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Kinetics of adrenal medullary cells*

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ABSTRACT

The adrenal medulla of mammals has a heterogeneous population of cells. In adults most are epithelial cells containing a particular type of cytoplasmic granule. Based on a variety of cytochemical and ultrastructural studies it is now accepted that 2 different adrenal medullary chromaffin cell types can be distinguished, i.e. noradrenaline (NA) and adrenaline (A) synthesising and storing cells. Other cell types present in the adrenal medulla include neuronal elements comprising either cell bodies or nerve fibres entering from outside the gland (extrinsic innervation).

It is assumed that adrenal medullary cells have a limited life span, i.e. they are replaced after a certain period. Data on this replacement process are scarce. Recently, we initiated an investigation into this question using cytochemical procedures that enable the detection of DNA duplication to measure mitotic activity in individual cells. Female Sprague–Dawley rats aged 22–36 wk received a single i.p. injection of BrdU or BrdU was administered continuously via an implanted mini-osmotic pump. Cell nuclei that had incorporated BrdU were demonstrated using an indirect immunoperoxidase staining technique. At 1 h after a single injection, $0.46 \pm 0.07\%$ of the adrenal medullary (chromaffin) cells were labelled. This increased to $0.77 \pm 0.08\%$ after 12 h with no further increase during the next 7–8 d. With continuous infusion of BrdU the fraction of labelled cells increased gradually to about 40% after 73 d (the longest period studied). These results show that in adult rats adrenal medullary cells are able to divide, although at a slow rate (renewal rate of about 1%/day).

INTRODUCTION

The distinct nature of the adrenal medulla was first noticed in 1865 by Henle, who observed that medullary tissue became brown if exposed to dichromate-containing fixatives, whereas the adrenal cortex remained unstained. The first evidence for the functional significance of the adrenal medulla was obtained at the turn of the century when it was shown that extracts of medullary tissue increase blood pressure and accelerate the heartbeat (Oliver & Schäfer, 1894, 1895*a*, *b*). Shortly afterwards Takamine (1901) and Aldrich (1901) isolated the active component from these extracts, which was named 'adrenalin' (A). Almost 50 y later noradrenaline (NA) was recognised as a second, independent hormone (von Euler & Hamberg, 1949). More recently, the adrenal medulla appeared to be able to synthesize and secrete a variety of peptides (Lundberg et al. 1979; Schultzberg et al. 1979; Viveros et al. 1979; Viveros & Wilson, 1983; see also Carmichael & Stoddard, 1989).

The adrenal medulla is composed of different cell types. The parenchymal cells, the adrenal medullary or chromaffin cells, are provided with characteristic cytoplasmic secretory granules. These cells are arranged in compact clusters surrounded by a typical network of reticular fibres and blood capillaries. In some mammalian species lymphatic vessels are also present (Verhofstad & Lensen, 1973). In addition, the adrenal medulla contains a limited number of ganglion cells and numerous intrinsic, as well as extrinsic, nerve fibres. In the past 4–5 decades knowledge on the structural and molecular organisation of the adrenal medulla has increased greatly (see e.g. Coupland, 1965*a*, 1989).

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ADRENAL MEDULLARY CELLS

In most mammals adrenal medullary cells are able to synthesise NA and A, although species-specific ratios of NA and A have been reported (see e.g. von Euler, 1963; Coupland, 1975). NA and A are derived from common precursor molecules (Fig. 1). The biosynthetic pathway of NA and A was first proposed by Blaschko (1939, 1942) but not finally proven until 1964. At that time Nagatsu et al. (1964) were able to isolate and characterise tyrosine hydroxylase, the first enzyme of the biosynthetic pathway. Although NA and A are almost identical in structure, they have different effects on the organism. For example, NA is more potent in increasing arterial blood pressure, whereas A is particularly effective in elevating blood glucose levels. These differences can be explained by the distinct behaviour of NA and A towards the various types of adrenergic receptor molecules present on the surface of the target cells.

It is now generally accepted that 2 different types of adrenal medullary cells are involved in the synthesis of NA and A, i.e. the NA and A-storing cells. Evidence for this 'dimorphism' is based on several cytochemical, electron microscopic and biochemical studies (e.g. Bänder, 1950; Eränkö, 1952, 1960; Hillarp & Hökfelt, 1953; Coupland, 1971). Among mammals, remarkable differences in the number and intramedullary distribution of the NA and A-storing cells have been described (e.g. Hökfelt, 1951; Eränkö, 1955; Palkama, 1962; Coupland, 1975).

