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Macromolecular Synthesis in the Urinary and Reproductive Systems

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1. Introduction

This chapter deals with the third parts of the application of microscopic radioautography to the organ systems, including the urinary organs, the reproductive organs and the endocrine organs.

2. Macromolecular synthesis in the urinary system

The urinary system consists of the kidney and the urinary tract. We studied only the macromolecular synthesis in the kidneys of several groups of aging mice by LM and EM RAG, while the localization of an anti-allergic agent was observed in the urinary bladders of adult rats (Nagata 2005).

2.1 Macromolecular synthesis in the kidney

We studied only the DNA, RNA and glucides syntheses in the kidneys of several groups of aging mice by LM and EM RAG.

2.1.1 The DNA synthesis in the kidney

The kidneys of mammals microscopically consist of the nephrons, which can be divided into two portions, the renal corpuscles and the uriniferous tubules. The renal corpuscles are composed of the glomeruli which are covered with the Bowman's capsules. They are localized in the outer zone of the kidney, the renal cortex, while the uriniferous tubules are composed of two portions, the proximal portions and the distal portions which can further be divided into several portions which run from the outer zone of the kidney, the renal cortex, to the inner zone, the medulla. We studied the DNA synthesis by ^{3}H-thymidine radioautography in 3 groups of ddY mouse embryos from prenatal day 13 (Fig. 16A), day 15 (Fig. 16B) to day 19 in vitro, as well as perinatal mice from embryonic day 19 to postnatal day 1, 8, 30, 60 and 365 (1 year) in vivo (Hanai 1993, Hani et al. 1993, Hanai and Nagata 1994a,b).

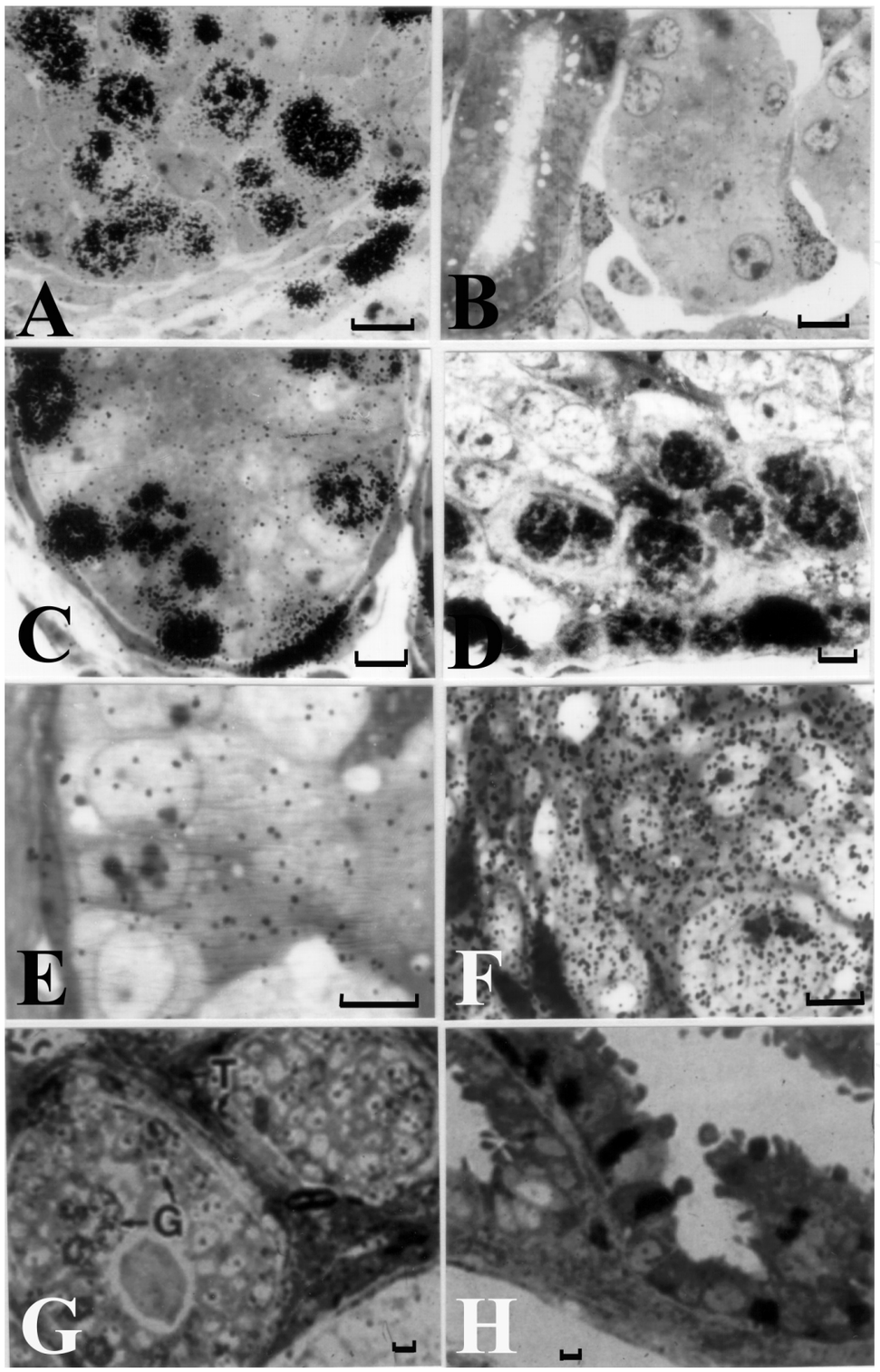


Fig. 16. LM RAG of the uro-genital organs. From Nagata, T.: Special Cytochemistry in Cell Biology, In, Internat. Rev. Cytol. Vol. 211, No. 1, p. 108, 2001, Academic Press, San Diego, USA, London, UK.

