The streaming adrenal cortex: direct evidence of centripetal migration of adrenocytes by estimation of cell turnover rate

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ABSTRACT

Thirty adult male rats were injected with 0·5 μCi [^{3}H]thymidine/g body weight (specific activity 5 Ci/mmol) and killed, in groups of five, 1 h and 14, 30, 60, 90 and 120 days after injection. The displacement of labelled adrenocytes with time was estimated in autoradiograms of adrenal sections. The radial distance of the labelled cell from the capsule was measured with an eyepiece micrometer and expressed in cell location units, i.e. the number of cells separating the labelled cell from the capsule. One hour after labelling, 95% of labelled cells were confined to the outer quarter of the cortex. During the following days, adrenocytes were displaced inwardly, approaching the medulla at a velocity of 0·24 locations/day. They traversed the three cortex zones, reaching the medulla after 104 days. The three adrenal zones represent three differentiation states of the adrenocyte. When young, the adrenocyte secretes aldosterone, after leaving the glomerulosa it produces corticosteroids and on reaching the reticularis it produces sex hormones. The adrenal cortex is a cell renewal system made of two compartments. A progenitor compartment extending between locations 1 and 15, and a functional compartment, covering locations 16–64. The first compartment produces 0·47 cells daily, which enter the second. Half of them die on their way while the rest are eliminated in the reticular zone. The cell stream is nourished by a subcapsular stem cell.

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INTRODUCTION

Cell turnover in the adrenal cortex has interested researchers ever since Gottschau's (1883) proposition that adrenocytes, nascent in the glomerulosa, migrate centripetally. Gottschau's (1883) 'cell migration theory' was opposed by the 'zonal' theory of Chester Jones (1948), according to which each zone replenished its cells independently. Since each zone produces different hormones, the cell migration theory implies that one cell is capable of producing them all. This contradicted the then prevailing dogma that each differentiated cell produces solely one end product.

Previous attempts to settle the above controversy have been inconclusive. Leblond (1964) regarded the adrenal cortex as an expanding cell population, proliferating solely during growth, or in response to damage. Other cell kinetic studies designed to prove the migration theory have failed to establish it. Some have been semiquantitative (Diderholm & Hellman, 1960*a,b*), while others have assessed cell turnover in growing animals when cell migration is difficult to estimate (Ford & Young, 1963; Reiter & Pizzarello, 1966; Wright, 1971; Wright, Voncina & Morley, 1973; Malendowicz & Jachimowicz, 1982). None has measured cell displacement *per se*, which has had to be inferred from cell turnover. In the present investigation, cells were labelled with [^{3}H]thymidine and their behaviour was studied in autoradiograms of histological sections.

MATERIALS AND METHODS

Thirty adult male rats, random bred Hebrew University strain, weighing between 250 and 300 g, were injected with 0·5 μCi [^{3}H]thymidine/g body weight (specific activity 5 Ci/mmol). Groups of five

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rats were killed 1 h and 14, 30, 60, 90 and 120 days after injection. The adrenals were fixed in formalin, embedded in paraffin and cut into 5 μm thick sections which were then dipped into liquid emulsion (Ilford K-5; Ilford, Sussex), exposed for 3 weeks and stained with haematoxylin.

In each adrenal cortex, 50 labelled adrenocytes were randomly selected by counting random visual fields. Their radial distance (d) from the capsule was measured with an eyepiece micrometer. The width (w) of the entire cortex and the number (n) of adrenal cells positioned along the ruler were measured in each section. All measured distances were expressed in cell location units. The entire cortex consisted of n locations. Since each cell location was w/n μm wide, the number of cell locations separating a cell from the capsule was $d/(w/n)$, or dn/w. This value served to specify the location of a cell in relationship to the adrenal capsule. Adrenocyte locations were thus represented by integers ranging between 0 and n. The subcapsular cell was defined as zero location, while the innermost cortical cell as location n.

The mean cell displacement velocity was estimated by linear regression. The significance of observed differences was tested by analysis of variance.

