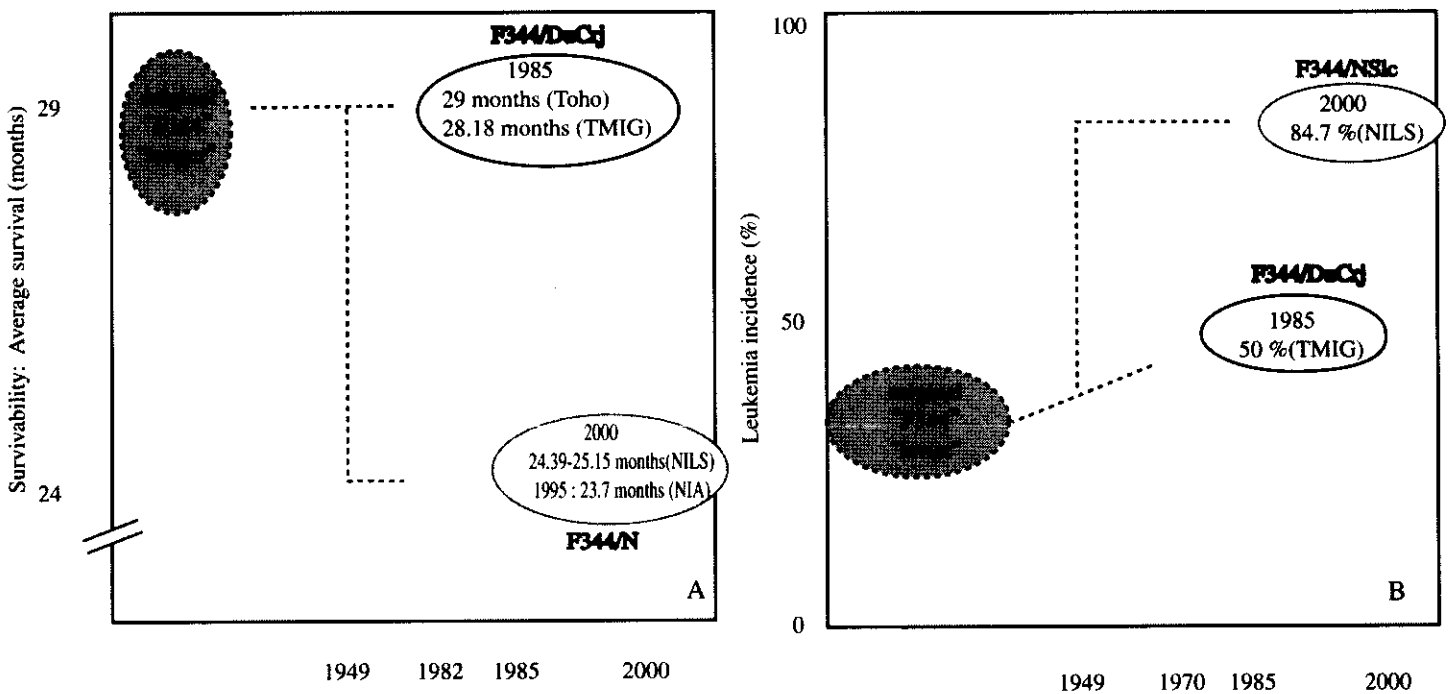


Fig. 7. Characteristics of original "F344" deduced from recent reports