Fig. 16A. LM RAG of the metanephros of a prenatal day 13.5 mouse embryo labeled with ^{3}H-thymidine, showing DNA synthesis. x1,200.
Fig. 16B. LM RAG of the metanephros cortex of a prenatal day 15.5 mouse embryo labeled with ^{3}H-thymidine, showing DNA synthesis. x1,200.
Fig. 16C. LM RAG of the testis of a postnatal day 7 male mouse labeled with ^{3}H-thymidine, showing DNA synthesis. x800.
Fig. 16D. LM RAG of the testis of a postnatal year 1 male mouse labeled with ^{3}H-thymidine, showing DNA synthesis. x800.
Fig. 16E. LM RAG of the testis of a postnatal day 3 male mouse labeled with ^{3}H-uridine in vitro, showing RNA synthesis. x1,500.
Fig. 16F. LM RAG of the testis of a male mouse at postnatal day 1 labeled with ^{3}H-leucine in vitro, showing protein synthesis. x1,125.
Fig. 16G. LM RAG of the ovary of a postnatal day 3 female mouse labeled with ^{3}H-thymidine in vitro, showing DNA synthesis in granulosa cells (G) and theca cells (T). x400.
Fig. 16H. LM RAG of the oviduct of a postnatal day 30 female mouse labeled with ^{3}H-thymidine in vitro, showing DNA synthesis in epithelial cells. x400.

The labeling indices by LM RAG in glomeruli (28 to 32%) and uriniferous tubules (31 to 33%) in the superficial layer were higher than those of labeling indices (10 to 12%) and (8 to 16%) in the deeper layer from the late fetal to the suckling period, then decreased with aging from weaning to senescence (Fig. 17). EM RAG revealed the same results (Hanai and Nagata 1994a,b,c). At the same time, immunocytochemical staining of PCNA/cyclin was carried out in the same animals in several aging groups as ^{3}H-thymidine RAG (Hanai 1993, Hanai et al. 1993). The results from the PCNA/cyclin positive indices in respective aging groups were almost the same as the labeling indices with ^{3}H-thymidine RAG. The incorporation of ^{3}H-thymidine was formerly observed by EM RAG in mitochondrial matrix of cultured kidney cells from chickens and mice in vitro demonstrating mitochondrial DNA synthesis (Nagata et al. 1967b).

2.1.2 The RNA synthesis in the kidney

The RNA synthesis by incorporation of ^{3}H-uridine into the kidneys of aging mice was studied by LM and EM RAG (Hanai and Nagata 1994a,b, Nagata 2002). When the kidneys of several groups of aging mice from embryo to postnatal 1 year were radioautographed with ^{3}H-uridine either in vitro (embryonic day 15, 19 and postnatal day 1) and in vivo (embryonic day 19, postnatal day 1, 7, month 1, 2, 12), RNA synthesis was observed in all the cells of the kidney at various ages. The numbers of silver grains demonstrating the incorporation of ^{3}H-uridine in glomeruli (34.6 per cell) and uriniferous tubules (56.4 per cell) were higher in the superficial layer than those (15.6 and 18.6 per cell) in the deeper layer at embryonic day 15 and decreased gradually with aging. These results demonstrated the aging changes of RNA synthesis in the kidney.

2.1.3 Glucide synthesis in the kidney

The incorporations of ^{3}H-glucosamine in the kidneys of aging mice were studies by LM RAG (Joukura 1996, Joukura and Nagata 1995) and EM RAG (Joukura et al. 1996). Silver grains were observed over all the cell type nephrons at embryonic day 19, i.e., glomerular epithelial cells, endothelial cells, mesangial cells, Bowman's capsular cells and tubule cells.

In newborn and suckling stages, from postnatal day 1, 3, 7 to 14, both the renal corpuscles and urinary tubules were well differentiated and the number of silver grains increased (Figs. 36 C, D, E F, G, H in Nagata 2002). The results from grain counting revealed that the numbers of silver grains in both the renal corpuscles and the uriniferous tubules were less in the embryonic stage, but increased postnatally and reached peaks at day 1 and 3, then decreased to senescence at 1 year. These results showed that glucide synthesis in the kidney cells also changed with aging of animals.

