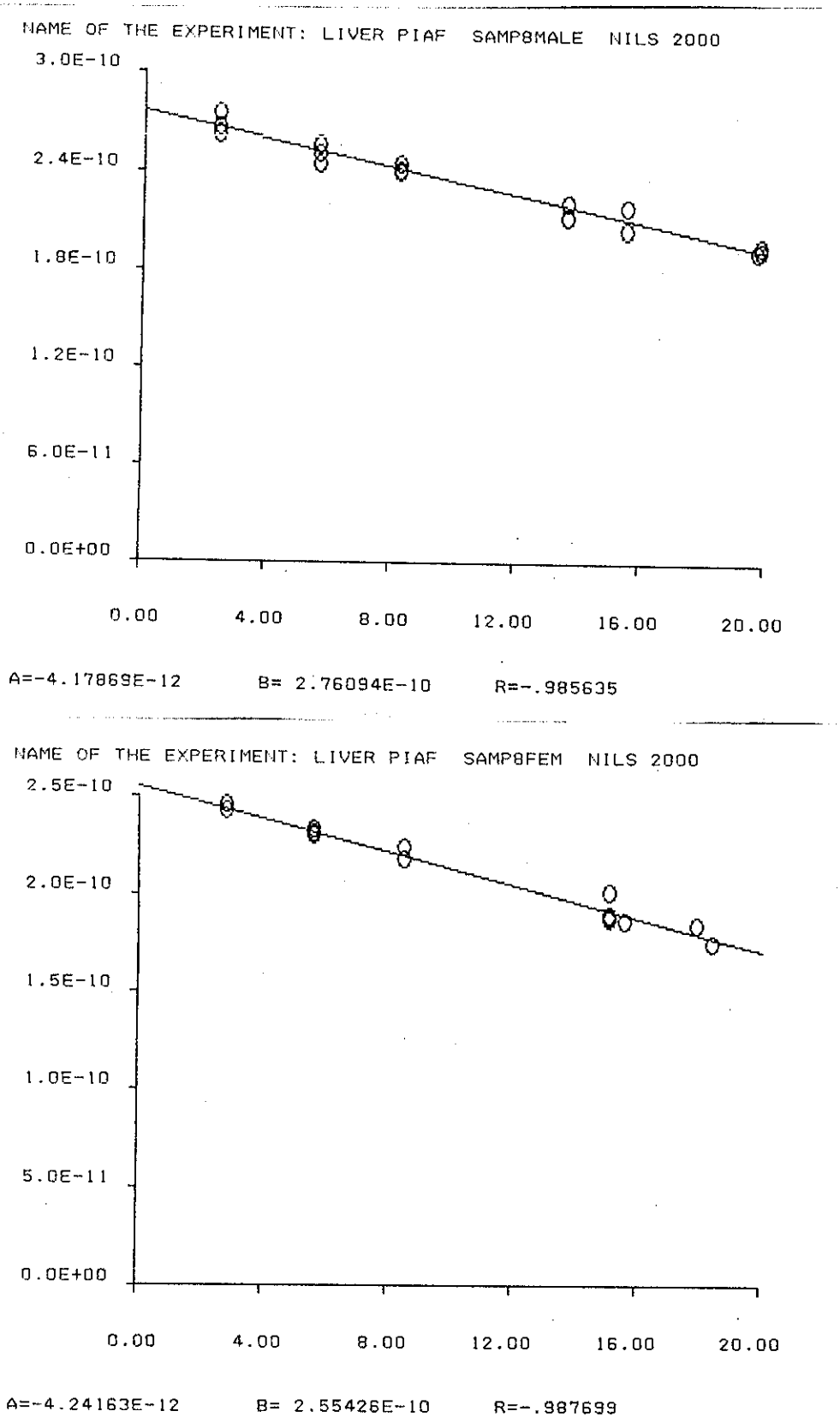
Table 6
INDIVIDUAL AVERAGES (± S.D.) OF D, FR% and DxFR MEASURED IN THE LIVER OF FEMALE SAMP8/Ta MICE BY PIAF

