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Archives of Gerontology and Geriatrics
19 (1994) 145–158

ARCHIVES OF
GERONTOLOGY
AND GERIATRICS

Aging and the liver: functional aspects

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Abstract

The drastic decline in the functions of the hepatic microsomal cytochrome monooxygenase system, initially reported in male rat livers, was shown to be due to a feminization of male rat livers with aging. In female rat livers as well as in mouse livers, this system was found to stay unchanged with age. Phase II reactions which showed some decline with aging in male rat livers again stayed fairly stable with age in female rat and mouse livers. Glutathione *S*-transferase (GST) enzyme activities, which are very stable with age in female rat and mouse livers, demonstrated highly age-dependent changes when dietary conditions were manipulated, suggesting a potential age difference in the homeostatic regulation of this enzyme system. Using the fluorescence recovery after photobleaching (FRAP) technique, unique studies revealed an age-dependent decline in the lateral mobility of proteins in hepatocyte surface membranes. The protease inhibitor model of aging, initially proposed by Ivy for brain cells, has been validated in hepatocytes, demonstrating an accumulation of lipofuscin-like granules in young animals treated with i.p. infusion of leupeptin for only 2 weeks. Antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT) in the liver were clearly demonstrated not to be reduced in general terms with aging. Rather, a clear increase in CAT enzyme activities with age was demonstrated in female rat livers, thus challenging the concept that intracellular enzyme activities generally decline with aging. In this paper, studies performed in Japan on aging and the liver over the past 30 years, with a focus on its functional aspects, are critically reviewed in terms of the clinical implications of these studies as well as on theories of aging in general.

Keywords: Aging; Drug metabolizing enzymes; Membrane alterations; Perturbation of proteolysis; Antioxidant enzymes and glutathione

1. Introduction

The liver is the heaviest organ in the body, even in humans having a heavy brain, and it performs a multitude of different physiological and biochemical functions. An

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SSDI 0167-4943(94)00584-T

alteration of liver functions with aging has been a subject of considerable interest for both clinical and basic scientists. The interests of clinical scientists have concentrated primarily on questions of how liver functions, especially drug metabolizing systems, are altered with aging, since this subject has a strong impact on pharmacotherapy for the elderly in general. This also relates to drug-hepatotoxicity in the elderly, as well as to medical care for liver diseases in the elderly, both of which should differ from those in the young. On the other hand, many basic experimental gerontologists have used the liver as a model organ for various reasons, in particular for generating their theories of aging. This review describes functional aspects of the aging liver which have been reported from Japan in the past three decades. Discussions on this subject in more general terms can be found in several past reviews (Kitani, 1988a, 1990a,b, 1991a, 1992).

2. Physiological functions

Unlike in humans, liver weight tends to increase with age in rodents, and in rats in particular. Those dealing with rodent livers in terms of the effect of aging must bear in mind that liver weight continues to increase with age in rodents, while in humans it tends to decrease (Kitani, 1988a, 1992). This difference becomes critically important when interpreting the data of in vivo studies, such as pharmacokinetic studies performed in rodents. The liver/body weight ratio is very high in immature (developing) rats and becomes stable with age after maturity, which is achieved, in general, after 6 months of age (Kitani, 1992). To this author's knowledge, only two studies examined blood flow changes with age in rat livers. Using different methodologies, both groups have come to the same conclusion: the hepatic blood flow stays stable with age in rats after maturity (Kitani, 1992). This differs from what is known for human livers, where the total flow rate and the flow rate per unit liver weight both steadily decline with age (Wynne et al., 1989).

It should be noted that so-called age-associated changes in physiological and biochemical parameters are very drastic only during the first 6 months of rat life but become fairly stable afterwards. Thus, the improper selection of developing animals younger than 6 months as a young adult group often leads to the conclusion that changes occurring during the developing stage are 'age-induced alterations'. Some examples are found in the past literature (Lauterburg et al., 1980).