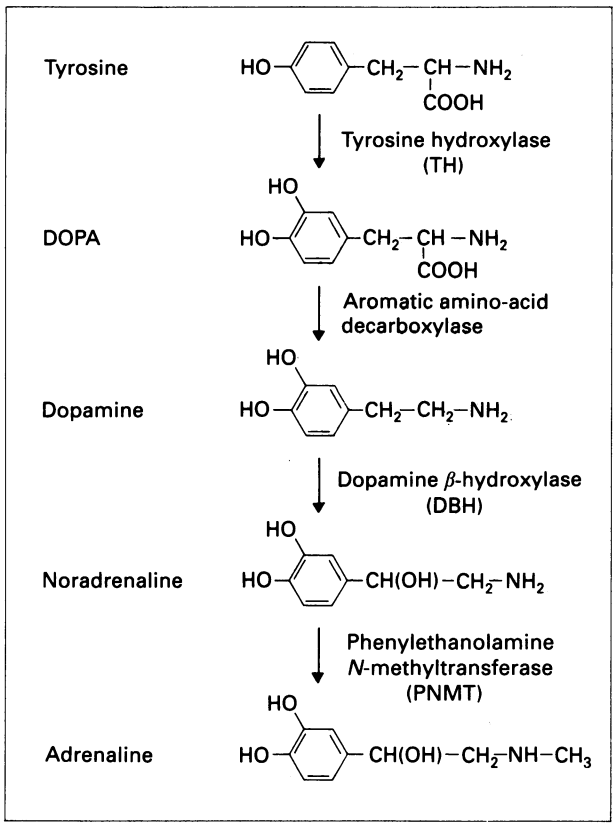

Fig. 1. Biosynthetic pathway of noradrenaline and adrenaline in the adrenal medulla. Four enzymes are involved: tyrosine hydroxylase (TH), aromatic amino-acid decarboxylase (dopa decarboxylase, DDC), dopamine β-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT). For references, see Blaschko (1972) and Stjärne (1972).

Several cytochemical procedures are available to demonstrate NA and A-storing cells at the light microscopic level (see e.g. Böck, 1982). Currently, immunocytochemical techniques based on the use of specific antibodies to NA, A and related biosynthesising enzymes (Hökfelt et al. 1973, 1980; Nagatsu, 1974; Verhofstad et al. 1980, 1983) (Table 1, Fig. 2) seem to be most reliable as far as specificity and sensitivity are concerned. These techniques have been employed to examine e.g. the prenatal and postnatal development of the NA and A-storing cells in rat and pig adrenal glands (Verhofstad et al. 1985, 1989) and the presence of NA and A in tumours derived from the adrenal medullary tissue (e.g. Lundberg et al. 1979; Lloyd et al. 1986; Lloyd, 1988). Other studies have addressed the possible costorage of NA or A with peptides (Kobayashi et al. 1983, 1984) or serotonin (Verhofstad & Jonsson, 1983). Ultrastructurally, NA and A-storing cells can be identified in a clear-cut way in tissues fixed with glutaraldehyde followed by osmification (Coupland et al. 1964; Coupland, 1965*b*; Coupland & Hopwood, 1966). Using this technique NA and A-storing cells show distinct types of storage granules.

Recently, Coupland and coworkers made a detailed quantitative analysis of the rat adrenal medulla from birth to adult age (Tomlinson et al. 1987; Coupland & Tomlinson, 1989; Tomlinson & Coupland, 1990). This analysis revealed remarkable differences between the 2 cell types with regard to the diameter of the storage granules, volume of individual cells and the total volume of the NA and A-storing cells. From these data the authors calculated that the adult rat adrenal medulla contains ~225000 NA and ~620000 A-storing cells.

CELL KINETICS

Cell kinetics is concerned with processes regulating the volume and composition of cell masses such as organs or tumours. In most instances these populations are composed of different subpopulations, each subpopulation representing a particular cell type. Cells composing these subpopulations may be either in the proliferating or nonproliferating cell com-

Table 1. *Immunoreactivity of adrenal medullary cells*

	Immunoreactivity to			
	DBH	NA	PNMT	A
NA-storing cell	+	+	−	−
A-storing cell	+	+	+	+

partment (Fig. 3). The proportion of cells within the proliferating compartment is indicated as the growth fraction. For a given cell type the number of cells present in a cell mass is determined by the balance between cell proliferation and loss due to cell death, migration to other parts of the organism or conversion into a different cell type (differentiation) (Fig. 3). If cell proliferation dominates the number of cells will increase, if cell loss dominates the number of cells will become smaller. On the other hand, if there is an equilibrium between cell proliferation and cell loss a steady state situation exists.

The steps involved in cell division are well known. Before mitosis takes place the amount of genomic material (DNA) doubles. This synthetic or S-phase is preceded by a period of variable duration, the first gap (G_1) phase, separating the previous mitosis from the S-phase. The S-phase is followed by another period of inactivity so far as DNA synthesis is concerned, the second gap (G_2) phase, preceding the actual mitosis of the cell. The phases separating 2 successive mitoses are part of the interphase of the cell cycle. Cells may cease dividing either permanently or temporarily, but they are able to enter the cell cycle again in response to certain stimuli, referred to as the G_0-phase (Fig. 3).

Several methods can be used to detect cell proliferation in tissue sections or cultured cells (e.g. Hall & Levison, 1990; Hall & Woods, 1990; Hall et al. 1992; Yu et al. 1992). First, during mitosis cell nuclei show characteristic morphological changes which can be used to identify dividing cells (Quinn & Wright, 1992). In addition, mitotic events can be blocked by substances such as colchicine. Under these conditions accumulation of mitotic figures takes place. Secondly, the amount of DNA present in cell nuclei can be measured by image cytometry or flow cytometry. The amount of DNA increases until it is doubled at the end of the S-phase and during the G_2-phase. Thirdly, newly synthesised DNA can be marked, e.g. by [^{3}H]thymidine or, more recently, by 5-bromo-2-deoxyuridine (BrdU) (Gratzner et al. 1975; Gratzner, 1982; Schutte et al. 1987*a*, *b*; Hayashi et al. 1988; Wilson & McNally, 1992). Incorporation of these compounds can be demonstrated by autoradiography and immunocytochemistry, respectively. In the past [^{3}H]thymidine autoradiography has been used frequently. However, BrdU-labelling has several advantages: time is not lost by the need for exposure of the autoradiographs, better optical resolution is achieved and, moreover, simultaneous detection of DNA-duplication and cell-specific marker substances is made possible. On the other hand, in order to give the antibody access to BrdU, tissue sections or cells have to be treated by acid hydrolysis with hydrochloric acid and proteolytic enzymes, for example, to denature DNA. These steps may cause several types of staining artifacts. Finally, antibodies to cell cycle-associated proteins can be used to recognise dividing cells, e.g. Ki-67, proliferating cell nuclear antigen (PCNA) and DNA polymerase α (see, e.g. Hall & Levison, 1990; Hall & Woods, 1990; Yu et al. 1992; Hall et al. 1992). However, whereas most of these antibodies work well on cells of human origin, there is little experience so far as application in other species is concerned.