RESULTS

One hour after labelling, 95% of labelled cells were confined to locations 0–15 (Fig. 1). The cell occupying the median of the labelled cell frequency distribution, defined here as the median labelled cell, was initially positioned at location 5. Sixty days later the median labelled cell advanced to location 19, at a velocity of 0·23 locations/day. Throughout the experiment the width of the cortex did not change (Table 1). When tested by analysis of variance, the observed differences did not differ significantly and were therefore pooled, yielding an average cortex width of $63{\cdot}7 \pm 2{\cdot}6$ (S.E.M.) locations. Although the median labelled cell reached location 19 on day 60, at least 5% of the labelled cells crossed location 45, where some of them were eliminated. For this reason, estimates of displacement velocity were derived solely from measurements made up to day 60.

The adrenal cortex is continuously renewing

Since [^{3}H]thymidine is incorporated solely into cells destined to divide, the distribution of labelled cells 1 h after labelling extends over locations in which cells are capable of DNA synthesis. Obviously not all cells switched their DNA-synthesizing machinery on. Some proceeded through G-1 and G-2 phases and did not therefore incorporate radioactivity, yet all are capable of synthesizing DNA when entering the S phase. The labelled cell distribution observed 1 h after labelling (Fig. 1) therefore outlines the locations in which DNA synthesis occurs, defined here as the progenitor or P compartment. Since 95% of labelled cells did not exceed location 15, the P compartment covers locations 0–15. It obviously does not extend beyond location 16, otherwise the cells there would be labelled. Adrenocortical cells or adrenocytes may thus be divided into two classes, progenitors or P cells extending up to location 15 and functional or Q cells residing between locations 16 and 64. The adrenal cortex thus consists of two compartments, a progenitor or P compartment which feeds a functional or Q compartment. Such a relationship is observed in renewing cell populations, e.g. in the epidermis in which P cells inhabit the basal layer, while the layers above are Q cells. In the epidermis, progenitors of the basal layer are displaced upward, maturing into spinous, granular and, finally, keratin-producing cells. Since the adrenal cortex is divided into three zones and since the adrenocyte traverses the entire cortex from its outer quarter inwards, it first assumes the appearance of the glomerulosa, turning later into a fasciculata constituent until at the end of its existence it is in the reticular layer.

While the mature adrenocyte keeps its label until death, in progenitors the label is continuously diluted. Following each cell division, the cell label content

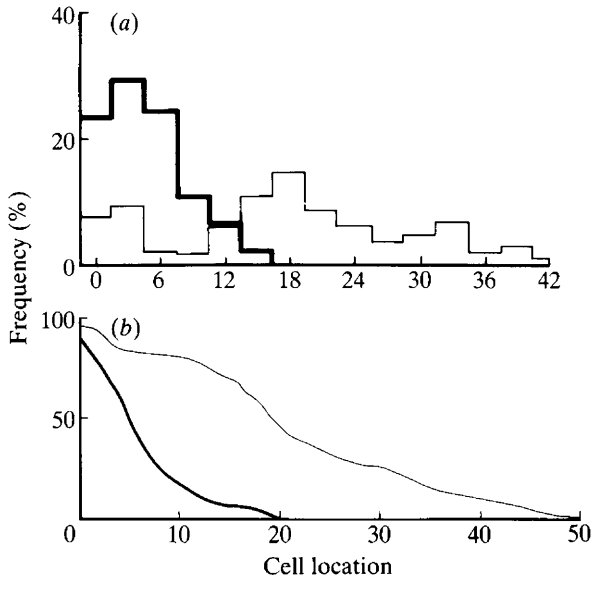

FIGURE 1. (*a*) Frequency and (*b*) cumulative frequency distributions of labelled rat adrenal cells 1 h (thick line) and 60 days (thin line) after labelling with [^{3}H]thymidine. The cell location gives the number of cells separating the labelled cell from the adrenal capsule.

TABLE 1. Animal weight and adrenocortical width from the time of labelling rat adrenal cells with [^{3}H]thymidine. Values are means ± S.E.M., $n = 5$; overall means are given in parentheses

Time (days)	Weight (g)	Cortex width (μm)	Cortex width (Cell locations)	Cell diameter (μm)
0	268 ± 24	651 ± 38	62·3 ± 4·2	10·5 ± 0·3
14	241 ± 9	563 ± 70	60·2 ± 5·9	9·6 ± 1·0
30	310 ± 9	678 ± 106	64·9 ± 9·3	10·5 ± 0·5
60	362 ± 13	762 ± 61	70·1 ± 4·4	11·0 ± 0·6
90	456 ± 25	710 ± 30	70·9 ± 4·7	10·2 ± 0·4
120	445 ± 5	617 ± 53	53·6 ± 7·4	12·2 ± 1·0
		(666 ± 26)	(63·7 ± 2.6)	(10·7 ± 0·3)