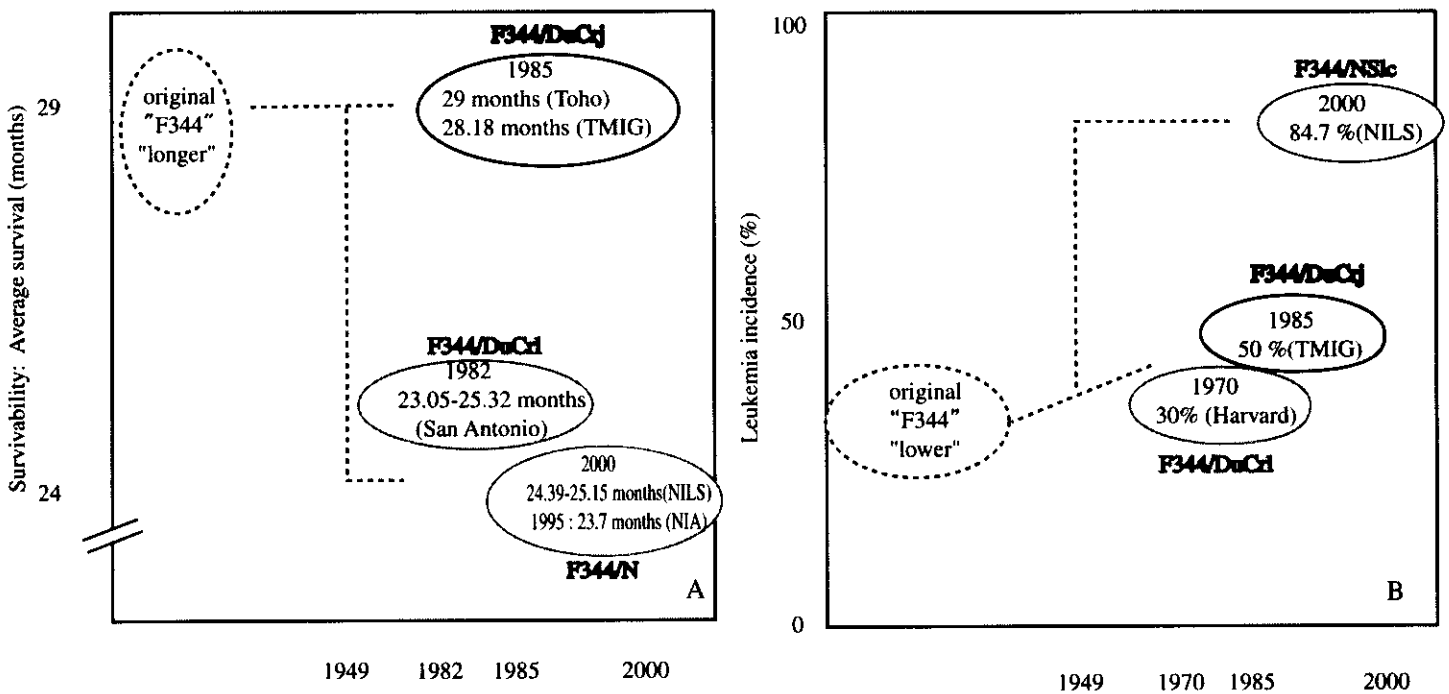


Fig. 8. Relation among "F344" substrains deduced from survival and leukemia incidence