Based on [^{3}H]thymidine autoradiography, Leblond and co-workers (Leblond & Walker, 1956; Messier & Leblond, 1960; Leblond, 1964) noticed different types of proliferative activity in tissues of adult experimental animals. In some tissues, referred to as 'static' tissues, no cell divisions were found, e.g. neuronal tissue. In others, such as muscle, liver, kidney and endocrine organs, only small numbers of proliferating cells were encountered. A 3rd group of tissues comprising, e.g. gastrointestinal epithelium, epidermis, blood-forming tissues and testes, a large number of dividing cells were seen.

In the past few decades most cell kinetic studies dealt with cell proliferation only, neglecting the counterpart, cell death. Recent evidence indicates that 2 modes of cell death occur (see e.g. Kerr & Harmon, 1991). One type of cell death, necrosis, is due to irreversible damage to groups of cells leading to clumping of chromatin, swelling of cytoplasmic organelles and leakage and breakdown of membranes. A 2nd type, indicated as apoptosis or programmed cell death, involves single isolated cells. These cells show characteristic morphological changes, i.e. shrinkage, together with fragmentation of the nucleus and cytoplasm into small particles surrounded by intact membranes that contain nuclear fragments as well as cytoplasmic organelles ('apoptotic bodies'). These particles are rapidly phagocytosed by neighbouring cells or macrophages. Interestingly, apoptosis seems to be an active process, starting with the activation of enzymes such as an endonuclease, which cuts DNA into fragments leaving intact its nucleosomal parts. In contrast, necrosis is accompanied