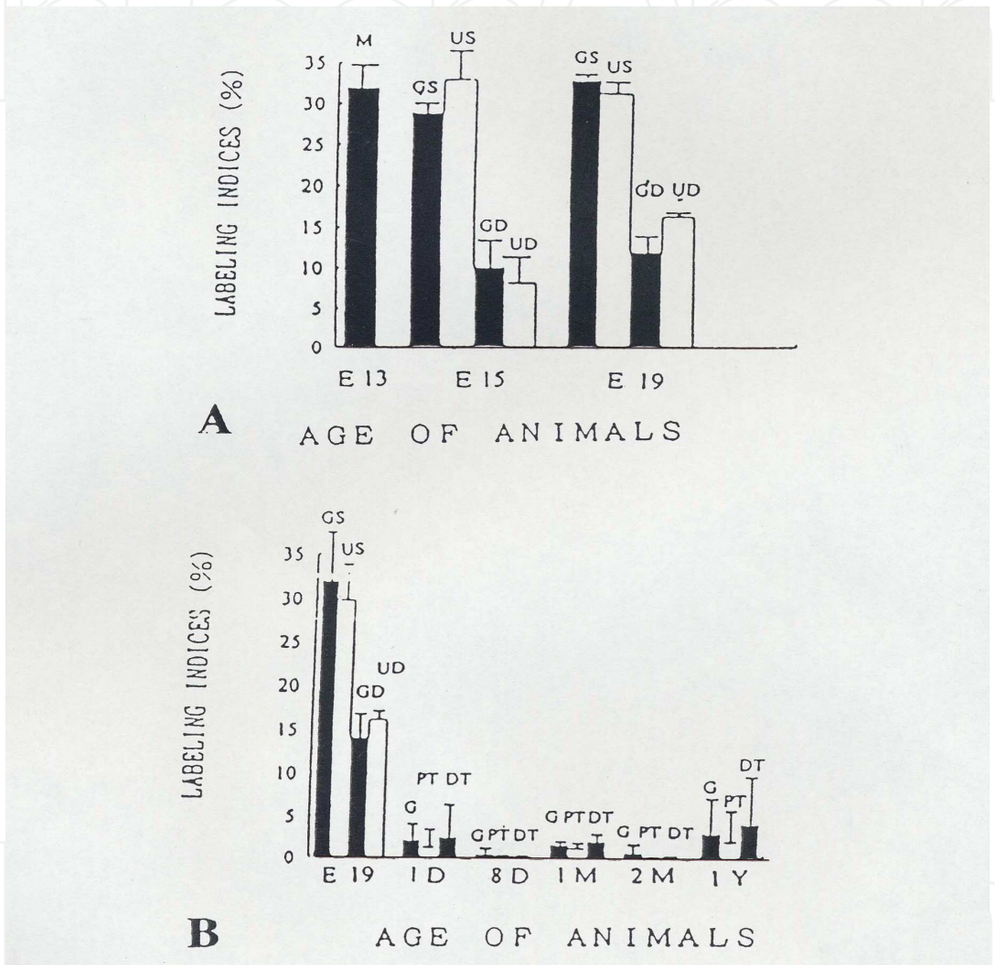


Fig. 17. Histogram showing aging changes of average labeling indices in respective cell types of the kidneys of aging mice labeled with ^{3}H-thyidine. Mean ± Standard Deviation. From Nagata, T.: Radioautographology, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p. 156, 2002, Urban & Fischer, Jena, Germany

Fig. 17A. Labeling indices of the glomeruli and the uriniferous tubules of of mouse embryo from prenatal day 13 and 19.

Fig. 17B. Labeling indices of the glomeruli and the proximal and distal tubules of mouse embryo from prenatal day 19 to postnatal year 1. Abbreviations: GS=glomeruli of the superficial layer. US=uriniferous tubules of the superficial layer. GD=glomeruli of the deeper layer. UD=uriniferous tubules of the deeper layer. G=glomeruli. PT=proximal tubules. DT=distal tubules.

2.2 Localization of drugs in the urinary tract

The urinary tract is composed of the ureter, the urinary bladder and the urethra. We studied the urinary bladder of adult rats by LM RAG after oral administration of ^{3}H-tranilast, an anti-allergic agent produced by Kissei Pharmaceutical Co. (Momose et al. 1989, Nishigaki et al. 1987, 1990a,b). It was found that this agent specifically localized over the transitional epithelium and the endothelium of the veins in the mucosa of normal adult rats. However, any study on the DNA synthesis in the ureter, the urinary bladder and the urethra was not carried out.

3. Macromolecular synthesis in the reproductive system

The reproductive system or genital organs can be divided into two parts, the male genital organs and female genital organs. We studied the DNA and RNA syntheses and protein synthesis in several groups of aging mice, both male and female, by LM and EM RAG (Nagata 2002).

3.1 Macromolecular synthesis in the male genital organs

The male genital organs consist of the testis and its excretory ducts such as ductuli efferentes, ductus epididymidis, ductus deferens, ejaculatory ducts, auxiliary glands and penis. We studied both DNA and RNA syntheses in these organs of several groups of ddY aging mice by LM and EMRAG using macromolecular precursors.

3.1.1 The DNA synthesis in the male genital organs

Among the male genital organs, the testis was the main target of the scientific interests. Formerly, Clermont (1958, 1963) demonstrated using ^{3}H-thymidine radioautography that several stages of development of the spermatogonia were found at different levels in the germinal epithelium of mature men and rodents, with the most primitive germ cells found at the base and the more differentiated cells located at higher levels. We studied the DNA synthesis in the testis of several groups of aging mice.

3.1.1.1 The DNA synthesis in the testis

The structure of the testis of mammals is a compound tubular gland enclosed in tunica albuginea, a thick fibrous capsule. The parenchyma of the testis is composed of around 250 pyramidal compartments in men and animals, named lobules. Each lobule is made of convoluted seminiferous tubules, consisting of many spermatogenic cells differentiatiang to sperms among the supporting cells of Sertoli in the seminiferous epithelium, surrounded by the interstitial cells of Leydig. We first studied the macromolecular synthesis in the testis of aging male ddY mice at various ages (Gao 1993, Gao et al. 1994,1995a,b). When testicular tissues were labeled with ^{3}H-thymidine and observed by LM and EM RAG, many spermatogonia and myoid cells as well as Leydig cells were labeled with ^{3}H-thymidine at various ages from embryonic day 19 to postnatal day 1, 3, 7 (Fig. 16C), 14, month 1, 2, 6, 12 (Fig. 16D) and 24 (2 years). Silver grains were localized over the nuclei and several mitochondria of the spermatogonia showing DNA synthesis. Among of the aging groups, we counted the numbers of mitochondria per cell profile area, the numbers of labeled mitochondria per cell of the spermatogonia from 4 aging groups, prenatal embryonic day 19, postnatal day 3, and adults at month 1 and 6, and the labeling indices were calculated.