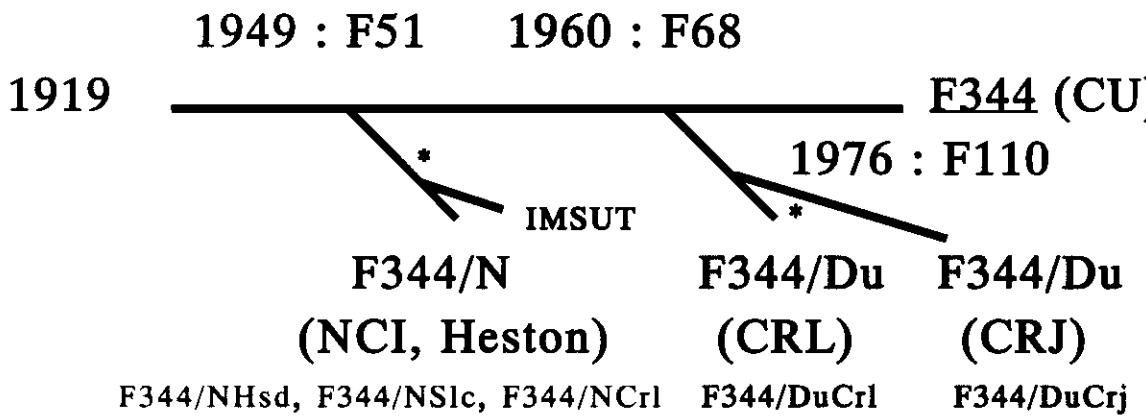


**Fig. 9. Pedigree of "F344" rats deduced from survival rate**

# Ⅱ．　研究成果の刊行に関する一覧表

別紙５

研究成果の刊行に関する一覧表

書籍

| 著者氏名 | 論文タイトル名 | 書籍全体の編集者名 | 書籍名 | 出版社名 | 出版地 | 出版年 | ページ |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |

雑誌

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| S. Tanaka, N. Tamaya, K. Matsuzawa and O. Miyaishi | Differences in survivability among F344 rats. | Exp. Anim. | 49 | 141-145 | 2000 |
| O. Miyaishi, S. Tanaka, R. Kanawa, K. Matsuzawa and K. Isobe | Anisocytosis precedes onset of the large granular lymphocyte leukemia in aged F344/N rats. | Arch. Geront. Geriat. | 30 | 161-172 | 2000 |
| S. Tanaka, T. Segawa, N. Tamaya and T. Ohno | Establishment of an aging farm of F344/N rats and C57BL/6 mice at National Institute for Longevity Sciences (NILS). | Arch. Geront. Geriat. | 30 | 215-223 | 2000 |
| O. Miyaishi, K. Matsuzawa, R. Kanawa, K. Isobe and S. Tanaka | The diagnostic significance of left auricular thrombus in F344/N rats | Arch. Geront. Geriat. | 31 | 107-113 | 2000 |

| | | | | | |
|---|---|---|---|---|---|
| I. Zs.-Nagy, S. Tanaka and K. Kitani | Comparison the lateral diffusion coefficient of hepatocyte plasma membrane proteins in 3 strains of senescence accererated mouse (SAM). | Arch. Geront. Geriat. | in press | | 2001 |
| S. Tanaka, T. Segawa, N. Tamaya, O. Miyaishi and T. Ohno | A group of five parameters as a new biological marker on F344/N rats. | Arch. Geront. Geriat. | in press | | 2001 |
| A. Shito, T. Ohno, N. Tamaya, O. Miyaishi, M. Nishimura and S. Tanaka | Difference in average survival between F344/Du and F344/N rats is not due to genetic contamination. | Arch. Geront. Geriat. | submitted | | |

# Ⅲ. 研究成果の刊行物・別刷

*Exp. Anim. 49(2), 141–145, 2000*

—Note—

# *Differences in Survivability among F344 Rats*

Shin TANAKA[1)], Norika TAMAYA[1)], Kaori MATSUZAWA[2)], and Osamu MIYAISHI[2)]

*[1)]Laboratory Animal Research Facilities (LARF) and [2)]Laboratory of Pathology, Department of Basic Gerontology of National Institute for Longevity Sciences (NILS), 36–3, Gengo Morioka-cho, Obu 474-8522, Japan*

***Abstract:*** *Important parameters to identify and develop appropriate animal models for longevity science include survivability, age-related disorders, and easy handling of aged individuals. It is found that F334/Du and F344/N have distinctive strain difference in these parameters. The finding suggests F334/Du and F344/N, even though they are historically siblings, need clearly separate identification when used as animal models for aging science, in particular, longevity science.*

***Key words:*** *aging model, F344/N and F344/Du, strain difference, survivability*

Laboratory strains of small rodents, mice and rats have been established as animal models for biomedical science with detailed controls over factors related to genetics, microbiology and nutrition but not necessarily specific time that has lapsed. In aging science, it is aged/aging laboratory rodents that are essential for understanding the mechanisms of aging at the individual level.