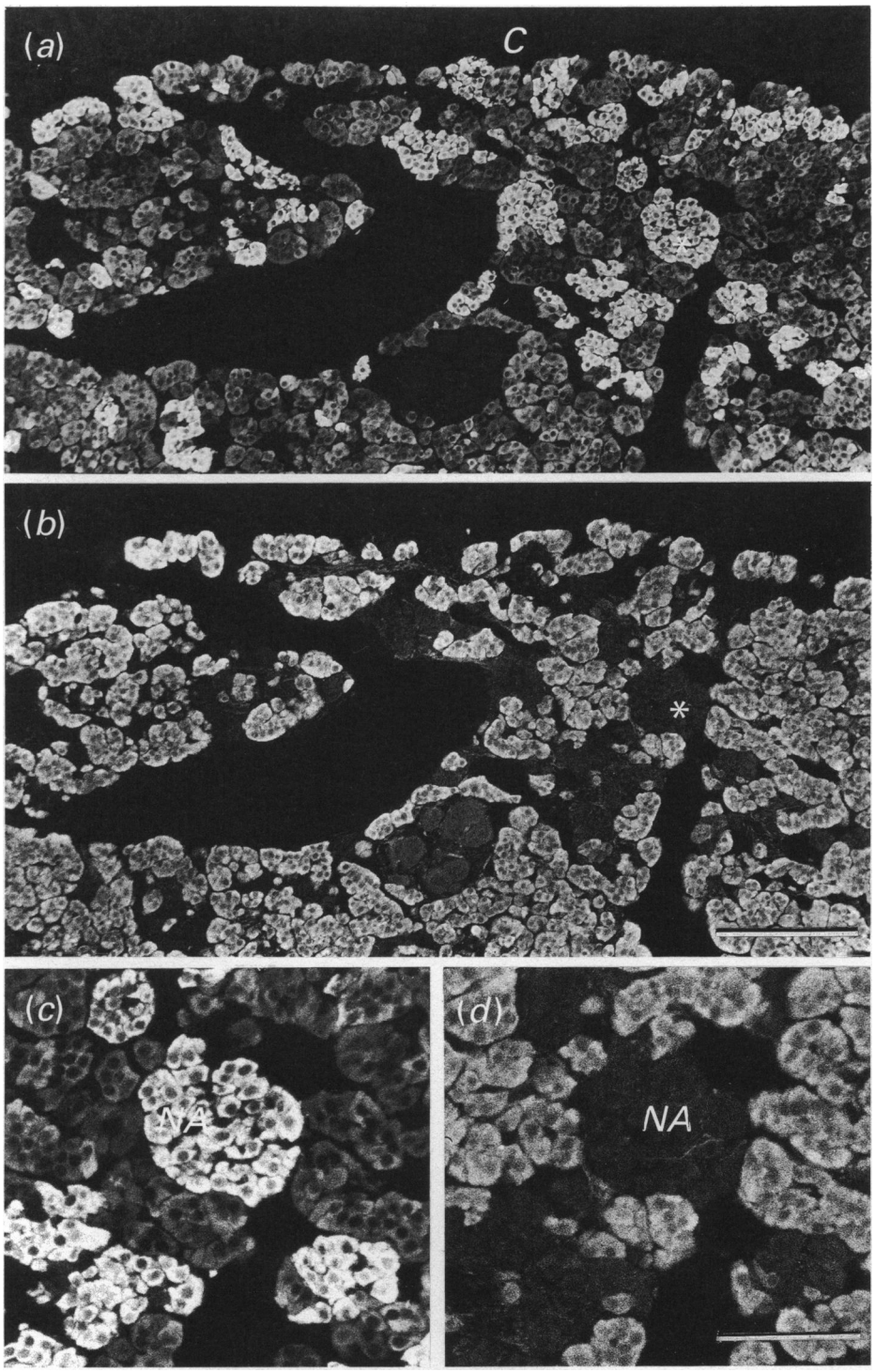

Fig. 2 For legend see facing page.

by random DNA breakdown. Recently, an immunocytochemical procedure to detect apoptosis in tissue sections or cultured cells was reported by Jonker et al. (1992) and Wijsman et al. (1993).

Cell death by apoptosis has been demonstrated in several tissues and is now considered to be an important mechanism in cell kinetic processes not only during development, but also in the 'adult stage' and during involution of organs. In addition, apoptosis plays a significant role in various pathological processes such as neoplastic growth (e.g. Kerr et al. 1972; Wyllie et al. 1980; Tomei & Cope, 1991; Williams, 1991; Alison & Sarraf, 1992).