The results showed that the LI of the spermatogonia increased from embryonic day 19 (17%) to postnatal day 7 (25%) and month 1 (30%), reaching the maximum, then decreased to month 6 (20%) to year 2.

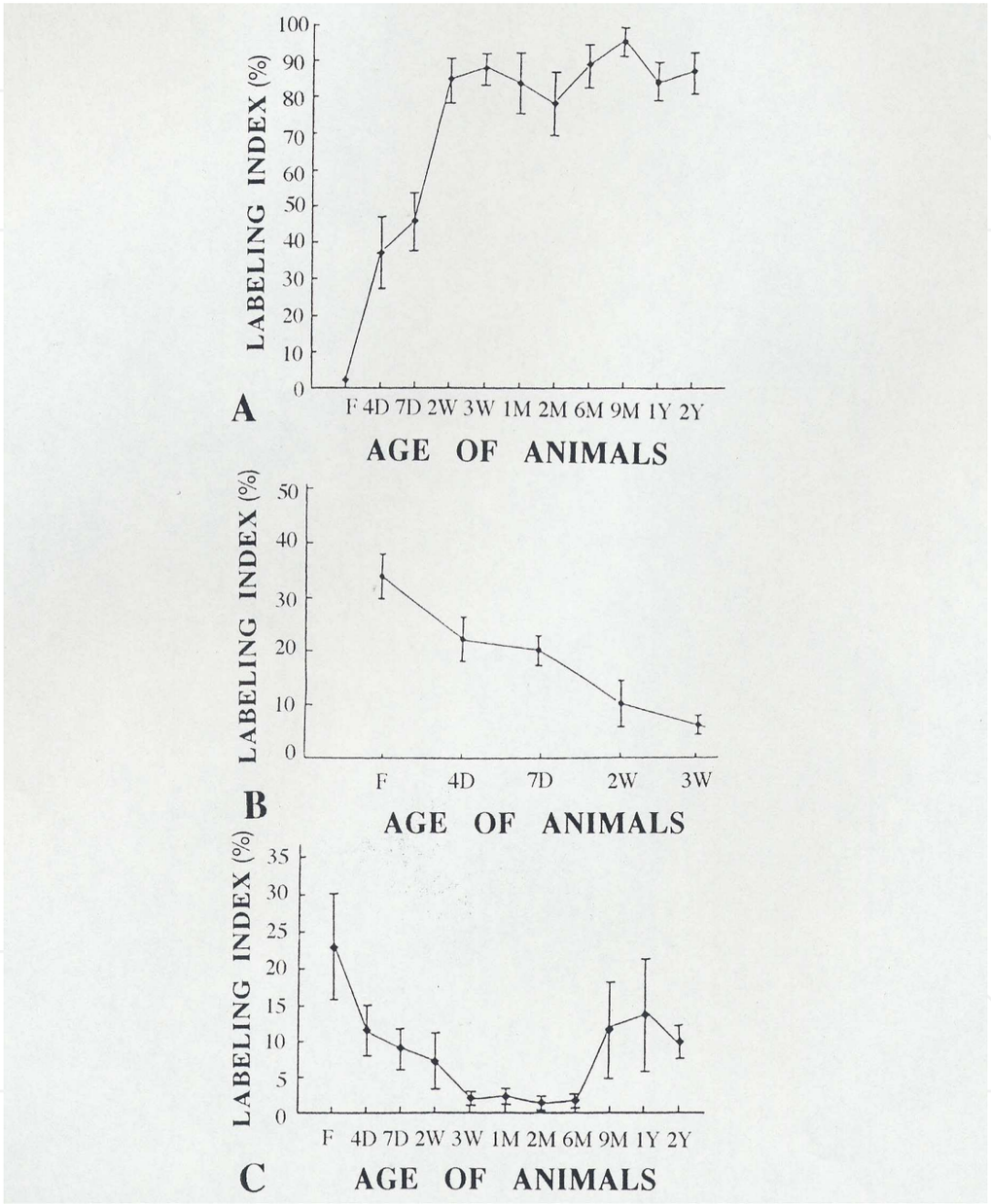


Fig. 18. Transitional curves of the labeling indices of respective cell types in the testis of aging mice labeled with ^{3}H-thymidine, showing DNA synthesis. Mean ± Standard Deviation. From Nagata, T.: Radioautographology, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p. 160, 2002, Urban & Fischer, Jena, Germany
Fig. 18A. The spermatogonia.
Fig. 18B. The Sertoli cells.
Fig. 18C. The myoid cells.

At embryonic and neonatal stages, DNA synthesis of spermatocytes was weak and only a few labeled spermatogonia could be observed during the perinatal stages. The labeled spermatocytes were recognized at postnatal day 4 and 7 (Fig. 16C) and the number of labeled spermatogonia and spermatocytes increased from 2 and 3 weeks, keeping high level from month 1 to year 1 and 2 until senescence (Fig. 18A). However, the Sertoli's cells (Fig. 18B) and myoid cells (Fig. 18C) labeled with ^{3}H-thymidine were frequently observed at perinatal stages from embryo to postnatal day 7, while the labeling indices of both cells decreased from young adulthood (postnatal 2 weeks) to senescence (Gao 1993, Gao et al. 1994,1995a). The interstitial cells of Leydig in the testis surrounding the seminiferous tubules shall be described in the following section of the endocrine system in detail.