Gen. number	Age (months)	D (x 1E-10) (cm^2/sec)	Number of cells/omitted	FR %	FRxD (x 1E-10) (cm^2/sec)
§ F96	2.8	2.4256 ± 0.5888	40/0	103.2 ± 17.93	2.4685 ± 0.5850
§ F96	2.8	2.4597 ± 0.6409	37/3	97.98 ± 15.03	2.4062 ± 0.6864
F107	5.6	2.3345 ± 0.6130	33/1	97.28 ± 14.59	2.2703 ± 0.7048
F107	5.6	2.2994 ± 0.4857	33/3	99.05 ± 13.77	2.2763 ± 0.5671
F107	5.6	2.3128 ± 0.6775	33/1	94.82 ± 17.38	2.1785 ± 0.6891
F107	5.6	2.3032 ± 0.6930	33/0	102.1 ± 13.93	2.3223 ± 0.6880
§ F95	8.5	2.1727 ± 0.5439	38/0	102.1 ± 13.60	2.2033 ± 0.5942
§ F95	8.5	2.1770 ± 0.6354	33/3	112.3 ± 11.76	2.4153 ± 0.6347
§ F95	8.5	2.2364 ± 0.4577	37/3	100.4 ± 13.53	2.2235 ± 0.4551
F104	15.1	1.8813 ± 0.4855	33/3	113.8 ± 14.13	2.0948 ± 0.4217
F104	15.1	2.0082 ± 0.4587	31/1	100.7 ± 13.69	2.0099 ± 0.4918
F104	15.1	1.8701 ± 0.4472	33/3	103.3 ± 18.10	1.9054 ± 0.4829
F104	15.1	1.8918 ± 0.3937	33/2	103.1 ± 15.05	1.9396 ± 0.4281
F104	15.6	1.8683 ± 0.4494	33/3	95.26 ± 15.95	1.7830 ± 0.5393
F103	17.9	1.8476 ± 0.4920	33/2	86.47 ± 13.92	1.5804 ± 0.4330
F103	18.4	1.7470 ± 0.4971	33/2	108.8 ± 15.41	1.8803 ± 0.4821

Note: § indicates mice analyzed in 1997.



Figures 7-8. The age-correlation of D values in male (upper part) and female (lower part) of SAMP8 mice. A= the slope, B= the intercept point, R= the correlation coefficient of the linear regression analysis. Detailed numerical data can be found in Tables 5-6 and 7, respectively.

Table 7
THE PARAMETERS OF THE LINEAR REGRESSION EQUATIONS SHOWN IN Figures 3-8 FOR VARIOUS TYPES OF MALE AND FEMALE SAM MICE
(data regarding C57BL/6 mice are also reported for comparison)

Type of mice	B Mean	B ± S.D.	A Mean	A ± S.E.M.	t for 0-hypoth.	Signif. p <
MALES:						
C57BL/6	2.813E-10	6.390E-12	-1.652E-12	1.336E-13	12.366	0.001
SAMR1/Ta	2.843E-10	6.032E-12	-3.312E-12	1.307E-13	25.342	0.001
SAMP6/Ta	2.720E-10	4.836E-12	-4.687E-12	1.491E-13	31.445	0.001
SAMP8/Ta	2.761E-10	5.137E-12	-4.179E-12	1.849E-13	22.603	0.001
FEMALES:						
C57BL/6	2.669E-10	7.097E-12	-2.000E-12	1.639E-13	12.209	0.001
SAMR1/Ta	2.793E-10	8.863E-12	-4.474E-12	2.280E-13	19.625	0.001
SAMP6/Ta	2.519E-10	7.366E-12	-5.036E-12	2.540E-13	19.828	0.001
SAMP8/Ta	2.554E-10	4.359E-12	-4.242E-12	1.795E-13	23.636	0.001

Statistical comparisons of the intercepts

	C57/R1	C57/P6	C57/P8	R1/P6	R1/P8	P6/P8
MALES	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
FEMALES	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
and the slopes (p <)						
MALES	0.001	0.001	0.001	0.001	0.001	0.05
FEMALES	0.001	0.001	0.001	N.S.	N.S.	0.01

Notes: General form of the equation: $y = B + Ax$, where x is the age in months, B is the intercept and A is the slope of the regression line. N.S.= not significant. For other explanations, see the text.