When aged/aging laboratory rodents were not commercially available, some groups of researchers on aging in Japan and US, including the National Institute on Aging (NIA), Maryland, tried to establish aging farms (A/F) to produce colonies of rodents for their research. NIA developed A/Fs under both private and contractual arrangement with funding by the National Institute of Health (NIH), Maryland (See the latest NIA catalogue for animal resources).

Longevity science pursues quality of life for human beings. It mainly consists of basic and social gerontology and geriatrics. Basic gerontology requires animal models that are aged in good health without substantial disorders, while geriatrics needs those that significantly develop age-related disorders. It is therefore important to identify and develop effective parameters for animal models to meet specific needs in basic gerontology and geriatrics. The parameters should also be defined sensitively by incorporating time and individuals.

In aging science a uniquely significant parameter, survivability (SvA), has been developed for laboratory rodents. SvA is the accumulated life span of individual rodents. If a factor that may modify SvA can be pathologically identified as an disorder, it will help to improve the quality of laboratory rodents as animal models, thus contributing to geriatric research. It also means that after such a disorder modifies SvA, the handling or maintenance of animals will become difficult, since SvA, disorders and ease of handling are closely related to one another, and are easy to make estimations exter-

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*Address corresponding: S. Tanaka, Laboratory Animal Research Facilities (LARF) of National Institute for Longevity Sciences (NILS), 36–3, Gengo Morioka-cho, Obu 474-8522, Japan*

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# Anisocytosis precedes onset of the large granular lymphocyte leukemia in aged F344/N rats

Osamu Miyaishi [a,*], Shin Tanaka [b], Rikako Kanawa [a], Kaori Matsuzawa [a,b], Ken-ichi Isobe [a]

[a] *Department of Basic Gerontology, National Institute for Longevity Sciences, (NILS), 36-3 Gengo, Morioka, Obu 474-8522, Japan*

[b] *Laboratory Animal Research Facilities, National Institute for Longevity Sciences, (NILS), 36-3 Gengo, Morioka, Obu 474-8522, Japan*

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**Abstract**

In investigations using experimental animals, the unexpected affection of certain diseases often cause great impairment to them especially when using aged animals. In F344 rats, large granular lymphocyte leukemia is the most frequent fatal disease which increases along their aging. The timely detection of rats at risk for leukemia is very important in order to exclude such animals and thus obtain precise results in many fields of investigation. In the process of assessing the main cause of death in F344/N rats of the aging farm of our institute, NILS, we found cases with no obvious contributory disease to death that showed anisocytosis in a peripheral blood smear. In such cases, histological examination of spleen revealed consistent features of leukemia and findings of the liver and kidney were considered due to this hematologic disorder. Anisocytosis was frequently seen in the advances stage of leukemia. Thus we concluded that anisocytosis is a prior condition of leukemia and its detection in a peripheral blood smear is predictive of the disease when using aged animals. 

*Keywords:* F344/N rat; Leukemia; Peripheral blood smear; Anisocytosis

* Corresponding author: Tel.: +81-562-445651, ext. 754; fax: +81-562-446591.
*E-mail address:* miyaishi@nils.go.jp (O. Miyaishi)

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# Establishment of an Aging Farm of F344/N Rats and C57BL/6 Mice at the National Institute for Longevity Sciences (NILS)

Shin Tanaka *, Takeshi Segawa, Norika Tamaya, Tamio Ohno [1]

*Laboratory Animal Research Facilities (LARF), National Institute for Longevity Sciences (NILS), 36-3, Gengo Morioka-cho, Obu, Japan474-8522*

Received 14 December 1999; received in revised form 22 February 2000; accepted 1 March 2000

**Abstract**

In 1996 the National Institute for Longevity Sciences (NILS) started an aging/aged farm (Aging Farm), an animal farm for producing aging/aged laboratory rodents on inbred strains, F344/N rats and C57BL/6 mice at its Experimental Animal Facility Wing, based on plans prepared by the Laboratory Animal Research Facilities (LARF). The NILS Aging Farm, being well established, began internal supply of aging/aged laboratory rodents in 1999 to promote both aging and longevity science. This report describes development of the NILS Aging Farm under NILS Aging Farm Guide and the effectiveness of the Guide. 