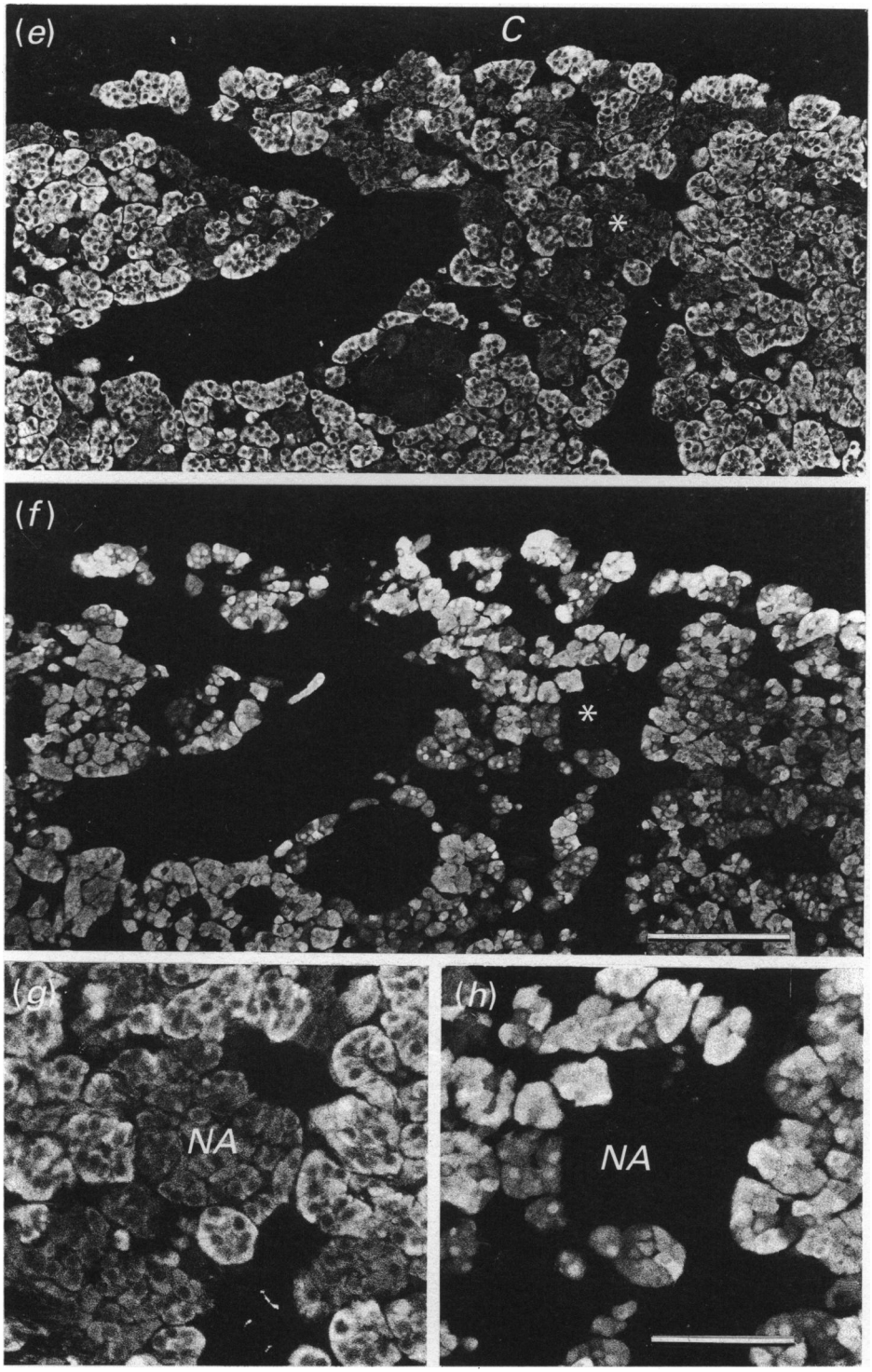

Fig. 2 (*a–d*). Immunofluorescence photomicrographs of the adrenal medulla of adult rat. Consecutive sections stained with an antiserum to noradrenaline (*a, c*) or adrenaline (*b, d*). (*c*) and (*d*) are higher magnifications of areas in (*a*) and (*b*) marked by asterisks. Note in (*a*) and (*c*) intensely (NA-storing) besides moderately (A-storing) fluorescing cell groups. In (*b*) and (*d*) only the A-storing cell groups are fluorescing. *C*, adrenal cortex; *NA*, noradrenaline-storing cell group. Bars: (*a, b*) 68 μm; (*c, d*) 144 μm. (*e–h*) Immunofluorescence photomicrographs of the adrenal medulla of adult rat. Consecutive sections stained with an antiserum to DBH (*e, g*) or PNMT (*f, h*). (*g, h*) Higher magnifications of areas in (*e*) and (*f*) marked by asterisks. Note in (*e*) and (*g*) moderately (NA-storing) besides somewhat more intensely (A-storing) fluorescing cell groups. In (*f*) and (*h*) only the A-storing cell groups are fluorescing. *C*, adrenal cortex; *NA*, noradrenaline-storing cell group. Bars: (*e, f*) 68 μm; (*g, h*) 144 μm.

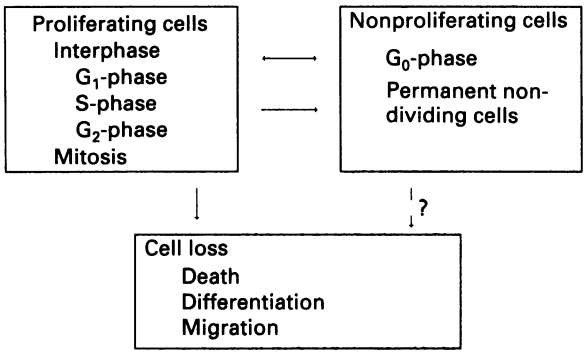

Fig. 3. Diagram indicating that a particular cell might be either in one of the phases of the cell cycle (G_1, S, G_2, mitosis) or within the compartment of nonproliferating cells (G_0-phase, permanent nondividing cells). Cell loss may be due to cell death (either programmed cell death or necrosis), cell differentiation or migration to another part of the organism. Most likely the conversion of a proliferating into a nonproliferating cell or to programmed cell death is established in the G_1 phase. (Based on Hall & Levison, 1990; Tomei & Cope, 1991; Hall et al. 1992; Yu et al. 1992.)

CELL KINETIC STUDIES OF ADRENAL MEDULLARY CELLS

As explained in the previous section, quantitative information on cell proliferation and cell loss is needed to understand why organs grow, decline or stay in a steady state situation. With regard to the adrenal medulla, only limited information on cell proliferation is available in the literature. No information on cell death by apoptosis has been found.

The main results of studies dealing with cell proliferation in the adrenal medulla of adult rats are summarised in Table 2. In most studies only a limited number of animals either for adults or developmental stages, were used, and a variety of techniques and experimental conditions have been employed. As a consequence, it is difficult to draw precise conclusions from these experiments. However, most data indicate that in the adrenal medulla of adult rats cell division seldom occurs. Interestingly, Tischler et al. (1988, 1989*a*, *b*) demonstrated a dose-dependent increase of the number of dividing cells following the administration of reserpine to 9-month-old rats. This increase could be prevented by denervation of the adrenal gland, indicating that cell proliferation of adrenal medullary cells is under nervous control (Tischler et al. 1991).

Only a few reports have been published dealing

Table 3. *Percentage of BrdU-labelled adrenal medullary cells following single injection*

Survival time (h)	BrdU-labelled cells (%)
1 (3)	0.46±0.07
12 (3)	0.77±0.08
24 (3)	0.77±0.07
48 (3)	0.86±0.05
96 (3)	0.86±0.12
192 (3)	0.92±0.09

Number of animals in parentheses. Values expressed as mean ±S.E.M.

Table 2. *Cell proliferation in adult rat adrenal medulla – References from the literature*

Author(s)	Method used	Findings
Jackson (1919)	Mitotic count	3 mitoses in 1 section in 56 d animal No mitoses in older (up to 340 d) animals
Mitchell (1948)	Mitotic count following colchicine	Average of 3 mitoses per section of 34-d-old animals No mitoses in animals aged 34–63 d
Messier & Leblond (1960)	[^{3}H]thymidine	0.5% medullary cells labelled
Malverdi et al. (1972)*	Mitotic count	Average of 20 dividing cells per adrenal (0.004%)
	Mitotic count following colchicine	Average of 20–37 dividing cells per adrenal (0.009%) – calculated mean life span 1388–3125 d
	[^{3}H]thymidine	1.16% labelled cells
Tischler et al. (1988)	Mitotic count following colcemid	0.3% of the cells divide (calculations based on Table 1)
Tischler et al. (1989*a*)**	Mitotic count following colcemid	1 cell or less per section
	BrdU 6 h before killing	Up to 7 labelled cells per section

* See also Viola-Magni (1966) and Malverdi et al. (1968).
** See also Tischler et al. (1989*b*) and Tischler et al. (1991).

with cell division in the adrenal medulla of the adult mouse. Whitehead (1933) found only a single mitosis in 3 sections, whereas Messier & Leblond (1960) showed that 0.24–0.88% of the medullary cells could be labelled with [^{3}H]thymidine. Jurecka et al. (1978), also using [^{3}H]thymidine autoradiography, counted less than 1% of labelled cells. More recently, Monkhouse & Fussell (1988) examined the effect of hydrocortisone on [^{3}H]thymidine uptake by adrenal medullary cells of newborn mice. These authors calculated that in untreated newborn mice the total cell cycle lasts 7 h, divided in an S-phase of 1.5 h, a G_2-phase of 1 h, an M-phase of 4 h and a G_1-phase of 0.5 h.

