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Studies on Lipid Peroxidation in Biological Systems. I. Effects of Various Factors on Lipid Peroxide Level in Blood[1]

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Plasma lipid peroxide concentration has been demonstrated to vary with the species, strain, sex and age of laboratory animals as well as with conditions of starvation and stress. Mice showed remarkably high plasma TBA levels as compared with other species of animals, and the ICR and ddY strains of mice, among other strains, may be useful as experimental models, since they proved to have relatively slight interindividual differences. The data obtained indicate that great care in the management and experimental handling of laboratory animals are essential because of the enhancing effect of stress on the plasma TBA level.

Keywords——lipid peroxide; species; strain; mice; sex; age; stress; starvation; plasma TBA value

In recent years, attention has been drawn to the relation of lipid peroxidation in biological systems to various diseases associated with degenerative changes, and since the report of Glavind *et al.*[3] in 1952 numerous studies have appeared in the literature.[4] In particular, the recent development of a microassay technique for lipid peroxides in the blood by Yagi *et al.*[5] has generated impetus toward clinical studies, and it has become increasingly clear that lipid peroxides play an important role in a number of diseases.[4] However, most of the laboratory investigations have been concerned almost exclusively with particular organs, and little work has been done on the plasma lipid peroxide level, except in connection with work on assay methods. It would be of great value, therefore, to prepare a new experimental animal model with maintained high plasma levels of lipid peroxides.

The present study was conducted to investigate variations of plasma lipid peroxide level caused by various factors in normal animals in order to select appropriate conditions for the preparation of a pathopharmacological experimental model in laboratory animals for lesions in humans.

Experimental

Materials——All chemical reagents used were of special grade: sulfuric acid (Kanto Chemicals), phosphotungstic acid (Wako Pure Chemicals), 2-thiobarbituric acid (Tokyo Kasei), acetic acid (Wako Pure Chemicals) and 1,1,3,3-tetramethoxypropane (Tokyo Kasei). In the experiments to assess interspecies differences, sexually mature male ddY mice, Sprague-Dawley rats, golden hamsters, rabbits (Jw-Csk), miniature pigs (Göttingen strain) and rhesus monkeys were used. All other experiments were carried out in mice of various strains, *i.e.* 5-week-old male mice of the ddY, C57-BL/6J, DBA/2, NZB, nu/nu (BALB/c), ICR and BDF_1 strains for the detection of interstrain variations, ddY mice at 5, 6, 9, 16 and 48 weeks of age for assessments of differences due to sex and age, and 5-week-old male ddy mice for other tests. In all the experiments,

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2) Location: *3-41-8 Takada, Toshima-ku, Tokyo.*
3) J. Glavind, S. Hartman, J. Clemmesen, K.E. Jessen, and H. Dam, *Acta. Pathol. Microb.*, **30**, 1 (1952).
4) K. Fukuzumi and T. Takagi, *Yukagaku*, **10**, 643 (1961); N. Yoshimine, F. Katsuya, and Y. Sato, *Pro. Jap. Soc. Geriat.*, **14**, 11 (1977); J. Noro, M. Kanemaru, and H. Takezawa, *ibid.*, **14**, 46 (1977); M. Sagai and A.L. Tappel, *Igaku-no-Ayumi*, **33**, 653 (1978).
5) K. Yagi, *Vitamin*, **49**, 403 (1975).

each group consisted of 5 animals. The animals were kept in a room with constant temperature (24±1°) and humidity (55±1%). Mice, rats and hamsters were housed five to a cage and other species of animals were individually caged. Food and water were provided *ad libitum*. Illumination was provided by standard fluorescent ceiling lights on a 14 hr light and 10 hr dark cycle.

Methods——1) Specimens and Assay: Immediately after drawing venous blood under ether anesthesia, the plasma was separated and assayed for lipid peroxides (plasma TBA) by the method of Yagi *et al.*[5] Blood samples were taken between 9 a.m. and 10 a.m.

2) Electrostimulation for Stress: A mouse was placed in a wooden chamber, 30×30×25 cm, with a 7.5 mm-spaced stainless steel grid laid on the floor *via* which rectangular wave electrostimuli (80V D.C., 0.1 Hz, 1 sec) were given for 15 minutes at intervals of 75 minutes over a period of 16—40 hours. Mice had free access to water and food throughout the test.