Cell locations, number of cells separating the labelled cell from the adrenal capsule.

represented by its grain count is halved, until the cell appears unlabelled. With time, more and more labelled cells become invisible and the number of P cells declines. The 'cell dilution' curve (Fig. 2) depicts this phenomenon and provides an estimate of the rate of progenitor cell division. The time at which 50% of progenitors had disappeared is proportional to the time at which their label was undetectable. It roughly equals the mean cell cycle time of adrenocyte progenitors, which was 32 days. As the P curve declines, the Q curve increases. Such a relationship is known in chemical kinetics as a precursor–product relationship, corroborating the above kinetic scheme, according to which P feeds Q.

Adrenocyte displacement velocity

In order to derive cell displacement velocities, the concept of the tissue radius has to be introduced. This is the trajectory along which the cell is displaced. The radius extends from location 0, which is defined here as its origin, towards location 64, which constitutes its periphery. Locations 0–15 and 16–64 are respectively the P and Q regions of the tissue radius. Each region exhibits an inner and an outer boundary. The respective inner and outer boundaries of P are locations 0 and 15, and of Q are locations 16 and 64. This kinetic description of adrenocyte displacement differs from the anatomical, according to which the medulla is the tissue centre and the capsule its periphery.

The change of the median and mean labelled cell with time provide estimates of the adrenocyte displacement velocity (Table 2). The estimated velocities were calculated from the following formula. When deriving velocity from the shift of the median, the median cell position 1 h after labelling is d(0), its position at time t is d(t) and the average daily velocity at time t is $(d(t) - d(0))/t$. When utilizing the mean position for velocity estimates, d(t) and d(0) represent the respective mean locations. The median and mean labelled cell positions increased up to day 60, after which they declined. Since most cells die at the cortex (kinetic) periphery (i.e. medullo-cortical junction), leaving the younger labelled cells behind, the mean and median shift backwards towards the smaller locations. For this reason, velocity estimates were based on measurements made up to day 60.

The change of d(t) with time was estimated with the linear least square method. The slope was significant at

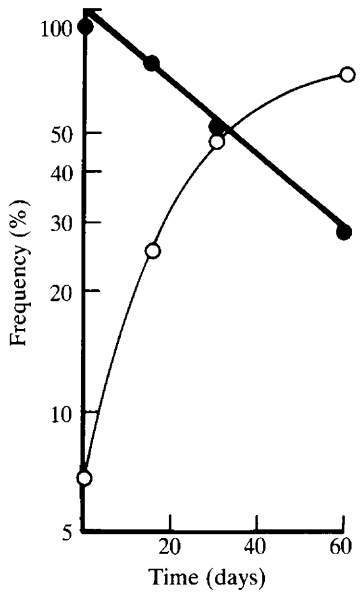

FIGURE 2. The rate of disappearance of labelled cells from the progenitor compartment (●) and accumulation of labelled cells in the functional compartment (○) of the rat adrenal cortex. Time is measured from the day at which [^{3}H]thymidine was injected.

TABLE 2. Median and mean cell distance of labelled rat adrenal cells at different times after [^{3}H]thymidine labelling. Values are means ± S.E.M., $n = 5$

Time (days)	Median distance from capsule	Daily velocity	Mean distance from capsule	Daily velocity
0	5	—	8·5	—
14	9	0·29	11·1	0·19
30	13·5	0·29	16·4	0·26
60	19·5	0·24	22·4	0·23
90	21	0·18	20·2	0·13
120	19·5	0·12	19·9	0·10

Daily velocity (measured in cell locations) = $(d(t) - d(0))/t$ where $d(t)$ is the mean or median distance from the cell capsule at time (t) after labelling and $d(0)$ is the distance at time 0. All distances are measured in cell locations.

$P < 0{\cdot}001$: $d(t) = 8{\cdot}5 + 0{\cdot}24t$ $(0 < t \leqslant 60)$. The initial cell position is at location 8·5 and it moves with a velocity of 0·24 locations/day.