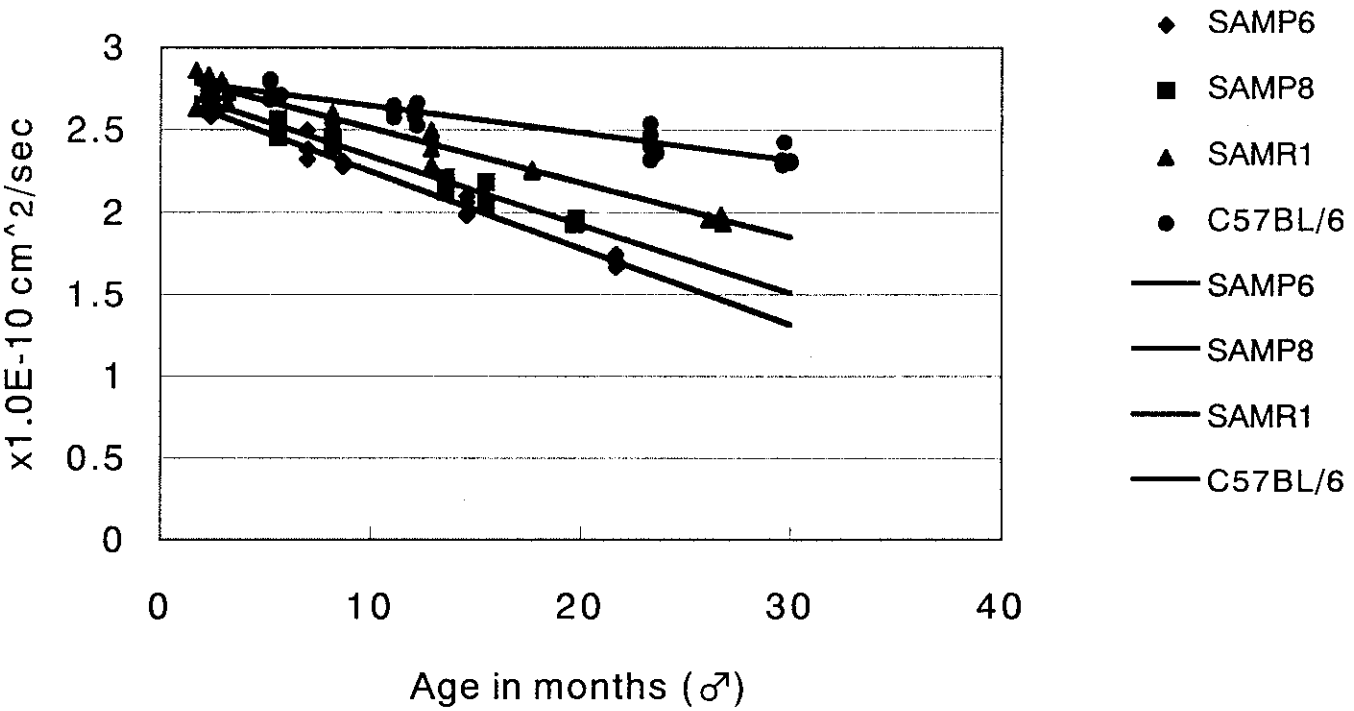


Figure 9. The comparison of the age-correlations of D values in male SAM mice, shown individually in Figs. 3, 5 and 7, with each other and that of the normal C57BL/6 mice, in the hepatocyte plasma membrane. The data regarding the C57BL/6 mice have been published earlier (Zs.-Nagy et al., 1989). Details of the regression equations and the respective significance values are given in Table 7.