Bile flow, bile salt excretion rate and bile salt composition in the natural bile may also be affected by aging. However, data in our own studies (Kitani, 1988b) in this regard suggest that after maturity the bile flow and bile salt excretion rate barely decline with age in rats, except for Fischer 344 (F-344) female rat bile. Other studies have shown bile flow decline with age on a unit body weight basis; however, this type of change should be interpreted with caution because the liver/body weight ratio declines with age in some rat strains such as Sprague–Dawley rats due to an ever-increasing body weight (Kitani, 1988b).

The uptake rate of i.v. injected indocyanine green (ICG) appears to decline with rat age; however, when the maximal ICG uptake rate was calculated, it stayed essentially unchanged with age (Kitani et al. 1978; Kitani, 1992), which agrees with the

report by Koff et al. (1973) that sulfobromophthalein (BSP) plasma retention values remain unchanged with age in healthy humans. These data, however, are at variance with results of many other studies which reported an age-dependent increase in BSP (and ICG) retention (e.g. Thompson and Williams, 1965). In contrast to the original contention that BSP relative storage capacity (S) declines, while the biliary transport maximum (T_m) stays unchanged with age in humans (Thompson and Williams, 1965), our own series of experiments in rats came to an opposing conclusion that BSP S stays unchanged, while the T_m declines with age (Kanai et al., 1985). The discrepancy between earlier clinical works and those in experimental animals have been fully discussed (Kanai et al., 1985). We believe that the earlier clinical information was biased by technical pitfalls. In contrast to dyes such as ICG, the hepatic uptake rates for ouabain (Ohta et al., 1988) and taurocholate (Ohta and Kitani, 1990) steadily decline with age in rat hepatocytes. This issue is discussed below in relation to physical–chemical alterations of hepatocyte surface membranes with aging (see section 7, for review; also see Kitani, 1991b).

3. Drug metabolism in the liver — I. Phase I reactions

The most extensively studied subject in terms of the liver and aging in the past has been an alteration of drug metabolism in the liver with age. Obviously, this issue has immediate clinical implications for pharmacotherapy in the elderly, since once drug metabolizing capacity in the liver is altered with age, the pharmacokinetic profiles of drugs may also be altered, eventually leading to an alteration of pharmacodynamics. The first report on this issue came from Italy, by a Japanese author. Kato et al. (1964) reported an age-dependent decline in metabolism of several drugs including hexobarbital and strychnine in male rats; all of these drugs are primarily metabolized by the liver.

Although subsequent studies of Kato and coworkers (Kato and Takenaka, 1968) as well as of others (Fujita et al., 1985a; Kamataki et al., 1985) showed that such a drastic decline in drug metabolism with age cannot be observed in either female rats or in mice (Fujita et al., 1986), the impact of the initial study of Kato et al. (1964) was so strong that it influenced the interpretation of subsequent studies in rodents and even in humans in the early seventies. In fact, when O'Malley et al. (1971) reported a significant age-difference in antipyrine $t_{1/2}$ in humans, the original data obtained from male rats (Kato et al., 1964) began to be even more strongly interpreted as experimental evidence for this type of clinical information. Thus, unfortunately, retrospectively speaking, the observations made in male rats have been overinterpreted in terms of their clinical implications, to the extent that studies reporting lowered hepatic clearances of drugs in elderly humans have been increasingly reported in the past 20 years (for review, see Kitani, 1988a).