3.1.2 The RNA synthesis in the male genital organs

Among of the male genital organs, we studied the RNA synthesis in the testis of several groups of aging mice.

3.1.2.1 The RNA synthesis in the testis

We studied the RNA syntheses in aging mouse testis by LM and EM RAG, demonstrating the incorporations of ^{3}H-uridine into various cells of the seminiferous tubules (Gao 1993, Gao et al. 1994, Nagata 2002). The RNA synthesis of various cells in the seminiferous tubules was studied using ^{3}H-uridine. Silver grains due to ^{3}H-uridine demonstrating RNA synthesis were observed over the nuclei and cytoplasm of all spermatogonia, spermatocytes, Sertoli's cells, myoid cells of immature mice at perinatal stages at day 1 and 3 (Fig. 16E), as well as in mature and senescent mice from month 1, 6 to year 1 and 2. The synthetic activities of spermatogonia, Sertoli's cells and myoid cells as shown by grain counting with ^{3}H-uridine, as expressed by grain counting, were low (2-8 grain counts per 10 mm^{2}) at the embryonic and neonatal stages but increased at adult stages and maintained high levels (10-20 grain counts per 10 mm^{2}) until senescence. These results showed that DNA synthesis in myoid cells and Sertoli's cells increased at the perinatal stages and decreased from postnatal 2 weeks as described previously (Fig. 16A), while the RNA synthesis (Fig. 16E) in spermatogonia increased from postnatal 2 weeks together with DNA and protein syntheses (Fig. 16F) to senescence.

3.1.3 The protein synthesis in the male genital organs

We studied the protein synthesis of the reproductive system in both the male and female reproductive organs.

3.1.3.1 The protein synthesis in the testis

We studied the protein syntheses in aging mouse testis by LM and EM RAG, demonstrating the incorporations of ^{3}H-leucine into various cells of the seminiferous tubules (Gao 1993, Gao et al. 1994, Nagata 2002). The protein synthesis of various cells in the seminiferous tubules was first studied after administration of ^{3}H-leucine into aging male mice at various ages from perinatal to senescence at postnatal 2 years. Silver grains due to ^{3}H-leucine incorporation demonstrating protein synthesis were observed over the nuclei and cytoplasm of all the cells, spermatogonia, spermatocytes, Sertoli's cells, myoid cells of all male mice at respective stages from perinatal to senescence. The synthetic activities of spermatogonia, Sertoli's cells and myoid cells as shown by the number of silver grains due to ^{3}H-leucine, as expressed by grain

counting, were low at the embryonic and neonatal stages but increased at adult stages and maintained high levels until senescence. These results showed that DNA synthesis in Sertoli's cells (Fig. 18B) and myoid cells (Fig. 18C) that increased at the perinatal stages and decreased from postnatal 2 weeks, while the DNA synthesis in spermatogonia increased from postnatal 2 weeks (Fig. 18A) together with RNA and protein syntheses to senescence.

3.2 Macromolecular synthesis in the female genital organs

The female genital organs consist of the ovary, the oviduct, the uterus, the vagina and the external genitals. We studied the macromolecular synthesis in the ovary, oviduct and uterus of several litters of ddY mice in aging.

3.2.1 The DNA synthesis in the female genital organ

Among the female genital organs, we studied the DNA synthesis in the ovary, oviduct and uterus of several litters of ddY mice in aging.

3.2.1.1 The DNA synthesis in the ovary

The ovary consists of the germinal epithelium covering the surface and the stroma containing many developing ovarian follicles depending upon the age of animals.

The nucleic acids, DNA and RNA, syntheses in the developing virgin mice ovaries of 6 litters, each 3 individuals, consisting of 36 female mice at various ages in respective precursors were studied by ^{3}H-thymidine and ^{3}H-uridine radioautography (Li 1994, Li and Nagata 1995, Li et al. 1992). The ^{3}H-thymidine incorporations were active in all surface epithelial cells, stromal and follicular cells of the ovaries between postnatal days 1 to 7 and decreased from day 14 (Fig. 16G) and maintained a lower level to day 60, while ^{3}H-uridine incorporations were active in all surface epithelial cells, stromal and follicular cells of the ovaries between postnatal days 1 to 7 and maintained medium levels from day 14 on.

The labeling indices with ^{3}H-thymidine showing DNA synthetic activity were high in all the surface epithelial cells, follicular cells and stromal cells of mice at neonatal stage from postnatal day 1 to 7, but decreased from day 40 to day 60 at mature stage (Fig. 19A). The grain counts showing RNA synthetic activity were high at neonatal stage from day 1 to day 7, and maintained medium levels from day 14 to day 60 at mature stage.

3.2.1.2 The DNA synthesis in the oviduct

The nucleic acids, DNA and RNA, syntheses in the oviducts of developing virgin mice at various ages were studied by ^{3}H-thymidine and ^{3}H-uridine radioautography (Li 1994, Li and Nagata 1995). The silver grains with ^{3}H-thymidine showing the DNA synthesis were observed over many nuclei in all surface epithelial cells, stromal and smooth muscle cells at neonatal stage between postnatal day 1 to 3 and decreased from day 7 to 30 (Fig. 16H) and 60, while the silver grains showing the RNA synthesis with ^{3}H-uridine were observed over the nuclei and cytoplasm of all the epithelial and stromal cells from postnatal day 1 to day 60. The labeling indices with ^{3}H-thymidine were high at neonatal stage from postnatal day 1 to 3 but decreased from day 7 to day 60 (Fig. 19C). The grain counts with ^{3}H-uridine were high at neonatal stage from postnatal day 1 to 3 and increased from day 7 to day 14 and decreased from day 30 to day 60. These results demonstrated an unparalleled alternation of DNA and RNA syntheses in the oviduct (Li and Nagata 1995).