Some authors have considered whether dividing cells are fully mature in the adult adrenal medulla. Thus Malverdi et al. (1968) showed that mitotic cells were stained in the chromaffin reaction. Tischler et al. (1988, 1989*b*), using electron microscopy, demonstrated chromaffin granules in the cytoplasm of dividing cells. In later studies (Tischler et al. 1989*a*, *b*, 1991) these authors found that medullary cells labelled with BrdU were either phenylethanolamine-N-methyltransferase (PNMT)-immunoreactive or without detectable amounts of PNMT. Finally, Jurecka et al. (1978), working with mouse adrenals, demonstrated that medullary cells labelled with [^{3}H]thymidine showed formaldehyde-induced fluorescence. Taken together, all data indicate that fully mature cells, both NA and A cells, are able to undergo mitotic division.

Recently we have also examined cell proliferation of adrenal medullary cells in adult rats. The initial results of these experiments will be presented here. For our study we used female Sprague–Dawley rats, 22–36 wk old and weighing 250–350 g. Eighteen animals received a single i.p. injection of BrdU (50 mg/kg body weight) dissolved in phosphate-buffered saline (PBS). In another 35 animals an ALZET mini-osmotic pump was implanted, under ether anaesthesia, subcutaneously in the neck region. The pumps were filled with sterilised BrdU dissolved in PBS (30 or 60 mg/ml). Using this pump system, BrdU was continuously infused with a dose of 1 μg/h. Animals that had received an i.p. injection were anaesthetised with ether and killed by exsanguination using the time intervals indicated in Table 3. Likewise, animals continuously exposed to BrdU were killed using the time schedule indicated in Table 4. To observe the rate of loss of labelled cells following continuous labelling, animals treated for 744 h (31 d) or 1752 h (73 d) were killed as indicated in Tables 5 and 6.

Table 4. *Percentage of BrdU-labelled adrenal medullary cells after continuous infusion*

Infusion time (h)	BrdU-labelled cells (%)
12 (3)	0.69±0.05
24 (3)	0.95±0.01
48 (3)	1.81±0.02
96 (3)	3.99±0.04
192 (3)	6.69±0.04
384 (3)	17.58±1.23
600 (3)	23.20±1.19
744 (3)	23.79±1.10
1752 (3)	41.96±1.43

Number of animals in parentheses. Values expressed as mean ±S.E.M.

Table 5. *Loss of BrdU-labelled adrenal medullary cells following continuous infusion during 744 h (31 d)*

Survival time (h)	BrdU-labelled cells (%)
0* (3)	23.79±1.10
192 (1)	17.65±1.85
384 (1)	10.23±1.23

* The same animals as used in Table 4.
Number of animals in parentheses. Values expressed as mean ±S.E.M.

Table 6. *Loss of BrdU-labelled adrenal medullary cells following continuous infusion during 1752 h (73 d)*

Survival time (h)	BrdU-labelled cells (%)
0* (3)	41.95±1.43
384 (3)	32.52±1.77
720 (3)	18.26±3.30

* The same animals as used in Table 4.
Number of animals in parentheses. Values expressed as mean ±S.E.M.

Adrenal glands were fixed at 4 °C by immersion in phosphate-buffered 4% formaldehyde for a maximum of 12 h. The glands were dehydrated and embedded in paraffin wax. Sections of 4 μm in thickness were used to demonstrate BrdU-incorporation in cell nuclei using the procedure described in detail by Schutte et al. (1987*a*). In brief, sections were dewaxed, treated with 0.3% H_2O_2 in absolute methanol to remove the endogenous peroxidase activity of the erythrocytes and then exposed to 0.02% pepsin in 0.1 N HCl followed by immersion in 2 N HCl to denature DNA. Following neutralisation of the pH with a borate buffer, sections were stained with a monoclonal

