《原　著》

CELL KINETICS OF THE ADRENAL GLAND, THE GONADS AND THE THYMUS IN PERINATAL RAT

A Whole Body Autoradiogram by The Use of Dry Mount Autoradiography

Toshiaki NAKAI*

Synopsis

Distribution of ^{3}H-thymidine in the perinatal rat, with special reference to cell dynamics of the gonads, the thymus and the adrenal cortex, was investigated with the use of our modified dry mounting autoradiographic techniques, which enabled us to observe the distribution of radioactivity in a whole body section of a rat. The thymus displayed intense labeling in a fetal rat in which the growth rate was rapid. It is, therefore, reasonable that there are considerable number of thymic cells engaged in cell division. The outerzone of the adrenal cortex and the testis displayed a moderate degree of synthetic activities. There were few labelled cells in the innerzone of the adrenal cortex. The observations seem to indicate the inward displacement of cells (cells derived from the outerzone move into the innerzone), supporting the centripetal cell displacement theory. The ovary showed a lower degree of synthetic activity than the testis. The difference of labelling index between the ovary and the testis may be partly due to the difference of hormonal activities,—the testis begins to function before birth, while the ovary is hormonally quiescent in fetal life.

* Department of Clinical Pathology and Metabolism, DOKKYO University School of Medicine, Mibu Town, Tochigi Prefecture

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中　井　利　昭

Since ^{3}H-thymidine has been reported to be incorporated specifically into newly synthesized DNA prior to the cell division (1, 2), a combination of the use of ^{3}H-labeled thymidine and the autoradiographic techniques has been extensively attempted to investigate the DNA-synthesis in cells and tissues for more than two decades. ^{3}H-thymidine is probably the most widely-used tracer in modern cell biology, stimulating numerous studies regarding the kinetics of cellular proliferation in a variety of tissues. Most of the autoradiographic studies hitherto reported have been carried out using early postnatal or young animals (3, 4, 5), but little observation has been made on cell growth dynamics in perinatal life. Development of the whole-body autoradiography (6, 7) has enabled us to observe the distribution of radioactivity in a whole-body section of a rat. The present study was undertaken to investigate cell growth dynamics of various tissues, particularly those of the gonads, the adrenal gland and the thymus in the perinatal rat, with the aid of our modified dry-mounting autoradiographic techniques.

Materials and Methods

Wistar strain female adult rats were mated and the onset of gestation was ascertained by finding the sperm in the vaginal smear. The day of finding the sperm was counted as the first day of pregnancy. On the 22nd day of pregnancy, 4 gravid females were subjected to a midventral laparotomy under ether anesthesia and their fetuses were given an intraperitoneal injection of 0.045 m*l* of solution containing 22.5 μCi of ^{3}H-thymidine (S. A. 5.0

Ci/mM, Daiichi pure chemical Co., Japan). 4 neonatal rats at 1 and 3 days of age were injected intraperitoneally with ^{3}H-thymidine of 5 μCi per g body weight. At thirty minutes after the injection, fetuses and neonatal rats were killed by freezing in a suitable container, cooled at −70°C in a mixture of carbon dioxide and acetone. Each of frozen rats was placed on the brass stage of a Leitz sledge microtome, Model 1300, in a cryostat kept at −20°C. The inferior side of the specimen was coated with a thin layer of 2% hydroxypropylmethyl cellulose solution, which had been cooled at 1°C. A further layer of the cellulose solution was coated on the previous layer as soon as it solidified. This procedure was repeated several times until the specimen was encased. The whole body of the specimen was cut at 7 μ at −20°C with the microtome. A section of the specimen being cut gently with the microtome was scooped up with a sharpened tip of paper card. The section was placed on a cooled glass slide which was previously smeared homogenously by a very small amount of albumin-glycerin mixture (2:1). A close contact of the section with the glass was made by warming slightly the reverse side of the slide with finger. Then it was transferred to a specimen chamber in the same cryostat. The specimen chamber (in which about 20 m*l* of water was frozen to prevent the water in the thin section from evapolating unequivalently as ice vapor) was made vacuous at about 10^{-2} mmHg. After the freeze-drying was completed, as described by Aoyagi et al (8), the specimen chamber was allowed to be warmed to room temperature and the vacuum was broken. The frozen-dried sections were removed and stored in a desiccator at room temperature until further use.