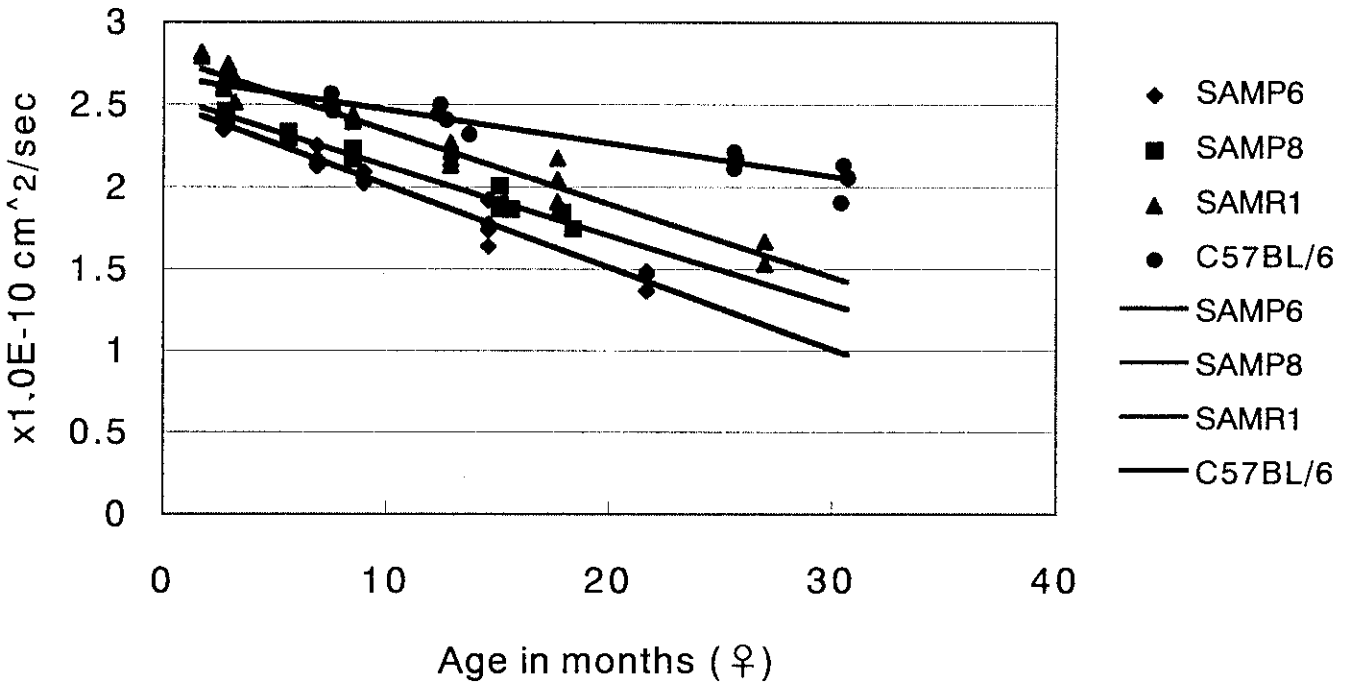


Figure 10. The comparison of the age-correlations of D values in female SAM mice, shown individually in Figs. 4, 6 and 8, with each other and that of the normal C57BL/6 mice, in the hepatocyte plasma membrane. The data regarding the C57BL/6 mice have been published earlier (Zs.-Nagy et al., 1989). Details of the regression equations and the respective significance values are given in Table 7.

1. Test-tube enzyme biochemistry generally suffers from the problem cuased by the impossibility of measuring enzyme quantities in the tissues. Therefore, we mostly refer to some enzyme effects (called activities), and express them on a "per mg of total protein" basis. One can obtain quantitative data in this way, but those data have very little to do with the intracellular reality of the enzyme functions, as explained below.

2. The MHA speaks about the general age-dependent decline of the intracellularly existing enzyme activities, due to the ever increasing intracellular physical density, accompanied by the considerable loss of intracellular water content. All this is the result of the altered cell membrane functions. However, true intracellularly existing enzyme efficiency (or activities) have never been and cannot be measured by test-tube biochemistry. Nevertheless, the existing enzyme kinetic models predict, and a great number of experimental evidence show that the enzymes slow down their performance, if the viscogen content of their environment increases. The changes in the physicochemical properties of the cell membrane during aging, revealed by the FRAP technique, are in agreement with the assumption that such changes may cause the increase of the intracellular density, leading to the functional losses with advanced age. The present author is actually writing a manuscript to answer the claims of Kitani (1999) in detail.

In spite of the discussions mentioned briefly above, the results obtained on SAM mice seem to follow the basic concepts of MHA. Namely, the shorter is the survival of a subclone, the steeper is the decline of the age-dependent regression line describing protein lateral mobility in the cell plasma membrane of hepatocytes. The situation is somewhat complicated by the fact that SAM mice display different longevities under conventional and SPF conditions, and furthermore, they are characterized by various disorders (pathologies?), especially in the SAMP6 subclone. Some of these disorders seem to be related to aging, but others do not. The genetic selection or selection pressure in inbred strains sometimes result in the fixation of specific disorders. As an example, one can quote also the F344 rats: in males of this strain, the leukemia incidence varies very strongly between various animal farms: wherever this incidence is high, the medium life span shortens, and vice versa.