Progenitor compartment exit velocity

The average progenitor cell cycle time, $t(c) = 32$ days (Fig. 2), meaning that every 32 days the average progenitor produces a new cell. Since the tissue radius consists of 15 progenitors, they produce 15 new cells in 32 days, or 0·47 cells/day. The newly formed cells leave the P compartment and enter the functional Q compartment. Since the P compartment produces 0·47 cells daily, it needs approximately 2 days in order to enter a new location in the Q compartment. Stated otherwise, at location 15, which represents here the outer progenitor compartment boundary, cells leave the P compartment and enter the functional compartment at a velocity of 0·47 locations/day. By defining cell displacement in units of cell locations, cell production and cell displacement are equal and one may be derived from the other. The production of a new cell is associated with displacement of all outer cells by one cell location. The more cells are produced, the faster do the distal cells travel and *vice versa*. Since, in this representation, cell locations are equivalent to cells, the term location may be omitted. Progenitor compartment exit velocity is thus 0·47 cells/day. It is twice as fast as the velocity estimates of 0·24 cells/day which were derived from the median shift (Table 2). The two estimates differ because they describe different cells. The first estimate accounts for the velocity of the progenitor cell, while the shifting median includes P as well as Q cells. The velocity difference of 0·23 cells/day between the two estimates results from cell elimination in the Q compartment. In order to slow down a Q cell by 0·23 locations/day, the same numbers of adrenocytes have to be eliminated. The newly formed cell thus leaves the P compartment at a daily velocity of 0·47 and slows down in the Q compartment to 0·24 cells/day.

Throughout the experiment the width of the adrenal cortex did not change (Table 1), so that cell elimination has to balance cell formation, and 0·47 cells are eliminated daily. Since 0·23 cells die daily in the Q compartment, the rest (0·24 cells) have to be eliminated at the tissue periphery.

Maximal adrenocyte life span

If all cells died solely at the tissue periphery, a cell at location 15, leaving the P compartment, would reach location 64 in 104 days. The maximal adrenocyte life expectancy is thus 104 days. However, since about 50% of the cells die along the way, the adrenocyte life expectancy is shorter.

DISCUSSION

This study settles a 100-year-old dispute between the migration and zonal theories. Gottschau (1883) was correct in claiming that cells are formed in the glomerulosa and migrate centripetally towards the medulla. The adrenal cortex is a cell renewal system made of two compartments. A progenitor, occupying the outer quarter of the cortex, and a functional, covering its inner three-quarters. Cells formed in the first compartment traverse the second. Half are eliminated on the way, while the rest die in the reticularis where they are eliminated by apoptosis (Wyllie, Kerr, Macaskill & Currie, 1973). The adrenal cortex kinetic structure conforms to other renewing tissues such as epidermis and gastrointestinal mucosa, consisting of an origin where cells are formed, a periphery where most of them die and a trajectory, the tissue radius, along which cells are displaced. Since all cells are displaced

in one direction (outwards) the more distant a cell from the origin the older it is. Cells in the fasciculata are older than those in the glomerulosa, and younger than reticularis cells; in the same way as a spinous keratinocyte of the epidermis is older than the basal cell and younger than the granular cell. Cell displacement along the tissue radius involves cell differentiation. Throughout its existence the displaced adrenocyte produces all three adrenocortical hormones. When young it secretes aldosterone, after leaving the glomerulosa it is engaged in corticosteroid production and upon reaching the reticularis it turns to producing sex hormones. The three cortical layers, together with their typical hormone products, constitute differentiation states of one and the same adrenocyte. Thus in the adrenal cortex the 'one cell one product' theory is not applicable.