As ^{3}H-thymidine was decomposed, metabolites of thymidine were found to be incorporated into such components other than DNA as RNA, water soluble materials, and organic solvents. The following procedures were used to remove such components.

1. fix in Carnoy's fluid for 1 minute
2. soak in 100% alcohol for 5 minutes
3. soak in chloroform-methanol solution (1:3) for 5 minutes
4. soak in 94% alcohol for 5 minutes
5. soak in 70% alcohol for 5 minutes
6. soak in 50% alcohol for 5 minutes
7. wash in running distilled water for 5 minutes
8. soak in 0.1% perchloric acid for 5 minutes
9. repeat the soaking of procedure 8
10. wash in running distilled water for 5 minutes
11. submit to the action of ribonuclease solution for 30 minutes at 50°C
12. wash 30 minutes in running distilled water
13. soak in 0.1% perchloric acid for 10 minutes
14. wash for 20 minutes in running distilled water

As proteins were not removable by these procedures, preliminary experiments were done to investigate whether ^{3}H-fragments, derived from ^{3}H-thymidine, were incorporated into proteins or not. Some of the sections were submitted further to the action of desoxyribonuclease. The obtained autoradiogram showed that there were few silver grains in the cell nuclei as well as cytoplasm. This fact indicates that silver grains obtained by our procedures show the incorporation of ^{3}H-thymidine into nuclear DNA alone, showing no incorporation into proteins. A dry mounting autoradiogram was prepared using the following procedures.

A 40×93 mm glass plate was covered with a 43×93 mm of thin plastic sheet (Saranwrap). The remaining part of the sheet was turned over on the opposite surface of the glass plate. Under a dark-room lamp, a photographic plate covered with stripping type of nuclear emulsion (Fuji, ET2F, Japan) was immersed in a cold NaCl-glycerin solution containing 1 g of NaCl and 30 g of glycerin per liter.

After kept in the solution for a few minutes, the emulsion layer was stripped off from the plate and turned inside out to spread on the saran-covered glass plate avoiding the wrinkling of the emulsion. The emulsion put on the glass plate was dried in a dark room by leaning the plate against the wall at 60° angle. Then, a section slide was put on the dried emulsion plate very gently. In order to reduce shifting of the section and improve adherence, the film face had been exposed to steam (about 50°C) for a second. After the section slide adhered to the emulsion completely, the saranwrap and the glass plate was removed from the section slide, leaving the emulsion put on the section slide. Overwrapped part of the emulsion was turned to the back of the section slide and pasted there with

a piece of water proof cemedine tape in order to prevent the emulsion from moving on the slide. Then the section slide was stored in a desiccator box at −4°C for exposure. After four weeks of exposure, the slide was placed directly in a developer (Fuji, Rendol) at 20°C for 5 minutes, fixed in a Fuji Super Fix Solution at 20°C for 10 minutes, rinsed for 20 minutes in running distilled water, stained with haematoxylin-eosin.

Results and Discussion

The whole body autoradiogram of a 22-day-old perinatal rat is presented in Fig. 1. Under a high magnification, silver grains were visible in cell nuclei of various organs, as displayed in Fig. 1-A–E. The highest mitotic activity was found in the thymus. The outerzone of the adrenal cortex and the testis displayed a moderate degree of synthetic activities. The ovary showed a lower degree of synthetic activity than the testis. The innerzone of the adrenal cortex showed few mitotic activity.

Thymus: Intense labeling of the thymus was found (Fig. 1-A). In an attempt to contribute to a better understanding of the cellular events which occur during growth and involution of the thymus, Berman (9) studied the proliferative activity of the thymus of Fisher rats with different ages by the use of ^{3}H-thymidine and autoradiographic techniques. He found that the youngest group had a greater degree of labeling than the old group and that a decrease in a percentage of labeled cells occurred between 60 and 90 days of postpartum age which appeared to initiate the onset of age involution of the thymus. As early as 1914, Hellman (10) suggested that the prepubertal growth of the thymus, which was followed by weight loss with age, was a reflection of an increase and subsequent decrease of lymphocyte in this tissue. Enesco and Leblond (11) studied the weight and DNA content of the thymus in young adult rats and concluded that during the first three months of life, thymic weight increased as a result of an increase of cells number and that later atrophy was due to decreased cellularity. The purpose of the present work was to obtain some informations concerning the cell dynamics of thymocytes during the perinatal life. The mitotic activity in the thymus of perinatal rats was much higher than those in other organs (Fig. 1-A). If the premise is accepted that the weight of the tissue reflects its cellularity (11), then it is reasonable that mitotic activity of the thymus during the rapid growth period of perinatal life is considerably high.