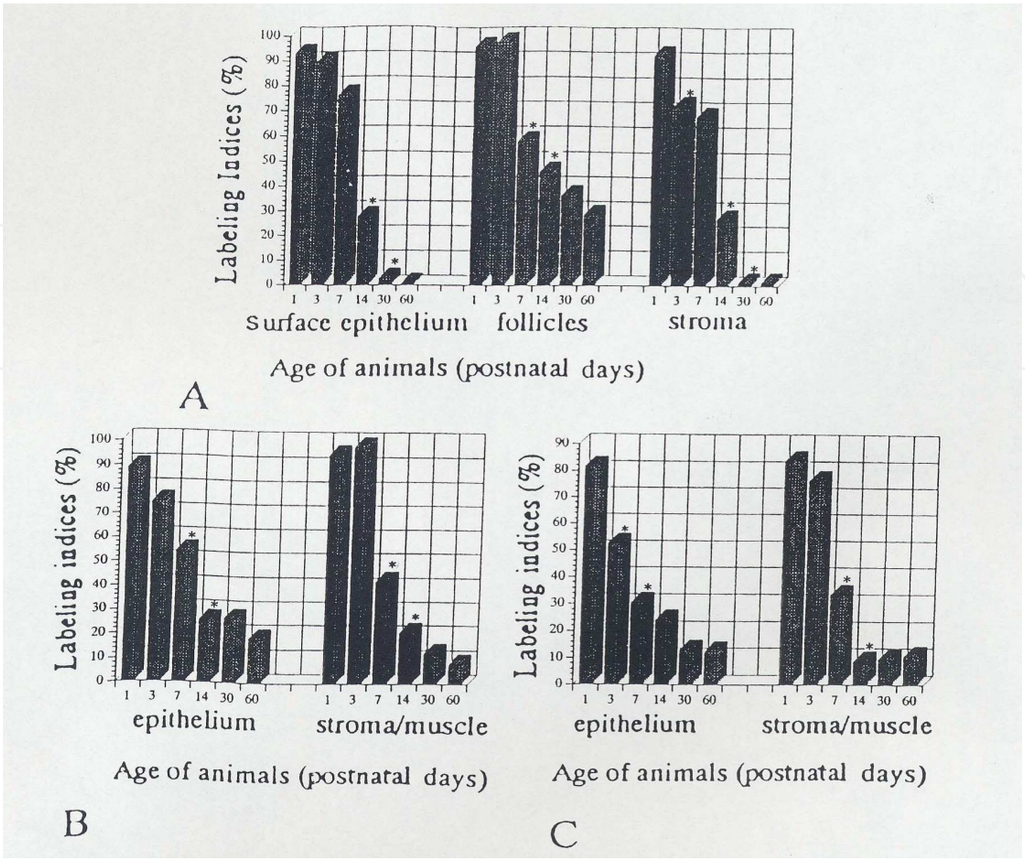


Fig. 19. Histogram showing aging changes of average labeling indices in respective cell types of female genital organs of aging mice labeled with ^{3}H-thymidine. Mean ± Standard Deviation. From Nagata, T.: Radioautographology, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p. 166, 2002, Urban & Fischer, Jena, Germany
Fig. 19A. The ovary.
Fig. 19B. The uterus.
Fig. 19C. The oviduct.

3.2.1.3 The DNA synthesis in the uterus

The silver grains with ^{3}H-thymidine showing DNA synthesis of the uterus was observed over some of the nuclei of all the cells in the epithelia, stroma and smooth muscles from postnatal day 1 to 60 (Li 1994, Li and Nagata 1995). The labeling indices with ^{3}H-thymidine (Fig. 19B) were high (80-95%) at postnatal day 1 and decreased from day 3 to 60 (>10%). The silver grains showing RNA synthesis of the uterus were observed over all the nuclei and cytoplasm of all the cells in the uterine epithelia, stroma and smooth muscles from day 1 to 60. The number of silver grains in the uterine epithelium increased from postnatal day 1 to 7 and decreased from day 14 to 60, while they increased in the stroma from day 1 to 3 and decreased from day 7 to 60.

These results from the female genital organs showed that both DNA and RNA syntheses, as expressed by labeling indices and grain counting, were active in all kinds of cells, such as surface epithelial cells, stromal cells and follicular cells of the ovaries between postnatal

days 1 to 7, then they decreased from day 14 to 60. However, the DNA synthesis in the epithelial cells and the stromal cells of both the uteri and the oviducts was active at postnatal day 1 and 3 and decreased from day 7 to 60. The RNA synthesis in the uteri and oviducts was active at postnatal day 1, increased from day 1 to day 14, and decreased from day 30 to 60. The unparalleled alteration of the DNA and RNA syntheses was shown between the ovary and the uterus or oviduct (Li and Nagata 1995).