*Keywords:* Aging farm; Aging farm guide; Survivability; F344/N rats; C57BL/6 mice

## 1. Introduction

NILS was founded to promote aging and longevity science on July 1, 1995. NILS decided to establish an aging/aged farm (Aging Farm), an animal farm for

* Corresponding author. Present address: Institute for Laboratory Animal Research, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Japan 466-8550. Tel.: +81-562-46-2311, ext. 874; fax: +81-562-44-6597.

[1] Present address: Institute for Laboratory Animal Research, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Japan 466-8550.

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# The diagnostic significance of left auricular thrombus in F344/N rats

Osamu Miyaishi [a,*], Kaori Matsuzawa [a,b], Rikako Kanawa [a], Ken-ichi Isobe [a], Shin Tanaka [b]

[a] *Department of Basic Gerontology, National Institute for Longevity Sciences (NILS), 36-3 Gengo, Morioka, Obu 474-8522, Japan*

[b] *Laboratory for Animal Research Facilities, National Institute for Longevity Sciences (NILS), 36-3 Gengo, Morioka, Obu 474-8522, Japan*

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**Abstract**

In the process of assessing the main cause of death in F344/N rats from the aging farm of our institute, we have often found left auricular thrombus in autopsy cases of moribund animals. In 319 autopsy cases, 45 were of left auricular thrombus and 44 were accompanied by hematopoietic neoplasms, including overt leukemia and a pre-leukemic condition of leukemia. In cases without splenomegaly, this lesion was found in 13 of 21 animals (61.9%) whereas, in cases with splenomegaly, 31 of 239 were positive for this lesion (13.0%). Thus, left auricular thrombus may be an important macroscopic diagnostic criteria of hematopoietic neoplasms, especially when splenomegaly is absent. Furthermore, this lesion tended to arise in aged animals despite the presence of splenomegaly. These results would therefore greatly contribute to aging science by confirming the health condition of experimental rats and the accuracy of subsequent results. 

*Keywords:* Left auricular thrombus; Leukemia in old F344/N rats; Splenomegaly

* Corresponding author. Tel.: +81-562-44-5651, ext. 754; fax: +81-562-44-6591.
*E-mail address:* miyaishi@nils.go.jp (O. Miyaishi).

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# COMPARISON OF THE LATERAL DIFFUSION COEFFICIENT OF HEPATOCYTE PLASMA MEMBRANE PROTEINS IN 3 STRAINS OF SENSESCENCE ACCELERATED MOUSE (SAM)

Imre Zs.-Nagy[§], Shin Tanaka and Kenichi Kitani

National Institute for Longevity Sciences (NILS)
Obu-shi, Aichi 474-8522, Japan

[§]Permanent address: Department of Gerontology (VILEG Hungarian Section), University of Debrecen, Medical and Health Science Center, P.O.B. 50, Debrecen, H-4012, Hungary

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# A group of five parameters as a new biological marker on F344/N rats

Shin Tanaka [a,*], Takeshi Segawa [a], Norika Tamaya [a], Osamu Miyaishi [b], Tamio Ohno [a,1]

[a] *Laboratory Animal Research Facilities (LARF), ~~Department of Basic Gerontology of~~ National Institute for Longevity Sciences (NILS), 36-3, Gengo Morioka-cho, Obu 474-8522, Japan*

[b] *Laboratory of Pathology, Department of Basic Gerontology of National Institute for Longevity Sciences (NILS), 36-3, Gengo Morioka-cho, Obu 474-8522, Japan*