Adrenal gland: Considerable labeling was seen in the outerzone of the cortex as shown in Fig. 1-B. In contrast, labeling was very low in the innerzone of the cortex. The number of labeled nuclei was estimated in approximately 500 nuclei selected at random on each slide of the adrenal gland and thus the percentage of the nuclei that were radioactive (labeled index) was calculated. The labeled index of the outerzone was 18% and that of innerzone was 3%.

Mitotic labeling of neonatal rats showed the same tendency. The origin and fate of cells within the growing adrenal cortex have been extensively studied autoradiographically in postpartum young rats given ^{3}H-thymidine. According to Diderholm and Hellman (12, 13), labeled cells were concentrated in the outerzone of the cortex in young rats shortly after the injection of ^{3}H-thymidine and six weeks after the injection, radioactive cells were found increasingly near the medulla, indicating that centripetal displacement had occurred. Brenner (14) reported that ^{3}H-thymidine labeled cells migrate centripetally in the adrenal cortex of young adult mice subjected to acute carbone tetrachloride stress. Ford and Young (15) found that cell proliferation was greatest in the zona glomerulosa, dropped off progressively deeper in the cortex and was negligible in the inner fasciculata and reticularis. Our results were well in harmony with the previous reports about young animals, suggesting an inward displacement of cells which ultimately would come to lie in the innercortical zone.

The gonads: The fetal rat testis showed a moderate degree of mitotic figures (Fig. 1-C). The mitotic figures of the fetal ovary was lower than those of testis (Fig. 1-D). In the neonatal rat high mitotic activity was observed in both the ovary and the testis (Fig. 1-E). Previous histological observation revealed that inspite of well developed fetal testis, the fetal ovary was not so well developed as the testis and the follicles did not begin to appear until two days after birth (16, 17). This may explain our obtained difference of cellular kinetics on the testis and the ovary during perinatal life.

In our previous paper (18), the developmental

cell dynamics in the hypothalamus, the anterior pituitary and the ovary of the rat was investigated by ^{3}H-thymidine autoradiography, and a close relationship between the functional development and morphogenesis of these endocrine organs was elucidated. Likewise, the above mentioned information on cell dynamics in various tissues not only indicate proliferation of these cells but also elucidate the relationship between the functional development and morphogenesis of these tissues in perinatal life. That is, the variations in the labeled index of different cell lines suggest some intracellular controls, perhaps related to specialized functions and synthetic activities of special cells.

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出生前後ラットにおける副腎皮質・性腺・胸腺の Cell Kinetics について
——ドライマウントオートラジオグラフィーを用いての全身オートラジオグラム——

独協医科大学臨床病理・代謝科
中井利昭

副腎皮質，性腺等の内分泌腺の cell kinetics については，成熟または幼若期の報告はいくつかみられるが，perinatal 期．とくに胎生期についての報告は今までみられない．今回われわれの改良，考案した dry mount autoradiography を用いての whole body autoradiogram により，perinatal 期のラットの内分泌腺の cell kinetics を検索し，機能的な発育との関連についていささかの知見を得たのでここに報告する．^{3}H-thymidine を 22 日齢胎仔および生後 1 日，3 日齢の新生仔ラットに注射し，30分後その体内分布を凍結乾燥切片による whole body autoradiogram により検討した．各臓器の ^{3}H-thymidine の labeling 状態を比べてみると，胸腺は最も細胞分裂が盛んであった．生後の胸腺については生直後 labeling index は高いが胸腺の退化がおこる 60 日齢頃に一致して labeling index の減少がおこるという報告があるが，われわれの結果も生前においては labeling index が高く，その旺盛な発育を示唆するものである．副腎皮質の外層は中等度の分裂像を示したが，副腎皮質の内層にはほとんど分裂像が認められなかった．このことは生後ラットの副腎皮質についての inward displacement theory——すなわち皮質外層細胞が内層へと移動する——を perinatal 期においても support する所見である．性腺については精巣の方が卵巣に比べより盛んな分裂像を示した．組織学的所見によると，胎生期精巣は分化が進んで seminiferous tubules もみられるが，卵巣は未だ未分化で follicle もみられないというが，精巣と卵巣の cell kinetics の違いはこの分化の違いに一因していよう．以上われわれの改良，考案した dry mount 法を用い，胎仔ラットに ^{3}H-thymidine を投与した autoradiogram を作成した結果，各臓器の細胞の分裂像のみならず，その臓器の機能との関連をも明らかにした．