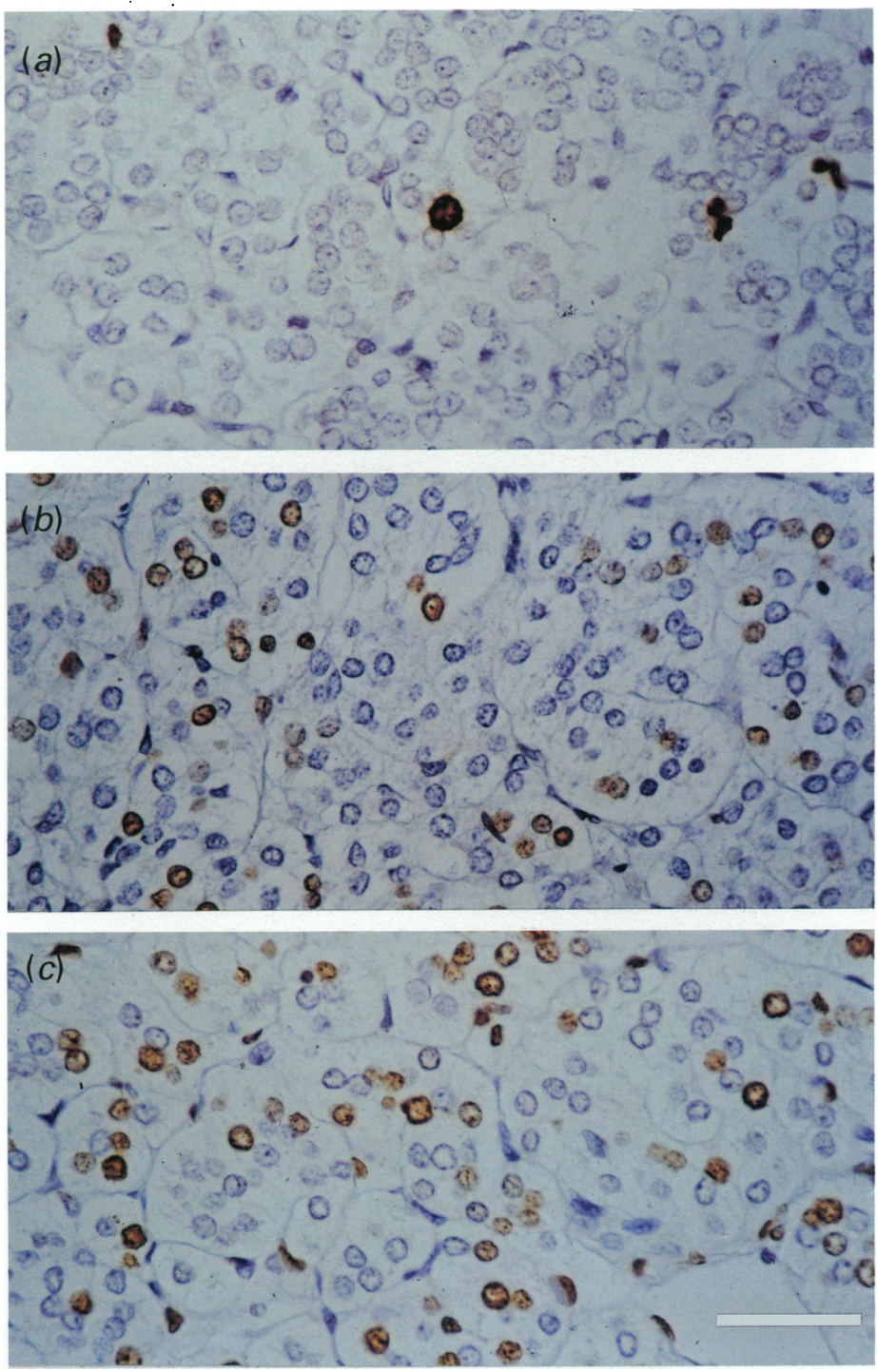

Fig. 4. Photomicrographs of adrenal medulla of adult rats after continuous infusion with BrdU for (*a*) 48 h, (*b*) 384 h (16 d) or (*c*) 1752 h (73 d). Cell nuclei with incorporated BrdU demonstrated by indirect immunoperoxidase staining using a monoclonal antiserum to BrdU. Sections counterstained with haematoxylin. Bar, 68 μm.

antibody to BrdU (clone II B5; Schutte et al. 1987*b*) using an indirect immunoperoxidase technique. Finally, the sections were counterstained with haematoxylin and mounted under a coverslip.

The fraction of adrenal medullary cells stained with the BrdU-antibody was determined using a Leitz microscope provided with a drawing tube, an objective 40 NP Fluotar 40/0.70 and an eyepiece Periplan GF 10 × M containing a graticule. Via the drawing tube BrdU labelled, as well as nonlabelled, cell nuclei were scored with the cursor of a semiautomatic image analysing device. In sections through the midportion of the adrenal glands nuclei of medullary cells were classified in 10 different areas each representing 10000 μm². From each animal 2 sections from the left as well as the right adrenal gland were examined. Data