ACKNOWLEDGEMENTS

Prof. I. Zs.-Nagy expresses his grateful thanks for the Organizers (Prof. Dr. K. Kitani and Dr. S. Tanaka) of his study trip to NILS, and to all their coworkers, who were extremely helpful in all situations. A particular thank goes to Dr. Michiko Koda (Department of Epidemiology, NILS) who helped in the computer-assisted preparation of the graphs presented in Figs. 9 and 10.

CITED REFERENCES

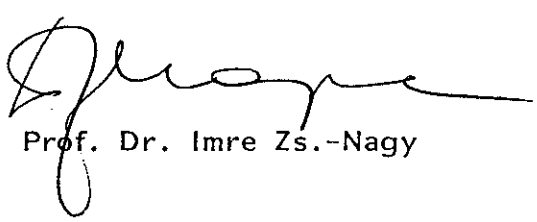
Kitani, K. (1999): Lateral mobility of proteins and lipids of cell surface membranes during aging: do the data support "The Membrane Hypothesis of Aging?" Mech. Ageing Dev., 107, 299-322.

Takeda, T., M. Hosokawa and K. Higuchi (1994): Senescence-Accelerated Mouse (SAM). A novel murine model of aging. In: T. Takeda (Editor): The SAM Model of Senescence. Internatl. Congress. Series, 1062, pp.: 15-22. Excerpta Medica, Amsterdam.

Zs.-Nagy, I. (1994): The Membrane Hypothesis of Aging. CRC Press, Boca Raton, USA.

Zs.-Nagy, I., K. Kitani and M. Ohta (1989): Age dependence of the lateral mobility of proteins in the plasma membrane of hepatocytes in C57BL/6 mice: FRAP studies on liver smears. J. Gerontol. Biol. Sci., 44, B83-B87.

Obu, February, 3, 2000.



Prof. Dr. Imre Zs.-Nagy

APPENDIX

The list of papers regarding the FRAP technique and the results obtained by this laboratory
(in chronological order of publications)

---

1. Zs.-Nagy, I., M. Ohta, K. Kitani and K.Imahori (1984): An automated method for measuring lateral mobility of proteins in the plasma membrane of cells in compact tissues by means of fluorescence recovery after photobleaching. Mikroskopie, 41, 12-25.
2. Zs.-Nagy, I., K. Kitani, M. Ohta, V. Zs.-Nagy and K. Imahori (1986a): Age-dependent decrease of the lateral diffusion constant of proteins in the plasma membrane of hepatocytes as revealed by fluorescence recovery after photobleaching in tissue smears. Arch. Gerontol. Geriatr., 5, 131-146.
3. Zs.-Nagy, I., K. Kitani, M. Ohta, V. Zs.-Nagy and K. Imahori (1986b): Age-estimations of rats based on the average lateral diffusion constant of hepatocyte membrane proteins as revealed by fluorescence recovery after photobleaching. Exp. Gerontol., 21, 555-563.
4. Kitani, K., I. Zs.-Nagy, S. Kanai, Y. Sato and M. Ohta (1988): Correlation between the biliary excretion of ouabain and the lateral mobility of hepatocyte plasma membrane proteins in the rat. The effects of age and spironolactone pretreatment. Hepatology, 8, 125-131.
5. Nokubo. M., I. Zs.-Nagy, K. Kitani and M. Ohta (1988): Characterization of the autofluorescence of rat liver plasma membranes. Biochim. Biophys. Acta, 939, 441-448.
6. Zs.-Nagy, I., Y. Ohno-Iwashita, M. Ohta, V. Zs.-Nagy, K. Kitani, S. Ando and K. Imahori (1988): Effect of perfringolysin O on the lateral diffusion constant of membrane proteins of hepatocytes as revealed by fluorescence recovery after photobleaching. Biochim. Biophys. Acta, 939, 551-560.
7. Zs.-Nagy, V., I. Zs.-Nagy, K. Kitani, M. Ohta and K. Imahori (1988): Possibilities and limitations of the smearing technique for cellular studies in compact tissues: A transmission electron microscopic analysis of liver smears. Tissue and Cell, 20, 229-237.

8. Zs.-Nagy, I., K. Kitani and M. Ohta (1989): Age dependence of the lateral mobility of proteins in the plasma membrane of hepatocytes in C57BL/6 mice: FRAP studies on liver smears. J. Gerontol. Biol. Sci., 44, B83-B87.