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**Abstract**

The National Institute for Longevity Sciences (NILS) established an aging farm (A/F) for producing aging/aged laboratory rodents at the Experimental Animal Facility Wing under the NILS A/F Guide planned by the Laboratory Animal Research Facilities (LARF). Five parameters, the average life span, the number of days of 75, 50, and 25% survival points, and average of the top 10 longest life span among laboratory strains of rodents at NILS-A/F, were reproducible for F344/N rats specifically by strain and sex under the LARF A/F guide. These five parameters may serve as an effective and practical biological marker, especially in aging science including longevity science, to evaluate characteristics of strains of laboratory rodents. The five parameters can identify clear substrain differences between F344/N and F344/Du and breeder differences between F344/DuCrj and F344/DuCrl. 

*Keywords:* Biological marker; F344/Du; F344/N; Survivability

* Corresponding author. Tel.: +81-562-462311, ext. 874; fax: +81-562-446597.

[1] Present address: Institute for Laboratory Animal Research, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.

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# Ⅳ. 添　付　資　料

肺の加齢変化に関わる解析は、“F344/N ラットの肺における加齢変化の形態学的研究”たる課題名で平成 12 年度の岐阜大学農学部獣医学科　田中　暁子の卒業論文として公表されたので資料として添付する

# Ⅰ.緒言

一般的に加齢動物の呼吸機能は低下するといわれている。すなわちヒト（Turner et al., 1968)、イヌ（Robinson et al., 1973)、ラット（Pinkerton et al., 1982: Sahebjami et al., 1979）など多くの哺乳動物で、加齢に伴う肺残気量の増加、一回換気量の低下、肺の伸展性を示す肺コンプライアンスの増加が起こることが報告されている。さらにヒトでは、胸壁の硬化や呼吸筋の筋力低下がおこるという報告もある（Pack et al., 1988)。このような加齢に伴って起こる呼吸筋力の低下やさらには肺組織自体の変化が、肺残気量の増加や換気量の低下の原因になっていると考えられている(Edward et al., 1998)。

加齢に伴って起こるこのような肺機能の変化が、肺のどのような組織学的変化に基づいているのかを検索するために、これまで様々な研究が行われてきた。特に肺の線維成分の主体である弾性線維と膠原線維の組織学的変化については多くの研究が行われている。D'Errico et al（1989）は、弾性線維および膠原線維に対する免疫組織化学によって、加齢個体では肺の弾性線維が減少するとともに膠原線維が増加することを示し、これらの変化が肺の弾性収縮力の低下をもたらすとしている。一方では、弾性線維、膠原線維の量はともに変化しない、あるいは逆に弾性線維は増加するが膠原線維は減少するという報告もあり（Pinkerton et al., 1982; Juan,

1997)、線維成分の加齢変化に関する報告は一定していない。

また、気管支や細気管支も呼吸機能に関わる重要な要素である。気管支の加齢による変化については Gabella(1991)がモルモットの気管平滑筋を電顕的に観察して加齢個体で平滑筋細胞の細胞膜に深い陥入が生じて輪郭が非常に不整になり、平滑筋細胞間に多くの結合組織が入る込むようになると報告している。しかし肺機能の加齢変化との関連性は明らかにされていない。

そこで今回、加齢に伴って機能低下を起こした肺の、形態学的変化を明らかにすることを目的とし、9ヶ月齢から 36ヶ月齢の F344/N ラットの肺を組織学的、免疫組織学的に検索した。

# Ⅱ.材料と方法

## 材料

F344/Nラット、計68匹を用いた。用いたラットはNational Institute of Aging (U.S.A.)または長寿医療研究センター（大府）内で飼育されたSPF動物であり、月齢、性別、例数、体重はTable. 1にまとめた。また本研究に用いたF344/Nラットは、9～12ヶ月齢、21～24ヶ月齢、27ヶ月齢～31ヶ月齢の3群に分けた場合、加齢に伴って一回換気量の減少、静肺コンプライアンスの増加、残気量の増加を示唆する動肺コンプライアンスの変化が起こるという研究結果が得られており、加齢に伴って肺機能が低下することがわかっていた（金丸ら,2000)。