Results

Interspecies Differences

The results are summarized in Table I. Mice showed a particularly high mean plasma TBA value of 8.51 nmol/ml, compared to those of other animals: 2.10 nmol/ml in rats, 2.41 nmol/ml in hamsters, 1.70 nmol/ml in rabbits, 1.31 nmol/ml in miniature pigs and 2.28 nmol/ml in rhesus monkeys.

Interstrain Differences

All the mouse strains tested were found to have uniformly higher plasma TBA levels (as shown in Fig. 1) than all other species of animals listed in Table I. There were conspicuous interstrain variations in the mouse plasma TBA level; significantly higher values were obtained for the DBA/2 and NZB strains as compared to the other five mouse strains. The data also disclosed relatively modest interindividual variations of plasma TBA value in the ddY (4.92±0.35) and ICR (3.58±0.33) mouse groups, compared to those in other strains: C57-BL/6J (4.15±0.94), DBA/2 (9.61±1.28), NZB (7.92±1.15), nu/nu (BALB/c) (5.12±1.00) and BDF_1 (3.19±0.39).

Table I. Plasma-TBA Level in Various Species of Normal Animals

Species	P-TFA (nmol/ml)
Mouse (ddY)	8.51±0.62
Rat (S.D.)	2.10±0.16
Hamster (Gölden)	2.41±0.09
Rabbit (Jw-Csk)	1.70±0.10
Miniature pig (Göttingen strain)	1.31±0.16
Rhesus monkey	2.28±0.22

Mean±S.E.

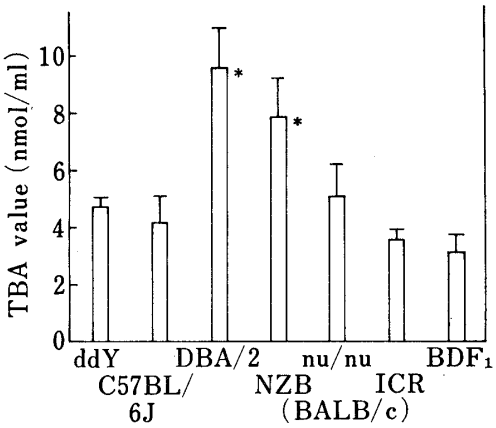

Fig. 1. Plasma-TBA Values in Various Strains of Mice

* Significantly different from ddy strains value ($p < 0.05$; student's t-test).
Mean±S.E.

Differences by Age and Sex

As shown in Fig. 2, male mice were noted to exhibit marked changes in plasma TBA level with aging; the average value increased sharply over the period up to sexual maturation at 9 weeks, followed by a decline to a level comparable to the 6-week level at 16 weeks. At 48 weeks they showed essentially the same value as at 16 weeks. In contrast, there was practically no influence of aging on the plasma TBA level in females, the values being significantly lower than those in males at all periods except at 5 weeks of age.

Effects of Starvation and Stress by Electrostimulation

Groups of mice were subjected to starvation or stress by electrostimulation for 16, 24 or 40 hours and their plasma TBA levels were compared with the level in untreated controls (at zero time in Fig. 3). Animals fasted for 16 hours showed a slight elevation, whereas the plasma TBA value decreased significantly in those deprived of food for longer periods of 24 and 40 hours. These latter groups were found at autopsy to have almost complete depletion of adipose tissue. Mice subjected to stress by electrostimulation for 16, 24 or 40 hours all showed higher plasma TBA values than the untreated controls, the elevation being most conspicuous in the 24-hour group. On necropsy, none of these groups were found to have such pronounced fat depletion of tissues as that in the starved groups.

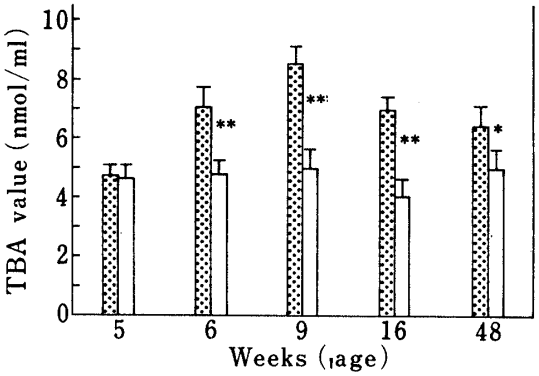

Fig. 2. Effects of Sex and Age Differences on Plasma-TBA Values in ddY Mice

*, ** Significant difference between male and female values by student's t-test (*: $p<0.05$, **: $p<0.01$).
Mean±S.E.
▨: male, □: female.