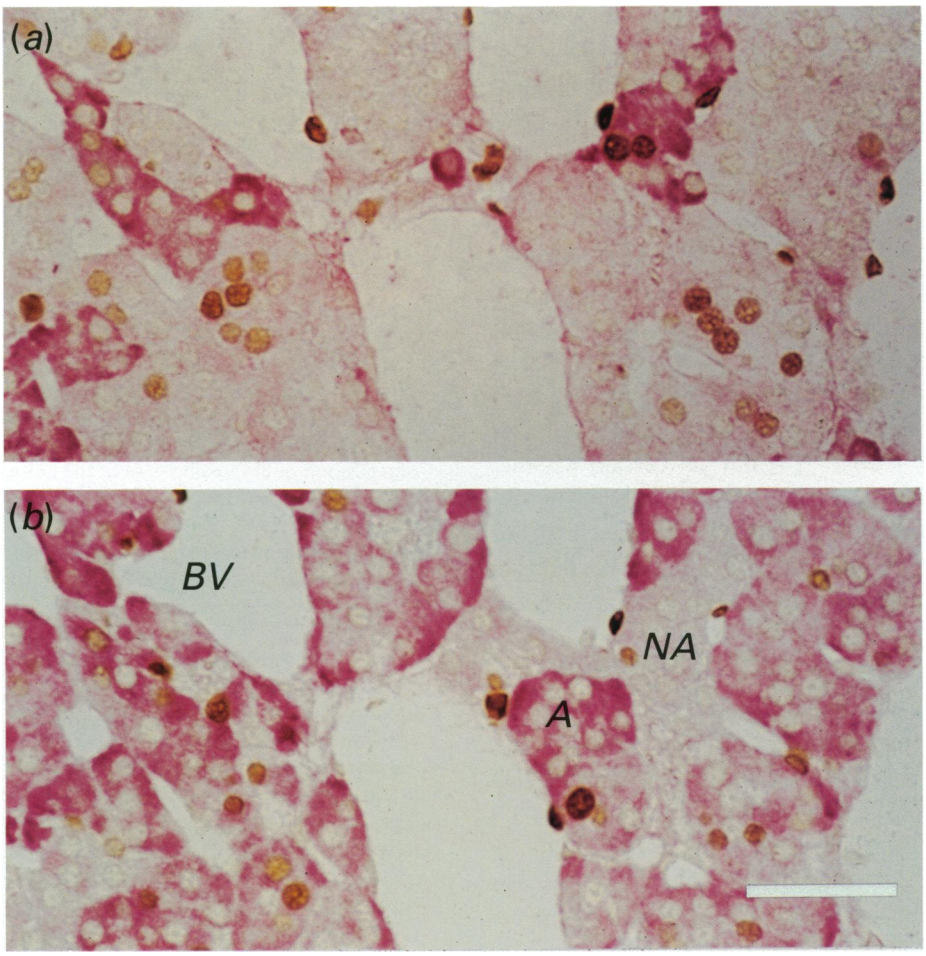

Fig. 5. Photomicrographs of adrenal medulla of 9 wk rat after continuous infusion with BrdU for 2 wk. Cell nuclei with incorporated BrdU demonstrated by indirect immunoperoxidase staining using a monoclonal antiserum to BrdU. Consecutive sections stained with an antiserum to noradrenaline (*a*) or adrenaline (*b*) using an indirect immuno-alkaline phosphatase staining. Note BrdU-incorporation (brown nuclei) in NA- and A-storing cells (red cytoplasm) as well as some endothelial cells. *NA*, noradrenaline-storing cell group; *A*, adrenaline-storing cell group; *BV*, blood vessel. Bar, 68 μm.

from animals with the same survival time, mostly 3 in number, were used for calculation of the labelling index (Tables 3–6).

Animals accepted BrdU-administration by mini-osmotic pumps well and were healthy even after treatment for 73 d. Control experiments using an i.p. injection of [^{3}H]thymidine in rats treated with BrdU for 73 d showed that cell proliferation was not influenced by the administration of BrdU.

Sections stained with the BrdU antibody revealed distinct brown cell nuclei. The intensity of the immunocytochemical reaction products varied among the cells even within a single section. These differences probably reflect different amounts of BrdU incorporated into DNA (Fig. 4). Besides adrenal medullary cells, endothelial cells were also stained. No staining was observed in the ganglion cells occasionally present in the sections.

Animals which received a single dose of BrdU showed 0.46 ± 0.07% of the medullary cells labelled 1 h after the injection (Table 3). This percentage increased to 0.77 ± 0.08 after 12 h. Essentially, the percentage of labelled cells remained constant during the next 7–8 d. It seems likely that medullary cells, marked 1 h after injection, represent cells in S- and/or G_2-phase of the cell cycle. Cells labelled with BrdU encountered 12 h after the injection, probably had already passed the M-phase, which explains the 2-fold increase of the labelling index between 1 and 12 h of survival. In adult rats the volume of the adrenal medulla changes little (e.g. Coupland & Tomlinson, 1989). It is also generally accepted that if a BrdU-labelled cell divides, both daughter cells contain a sufficient amount of BrdU to be stained. It is therefore tempting to assume that every day 1% of the adrenal medullary cells are 'born' and another 1% dies.

During the first 600 h (25 d) of continuous infusion with BrdU each interval showed a doubling of labelling percentages, slowing during the subsequent intervals (Table 4; see also Fig. 4). After the latest

interval studied (1752 h or 73 d) about 40% of the adrenal medullary cells were labelled, i.e. about 336000 cells. The slowing of the increase of labelled cells may indicate that 2 subpopulations of cells exist, differing either in proliferative activity or in lifespan.

In order to estimate the rate of loss of BrdU-labelled cells, animals continuously treated for 31 or 73 d, respectively, were allowed to survive for various periods (Tables 5, 6). Based on the findings obtained from these animals it can be estimated by linear extrapolation that each day 0.5–1% of the BrdU-labelled cells are lost. The present study has indicated that approximately 40% of the medullary cells are replaced within ~ 70 d. It is not yet certain whether the adult adrenal contains medullary cells in the G_0-phase or permanent nondividing category. If this turned out not to be the case it might be inferred that total renewal of adrenal medullary cells takes about 175 d.

To study whether NA- and A-storing cells have a similar proliferative activity, multiple staining techniques are required to enable the simultaneous demonstration of BrdU-incorporation and NA and A or DBH and PNMT (see Table 1). Recently, such a technique has been developed by W. R. Ubink & A. A. J. Verhofstad (unpublished observations). Examples of sections stained with this procedure are presented in Figure 5.

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