## 方法

ラットをペントバルビタールナトリウム（50mg/kg, i.p）で麻酔した。４％パラホルムアルデヒドで灌流固定した後、肺を採取した。一部の個体では灌流固定をせずに、気管に挿入したカニューレからブアン液を注入し、肺を胸腔壁に密着するまで膨らませて固定した。固定した左右の肺は、肺の上部から6部位に等分し、上部から2番目と5番目の組織をパラフィン包埋した。これらの材料を4μmの切片として以下の染色を行い、光学顕微鏡的に観察するとともに肺胞断面積の形態計測を行った。

## 染色

### ① Hematoxylin-Eosin(H.E.)染色

### ② Resorsin-Fuchsin 染色

### ③ Avidin-biotin-peroxdase complex (ABC) 法による免疫組織化学

使用した抗体は、α-smooth muscle actin (1 : 1,000)、γ-smooth muscle actin (1 : 1,000)、desmin (1 : 500)、vimentin(1: 1,000)collagen type Ⅰ(1 : 100)、collagen type Ⅲ(1:100)、collagen type Ⅳ(1:10)(詳細は Table.1 にまとめた)に対するもので ABC 法の手順と反応時間を以下に示した。

1) 脱パラフィン

2) collagen typeⅠ,Ⅲ,Ⅳに対する特異抗体を用いる切片に限っては、0.1%ペプシンを含む 0.5M 酢酸により 37℃で 2 時間処理した後、0.01%リン酸緩衝生理食塩水(PBS)で洗浄した。

3) 0.3%過酸化水素水を含むメタノールに室温で 15 分間浸漬した。その後 PBS で洗浄した。

4) α、γ-smooth muscle actin および vimentin の場合はブロッキング抗体として正常ウマ血清 (1 : 50) により、desmin、collagen type Ⅰ、collagen type Ⅲおよび collagen type Ⅳの場合は正常ヤギ血清 (1 : 50) により室温で 30 分間反応させた。

5) 特異抗体により 4℃で一晩反応させた。その後 PBS で洗

浄した。

6） 一次抗体の免疫動物がマウスの抗体を用いた切片はビオチン化抗マウス IgG ウマ血清（1：200）を用い、免疫動物がウサギのものにはビオチン化抗ウサギ IgG ヤギ血清（1：200）を用いて室温で 30 分間反応させた。その後 PBS で洗浄した。

7） Vectastein Elete ABC Kit（Vector Laboratories）に室温で 30 分浸漬。その後 PBS で洗浄した。

8） 0.3％過酸化水素水を加えた 3,3'-diaminobenzidine tetrahydrochloride（DAB）で室温にて 7 分間発色後、水洗した。

9） Hematoxylin で後染色後、脱水、透徹、封入した。

## 形態計測

ブアン液で固定し、H.E.染色を施した切片を用いて肺胞断面積の計測をおこなった。このとき、条件を一定にするためにラットの肺の上部から 5 番目のブロックから薄切した切片に統一して選択した。手順は以下に示した。

1） 各個体につき無作為に選んだ 10 視野の画像を 100 倍の拡大率で画像解析ソフトの Photoshop によってパーソナルコンピューターに取り込んだ（Fig. 1a）。

2） 取り込んだ画像の肺胞表面を Photoshop のフィルタを用いてトレースした（Fig. 1b）。

3） 肺胞のみの内腔の断面積をNIHimageによって測定した（Fig. 1c）。

4） 全ての肺胞断面積の平均値を取り散布図に表した。

また、肺胞の断面積と月齢の相関については、Spearmanの順位相関係数を用いて検定を行った。