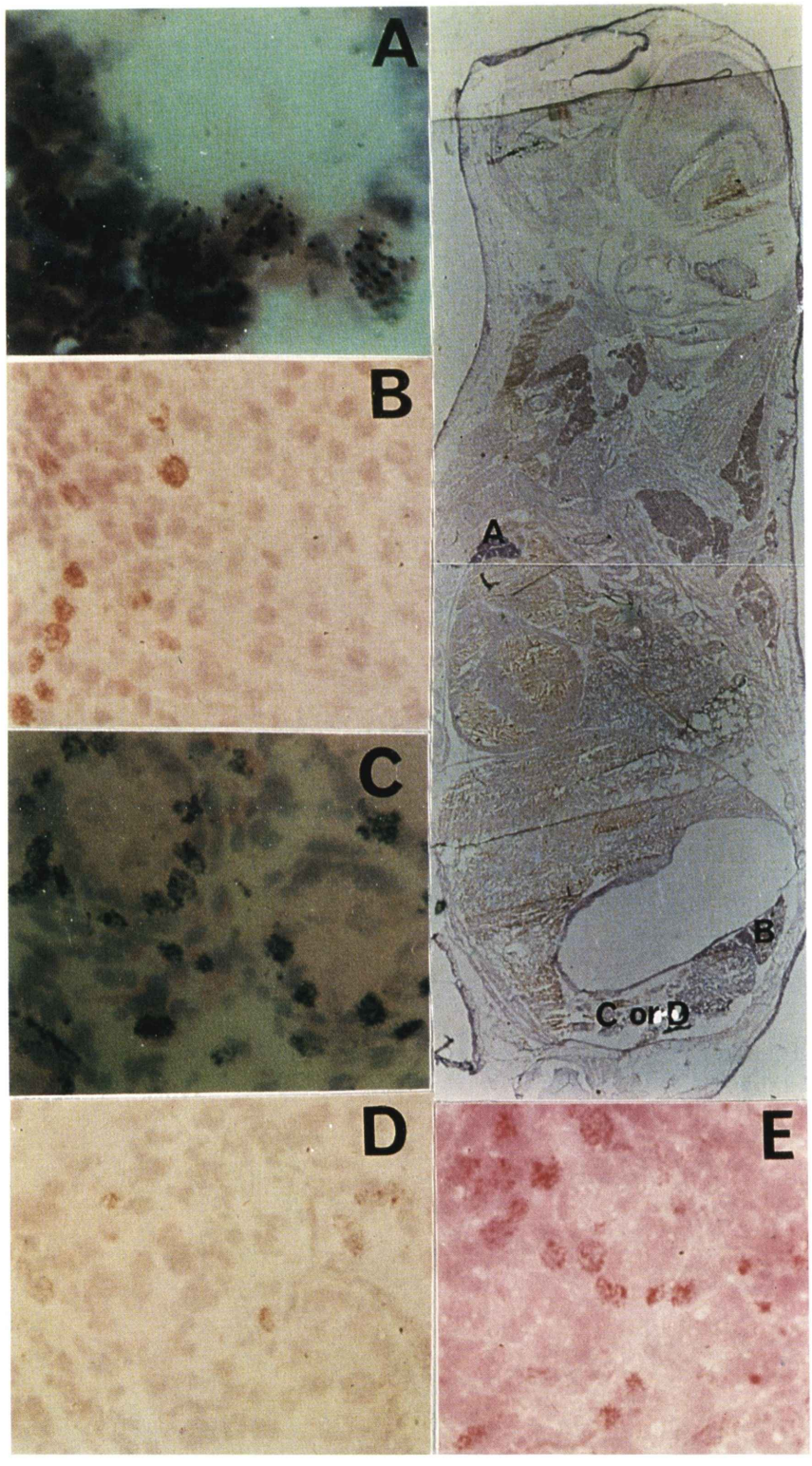

Fig. 1

Whole Boby Autoradiogram of a fetal rat

A: Thymus (×1000)
B: Adrenal gland (×400)
C: Testis (×400)
D: Ovary (×400)
E: Ovary (3 days old rat ×400)