We also studied PCNA/cyclin immunostaining in the ovary, oviduct and uterus (Li 1994). It was demonstrated that PCNA/cyclin positive cells were observed in the ovarian follicular epithelium, ovarian interstitial cells, tubal epithelial cells, tubal interstitial cells, uterine epithelial cells and uterine interstitial cells. The positive cells increased from postnatal 1 day to 3 and 7 days, then decreased from 14 days to senescence. These results accorded well with the results obtained from the ^{3}H-thymidine radioautography (Li 1994, Li and Nagata 1995). Moreover, the mucosubstance synthesis incorporating sulfuric acid was also carried out (Oliveira et al. 1991, Li et al. 1995).

3.2.1.4 The DNA synthesis in gametogenesis

The gametogenesis consists of both spermatogenesis in male germ cells and the oogenesis in female germ cells, leading to the implantation and further development of blastcysts. The macromolecular synthesis, DNA, RNA and protein synthesis, in both the testis and the ovary were already described in the sections of male and female reproductive systems (3.7.1. and 3.7.2.) previously.

3.2.1.5 The DNA synthesis in implantaion

In order to detect the changes of DNA, RNA and protein synthesis of the developing blastcysts in mouse endometrium during activation of the implantation, ovulations of female BALB/C strain mice were controlled by pregnant mare serum gonadotropin and human chorionic gonadotropin, then pregnant female mice were ovariectomized on the 4th day of pregnancy (Yamada 1993, Yamada and Nagata 1992a,b, 1993). The delay implantation state was maintained for 48 hrs and after 0 to 18 hrs of estrogen supply ^{3}H-thymidine was injected. The three regions of the endometrium, i. e. the interinplantation site, the antimesometrial and mesometrial sides of implantation site, were taken out and processed for LM and EM RAG. It was well known that the uterus of the rodent becomes receptive to blastcyst implantation only for a restricted period. This is called the implantation window which is intercalated between refractory states of the endometrium whose cycling is regulated by ovarian hormones (Yoshinaga 1988). We studied the changes of DNA synthesis by ^{3}H-thymidine (Yamada and Nagata 1992a,b) incorporations in the endometrial cells of pregnant-ovariectomized mice after time-lapse effect of nidatory estradiol. As the results, the endometrial cells showed topographical and chronological differences in the nucleic acid synthesis. The cells labeled with ^{3}H-thymidine increased after nidatory estradiol effects in the stromal cells around the blastocyst, but not in the epithelial cells. The results suggested that the presence of the blastocysts in the uterine lumen induced selective changes in the behavior of endometrial cells after nidatory estradiol effect showing the changes of DNA synthesis.

As for a lower vertebrate, cell proliferation and migration of scleroblasts and their precursor cells during ethisterone-induced anal-fin process formation of the medaka, orizias latipes, was studied by LM RAG labeled with ^{3}H-thymidine (Uwa and Nagata 1976). The results

showed that the labeling index in the posterior margin of the joint plate rapidly increased and the scleroblast population in the central portion increased simultaneously from the 3rd to 5th day of ethisterone treatment. These results indicated that the scleroblasts and their precursor cells migrated from the peripheral portion to the central portion along the proxi-distal axis of the joint plate.

3.2.2 The RNA synthesis in the female genital organs

We studied the RNA synthesis of female reproductive organs of aging mice after the administration of ^{3}H-uridine at various ages.

3.2.2.1 The RNA synthesis in the ovary

The ovary consists of the germinal epithelium covering the surface and the stroma containing many developing ovarian follicles depending upon the age of animals.

The nucleic acids, DNA and RNA, syntheses in the developing virgin mice ovaries of 6 litters, each 3 individuals, consisting of 36 female mice at various ages in 2 groups were studied by ^{3}H-thymidine and ^{3}H-uridine radioautography (Li 1994, Li and Nagata 1995, Li et al. 1992). The ^{3}H-thymidine incorporations were active in all surface epithelial cells, stromal and follicular cells of the ovary between postnatal days 1 to 7 and decreased from day 14 (Fig. 16G) and maintained a lower level to day 60, while ^{3}H-uridine incorporations were active in all surface epithelial cells, stromal and follicular cells of the ovary between postnatal days 1 to 7 and maintained medium levels from day 14 on.

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3.2.2.3 The RNA synthesis in the uterus

The silver grains with ^{3}H-uridine showing RNA synthesis of the uterus was observed over almost all the nuclei and cytoplasm of all the cells in the epithelia, stroma and smooth muscles from postnatal day 1 to 60 (Li 1994, Li and Nagata 1995). The labeling indices with

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3.2.3 The protein synthesis of the female genital organs

We studied the protein synthesis of female reproductive organs of aging mice after the administration of ^{3}H-leucine at various ages.

3.2.3.1 The protein synthesis in the uterus

We studied the protein synthesis of the developing blastocysts in female mouse endometrium during activation of the implantation. The ovulations of female BALB/C strain adult mice were controlled by pregnant mare serum gonadotropin and human chorionic gonadotropin, then pregnant female mice were ovariectomized on the 4th day of pregnancy (Yamada 1993, Yamada and Nagata 1992a,b, 1993). The delay implantation state was maintained for 48 hrs and after 0 to 18 hrs of estrogen supply. After the mice were injected with ^{3}H-leucine, they were sacrificed and the uteri were processed for LM and EMRAG. We studied the changes of protein synthesis by ^{3}H-leucine incorporations (Yamada 1993, Yamada and Nagata 1992a). As the results, the endometrial cells showed topographical and chronological differences in the protein synthesis. The cells labeled with ^{3}H-leucine were observed in both epithelial cells and stromal cells. Quantitative analysis revealed that the number of silver grains increased from 0 hr to 3 and 6 hr, reaching the peak at 6 hr and decreased from 12 to 18 hr. The protein synthesis in the decidual cells of pregnant mice uteri was compared to the endometrial cells of virgin mice uteri using ^{3}H-proline and ^{3}H-tryptophane incorporations. The results demonstrated that silver grains were localized over the endoplasmic reticulum and the Golgi apparatus of fibroblasts and accumulated over collagen fibrils in the extracellular matrix suggesting that the decidual cells produced collagen in the matrix. The collagen synthesis in the mouse decidual cells by ^{3}H-proline showed that silver grains were localized over the endoplasmic reticulum and Golgi apparatus of fibroblasts and accumulated over collagen fibrils in the extracellular matrix (Oliveira et al. 1991, 1995). However, analytical studies on protein synthesis in aging mice at various ages were not yet carried out.