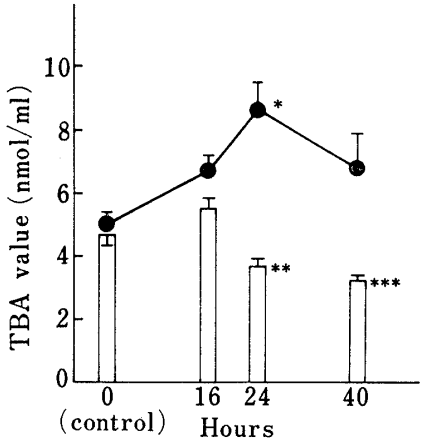

Fig. 3. Effects of Starvation and Stress by Electrostimulation on the Plasma-TFA Values in Mice

*, **, *** Significantly different from control values by student's t-test (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$).
Mean±S.E.
□: starvation, ●: stress.

Effects of Other Factors

There was no significant change in plasma TBA level that could be attributed to the influence of anesthesia at blood drawing, to any particular type of anesthetic used or to the site of blood drawing (posterior vena cava, heart, ocular).

Discussion

There were no marked differences in plasma TBA level among the various species of laboratory animals with the exception of the mouse, which showed a remarkably higher value. The reason for this incongruity is not known, but it may be presumed that the lipid peroxide anabolic and/or catabolic function of mice differs markedly from that of other species. In any case, it appears that the effect of peroxidation in tissues is more liable to be reflected in the plasma TBA level in the mouse than in other species, in that plasma lipid peroxides are derived largely from the peroxidation of tissue lipids.[6] Furthermore, there were significant variations of plasma TBA value among the mouse strains studied. The two strains with congenital functional abnormalities, DBA/2 (auditory system) and NZB (immunologic system), showed much higher values than other mouse strains. This might be ascribed to stress by phonic stimulation in the case of the DBA/2 strain, which is known to be highly susceptible

6) K. Yagi, *Saishin-Igaku*, 33, 653 (1978).

to sound stimuli, with a high incidence of audiogenic convulsion.[7] With the NZB strain, it is of considerable interest that the animals proved to exhibit elevation of plasma TBA even at ages prior to the development of clinical signs of immunologic disorder, thus suggesting the possibility of involvement of plasma lipid peroxides in the development of the clinical signs. The ddY and ICR strains exhibited only comparatively slight interindividual variations and, therefore, were considered to be suitable for use in the study. It was decided, accordingly, to use the ddY strain in the following experiments. It is also noteworthy that pronounced changes with advancing age were noted in male mice, showing some resemblance to the pattern of plasma TBA variations with aging in humans (a peak at age between 40 and 59 years, followed by a gradual decline) (Yamazaki *et al.*, 1978[8]); hence these mice are of interest as a possible experimental model for aging. Female mice of this strain, however, showed no variations with advancing age at all; the plasma TBA value remained at a constant, lower level throughout observation up to an advanced age of 48 weeks. It is possible that females are endowed with a system for inhibiting lipid peroxide production, or accelerating its removal, which differs from that in males. In view of the finding that DBA/2 mice displayed remarkably high plasma TBA values, suggesting the possibility of plasma TBA elevation by stress, we performed experiments to assess the effects of starvation and electrostimulation stress. In starved mice, the plasma TBA exhibited a slight increase at 16 hours and declined significantly at 24 hours and thereafter, possibly due to diminution of fatty acids. Mice with free access to food and water, on the other hand, showed a marked elevation of plasma TBA in response to imposed stress by electrostimulation. It can, therefore, be presumed that the *in vivo* lipid peroxidation process sensitively reflects the effect of stress, though varying in degree with the type of stress imposed.

In view of the finding that the plasma TBA level fluctuates markedly in response to various factors, the following three points require attention in carrying out studies with plasma lipid peroxides as a parameter of biological response.

1) Much caution must be exercised in laboratory animal care, as well as in performing experiments, since there is a likelihood that lipid peroxidation will be enhanced by stress.

2) The effect of peroxidation in the tissues on the plasma TBA level seems to be much greater in mice than in other common species of laboratory animals, and mice are therefore considered to be suitable for use in experiments. The data indicate that the ddY and ICR strains are preferable in that they show relatively modest interindividual variations of plasma TBA value.

3) It is also considered essential to use mice having a strictly uniform distribution of age and sex among experimental groups inasmuch as marked variations of plasma TBA level with age and sex have been demonstrated.

7) E.M. Vicari, *J. Psychol.*, **32**, 79 (1951).
8) S. Yamazaki and S. Kondo, *Saishin-Igaku*, **33**, 682 (1978).