9. Nokubo, M., M. Ohta, K. Kitani and I. Zs.-Nagy (1989): Identification of protein-bound riboflavin in rat hepatocyte plasma membrane as a source of autofluorescence. Biochim. Biophys. Acta, 981, 303-308.

10. Zs.-Nagy, I., M. Ohta and K. Kitani (1989): Effect of centrophenoxine and BCE-001 treatment on the lateral diffusion constant of proteins in the hepatocyte membrane as revealed by fluorescence recovery after photobleaching in rat liver smears. Exp. Gerontol., 24, 317-330.

11. Zs.-Nagy, I., M. Ohta and K. Kitani (1990): The effect of idebenone on the lateral mobility of proteins in hepatocyte plasma membrane of old rats as revealed by fluorescence recovery after photobleaching (FRAP) technique using an endogenous label. Arch. Gerontol. Geriatr., 11, 243-250.

12. Kitani, K., M. Ohta, S. Kanai, Y. Sato, M. Nokubo and I. Zs.-Nagy (1991): Age-induced restricted mobility of proteins in the hepatocyte surface membranes. A possible determinant of membrane transport functions. In: Liver and Aging - 1990, ed. by K. Kitani, pp.: 305-317, Elsevier Science Publishers, Amsterdam.

13. Kitani, K., I. Zs.-Nagy, M. Ohta, S. Kanai, Y. Sato and G.O. Ivy (1992): Effect of leupeptin on the lateral mobility of proteins in the plasma membrane of hepatocytes of C57BL/6 mice: FRAP studies on liver smears. Arch. Gerontol. Geriatr., 14, 27-45.

14. Zs.-Nagy, I. (1992): A review on the lateral mobility of cell membrane proteins as a measure of aging: FRAP studies and their significance. In: Biomarkers of Aging: Expression and Regulation. Editors: F. Licastro and C.M. Caldarera. pp. 85-102, CLUEB, Bologna, Italy.

15. Zs.-Nagy, I., R.G. Cutler, K. Kitani and M. Ohta (1993): Comparison of the lateral diffusion constant of hepatocyte membrane proteins in two wild mouse strains of considerably different longevity: FRAP studies on liver smears. J. Gerontol. Biol. Sci., 48, B86-B92.

16. Zs.-Nagy, I., K. Kitani, M. Ohta and R.G. Cutler (1993): The effect of caloric restriction on the lateral diffusion constant of hepatocyte membrane proteins in C57BL/6 male mice of various ages: FRAP studies on liver smears. Mech. Ageing Dev., 71, 85-96.
17. Zs.-Nagy, I. and K. Kitani (1996): Age-dependence of the lateral mobility of lipids in hepatocyte plasma membrane of male rats and the effect of life-long dietary restriction. Arch. Gerontol. Geriatr., 23, 81-93.
18. Kitani, K. and I. Zs.-Nagy (1996): The effect of ethinyl-estradiol treatment on the lateral mobility of lipids and proteins in hepatocyte plasma membrane of male rats (FRAP studies on liver smears). Int. Hepatol. Commun., 5, 236-243.
19. Zs.-Nagy, I., S. Tanaka and K. Kitani (1998): Age-dependence of the lateral diffusion coefficient of Con-A-receptor protein in the skeletal muscle membrane of C57BL/6J mice. Mech. Ageing Dev., 101, 257-268.
20. Kitani, K., S. Tanaka and I. Zs.-Nagy (1998): Age-dependence of the lateral diffusion coefficient of lipids and proteins in the hepatocyte plasma membrane of BN/BiRijHsd rats as revealed by the smear-FRAP technique. Arch. Gerontol. Geriatr., 26, 257-273.
21. Kitani, K. and I. Zs.-Nagy (1998): Effect of spironolactone on lateral mobility of lipids in hepatocyte plasma membranes in the rat. Hepatol. Res., 12, 131-139.
22. Zs.-Nagy, I., S. Tanaka and K. Kitani (1999): Age-dependence of the lateral diffusion coefficient of Concanavalin-A receptors in the plasma membrane of ex vivo prepared brain cortical nerve cells of BN/BiRijHsd rats. Exp. Brain Res., 124, 233-240.
23. Kitani, K. (1999): Lateral mobility of proteins and lipids of cell surface membranes during aging: do the data support "The Membrane Hypothesis of Aging?" Mech. Ageing Dev., 107, 299-322.

Obu, 3 February, 2000.