Streaming tissues

The present kinetic study is part of a wider endeavour intended to demonstrate that all proliferating tissues renew their cells and are composed of two compartments, a progenitor where cells are formed and a functional where they perform their metabolic tasks. Leblond (1964) classified tissues into three groups according to their proliferative potential: (1) renewing cell populations, e.g. the epidermis, gastrointestinal mucosa and bone marrow; (2) expanding populations, e.g. liver and kidney, and (3) non-proliferating cells, e.g. nerve cells. We have recently shown that the submandibular gland and liver are also renewing and proceed along similar tissue radii to the adrenocyte. The submandibular stem cell resides in the intercalated duct. Its progeny are displaced in two opposite directions forming respectively acini and intralobular duct epithelia (Zajicek, Yagil & Michaeli, 1985). The hepatocyte progenitor resides at the portal space, from where its progeny are gradually displaced towards the central vein, traversing the three zones known as Rappaport's acinus (Zajicek, Oren & Weinreb, 1985). Since the hepatocyte produces different enzymes in each zone, they represent differentiation states of one cell, exactly as in the adrenal cortex. The same probably holds for other exocrine glands, e.g. pancreas, mammary gland and prostate, since all are built like the submandibular.

Cell turnover in renewing tissues is generally associated with cell displacement, which should better be regarded as cell streaming since the advancing cells always keep the same neighbours. After leaving the basal layer, keratinocytes are joined together by desmosomes and advance jointly outwards. Hepatocytes are joined together by tight junctions forming intricate structures known as muralium; the cells progress towards the central vein *en masse*. The same probably applies to the adrenal cortex where cells advance together in a viscous stream of matter. The stream carries with it the cell as well as its microenvironment, as has been demonstrated in the submandibular gland (Zajicek *et al.* 1985). The streaming intercalated cell is accompanied along its entire journey by a lamina propria, forming a unit which was previously denominated proliferon (Zajicek, 1977, 1979). A similar displacement in the adrenal cortex was described by Brenner (1963) who studied adrenocyte migration following carbon tetrachloride stress. He found that a large number of labelled fibroblasts and endothelial cells slowly penetrated the boundary region between cortex and medulla, known as the *X* region. Since this region represents the site of adrenocyte death, these observations suggest that stroma may accompany the streaming adrenocyte.

Apparent cell displacement could also occur during organ growth. If during adrenal growth, cells were added only to the zona reticularis, they would appear as if being displaced away from the capsule. This is why cell displacement has to be studied during steady-state conditions, a requirement which most kinetic studies do not meet. Nearly all studies have reported cell turnover in infants or in growing animals, when the cortex becomes wider and the distance of a peripheral cell from the capsule increases (Ford & Young, 1963; Wright, 1971; Bertholet, 1980). The present study met this requirement entirely and although the rats grew throughout the experiment, the width of the adrenal cortex did not change (Table 1).

The streaming concept introduces a novel way of analysing cell proliferation. The traditional approach studies mainly cellular proliferation. Its techniques are inadequate for measuring cell displacement *per se* and could not resolve the controversy between the migration and zonal theories. The present method, by dealing with displacement, is kinetic in the true sense. It follows the streaming cell from its origin where the stem cell is located, along the radius, until it reaches the tissue periphery. Generally the stem cell at origin divides asymmetrically. One of its progeny replaces the parent cell while the other streams outwards, proliferating as it goes. When reaching the periphery, it is eliminated. The stream may be appreciated only if cell positions are measured in relationship to a fixed point situated outside the system, such as the adrenal capsule. Since cell streams are nourished solely by proliferating progenitors, their velocities depend entirely upon progenitor cell production. By choosing cell location as the unit for displacement, cell production may be derived from cell displacement.

Stem cell site

The subcapsular stem cell forms the origin of the

entire system. It probably resembles an immature fibroblast, which generally proliferates extremely slowly. Only during regeneration does it start dividing vigorously. After enucleating the adrenal cortex and leaving the capsule behind, stem cell adrenocytes which remain attached to the capsule, generate a new adrenal cortex (Skelton, 1959; Taki & Nickerson, 1985). These stem cell remnants are known also as a capsular blastema. Regeneration is probably regulated neurally (Engeland & Dallman, 1975). 'Neither a hypocorticoid signal nor hypersecretion of adrenocorticotrophic hormone is required for compensatory adrenal growth after unilateral adrenalectomy' (Engeland & Dallman, 1975), yet compensatory growth could be prevented by inducing hypothalamic lesions. In order to be neurally controlled the adrenocyte has to be innervated and, since streaming, it probably carries its innervation along with its microenvironment. It is proposed that nerve fibres penetrating the adrenal capsule join the newly formed stem cell descendant at location zero, accompany it and elongate as it goes, until it has reached the tissue periphery, when the cell dies and the fibre also degenerates (Zajicek, 1979).

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