3.2.3.2 The protein synthesis in the implantation

In order to detect the changes of DNA, RNA and protein synthesis of the developing blastocysts in female mouse endometrium during activation of the implantation, ovulations

of female BALB/C strain mice were controlled by pregnant mare serum gonadotropin and human chorionic gonadotropin, then pregnant female mice were ovariectomized on the 4th day of pregnancy (Yamada 1993, Yamada and Nagata 1992a,b, 1993). The delay implantation state was maintained for 48 hrs and after 0 to 18 hrs of estrogen supply and ^{3}H-leucine was injected. The three regions of the endometrium, i. e. the interinplantation site, the antimesometrial and mesometrial sides of implantation site, were taken out and processed for LM and EM RAG. It was well known that the uterus of the rodent becomes receptive to blastocyst implantation only for a restricted period. This is called the implantation window which is intercalated between refractory states of the endometrium whose cycling is regulated by ovarian hormones (Yoshinaga 1988). We studied the changes of protein synthesis by ^{3}H-leucine (Yamada 1993, Yamada and Nagata 1992a) incorporations in the endometrial cells of pregnant-ovariectomized mice after time-lapse effect of nidatory estradiol. As the results, the endometrial cells showed topographical and chronological differences in the nucleic acid and protein synthesis. The cells labeled with ^{3}H-leucine were observed in both epithelial cells and stromal cells. Quantitative analysis revealed that the number of silver grains as expressed by grain counting per mm^{2} in both the stromal and epithelial cells on the antimesometrial side with ^{3}H-leucine increased from 0 hr to 3 and 6 hr, reaching the peak at 6 hr and decreased from 12 to 18 hr. These results suggested that the presence of the blastocysts in the uterine lumen induced selective changes in the behavior of endometrial cells after nidatory estradiol effect showing the changes of DNA, RNA and protein synthesis. The time coincident peak of RNA and protein synthesis detected in the endometrial cells at the anti-mesometrial side of the implantation site, probably reflected the activation moment of the implantation window. The protein synthesis in the decidual cells of pregnant mice uteri was compared to the endometrial cells of virgin mice uteri using ^{3}H-proline and ^{3}H-tryptophane incorporations. The results demonstrated that silver grains were localized over the endoplasmic reticulum and the Golgi apparatus of fibroblasts and accumulated over collagen fibrils in the extracellular matrix suggesting that the decidual cells produced collagen in the matrix. On the other hand, collagen synthesis in the mouse decidual cells was studied by LM and EM RAG using ^{3}H-proline (Oliveira et al. 1991, 1995). Silver grains were localized over the endoplasmic reticulum and Golgi apparatus of fibroblasts and accumulated over collagen fibrils in the extracellular matrix. The results suggested that the decidual cells produced collagen into the matrix. The quantitative analysis showed that both incorporations in the decidual cells and the matrix increased in the pregnant mice than the endometrial cells in virgin mice.

3.2.4 The glucide synthesis in the reproductive system

Among the reproductive organs, only the mucosubstance synthesis with radiosulfate, $^{35}SO_4$, was studied in the ovaries of mice during the estrus cycle.

3.2.4.1 The glucide synthesis in the ovary

Litter mate groups of female ddY mice, aged 8-10 weeks, were divided into 4 groups, diestrus, proestrus, estrus and metestrus according to the vaginal smears. The ovaries were taken out, labeled with $^{35}SO_4$ in vitro and radioautographed. In all the animals, silver grains were localized over the granulosa and theca cells. Almost all compartments of the ovaries were labeled. The grain counts per cell changed according to cell cycle. From the results, it was concluded that all the cells of the ovary incorporated mucosubstances throughout the estrus cycle (Li et al. 1992).

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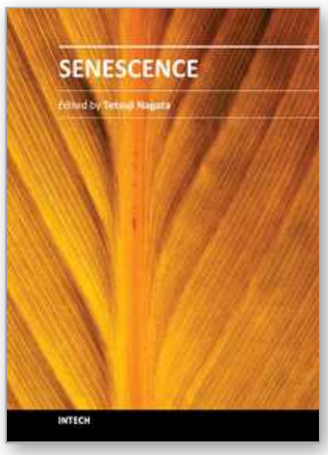
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Senescence
Edited by Dr. Tetsuji Nagata

ISBN 978-953-51-0144-4
Hard cover, 850 pages
Publisher InTech
Published online 29, February, 2012
Published in print edition February, 2012

The book "Senescence" is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Tetsuji Nagata (2012). Macromolecular Synthesis in the Urinary and Reproductive Systems, Senescence, Dr. Tetsuji Nagata (Ed.), ISBN: 978-953-51-0144-4, InTech, Available from: http://www.intechopen.com/books/senescence/macromolecular-synthesis-in-the-urinary-and-reproductive-systems

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