann'schen Zellen die Achsenzylinder konstant umhüllen, beginnen sie sich in der späten Foetalperiode erst zu entwickeln; aus diesem Grunde sind vor der Geburt die meisten Achsenzylinder noch nackt.

7. Die beschriebenen morphologischen Unterschiede sprechen für eine herabgesetzte Funktion des Nebennierenmarkes vor der Geburt.

The authors are greatly indebted to Dr A. M. Stadhouders, Director of the Electron Microscopic Laboratory of the University of Nijmegen, the Netherlands, for his generous support, and Dr R. D. Yates, Department of Anatomy, Galveston, Texas, for critical reading and revision of the English of the manuscript.

REFERENCES


Adrenal medulla of prenatal rat


(Manuscript received 18 November 1966, revised 13 February 1967)