In 1985, Kamataki et al. (1985) and Fujita et al. (1985a) independently reported that such a drastic decline reported by Kato et al. (1964) in male rat livers cannot be observed in female rats of an even much older age (up to 30 months) than initially examined by Kato and Takenaka (1968). Using different methodologies these two groups have reached the same conclusion: alterations that were found in the P-450

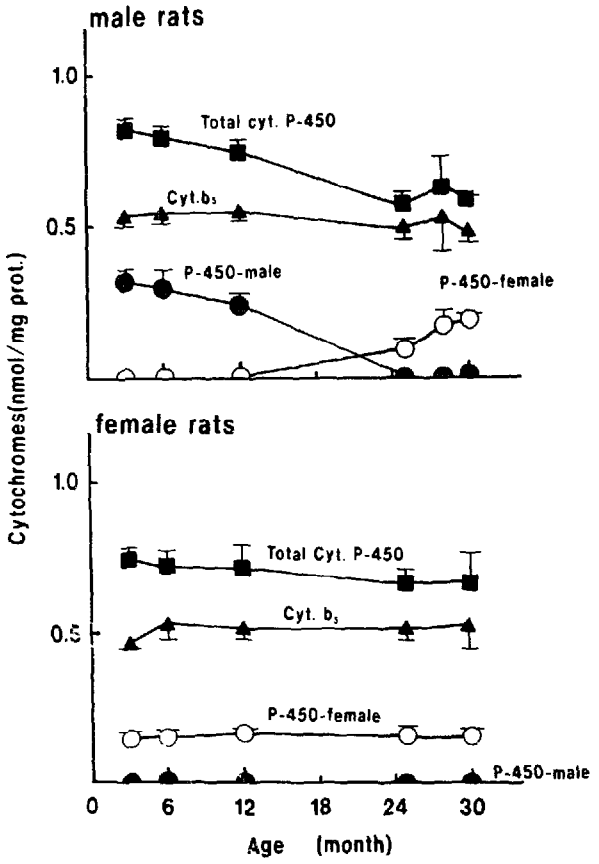

Fig. 1. Age-related changes in microsomal P-450 concentrations in male (upper panel) and female (lower panel) F-344 rats. (Reproduced with permission of the publisher from Kamataki, T., Maeda, K., Shimada, M., Kitani, K., Nagai, T. and Kato, R. (1985): Age-related alteration in the activities of drug-metabolizing enzymes and contents of sex-specific forms of cytochrome P-450 in liver microsomes from male and female rats. J. Pharmacol. Exp. Ther., 233, 222–228.)

isozyme proportion which occurred during aging in male rat livers were only a feminization of their proportions and in female rat livers nothing specific happened during normal aging (Fig. 1). All enzyme activities in old female rat livers examined were identical to respective values in young female rat livers, which agrees with the initial report by Kato and Takenaka (1968).

Although there are some studies reporting a general (and drastic) decline in mono-oxygenase activities with age in mouse livers, at least in C57BL mice, the functions of this system are generally well maintained up to an old age in both sexes (Fujita et al., 1986) as they are in female rats. The functional stability of this system with age in rodents (except for in male rats) corresponds to what has been reported for non-human primates, i.e. there is no evidence that this system declines with age

(Maloney et al., 1986; Sutter et al., 1985). Since in human livers as well, there is no evidence that monooxygenase activities show a negative correlation with age (Boobis and Davies, 1984; Wynne et al., 1988), it has been repeatedly suggested by this author that the initial information obtained from male rat livers showing that monooxygenase activities decline with age should not be generalized, especially to human livers (for review, see Kitani, 1988a).

4. Drug metabolism — Phase II reactions

In contrast to data on the effect of aging on Phase I reactions, Phase II reactions have scarcely been studied in the past. The first study from Japan could be that of Kitahara et al. (1982) who showed that glutathione *S*-transferase (GST) enzyme activities toward 1,2-dichloro-4-nitrobenzene (DCNB) decreased with age, while that toward 1-chloro-2,4-nitrobenzene (CDNB) remained unchanged with age in male Wistar rat livers. Subsequent studies on GST enzyme activities by Fujita et al. (1985b) in F-344 rats demonstrated that changes in enzyme activities are substrate-selective as well as sex-dependent. They found that GST enzyme activities toward different substrates tended to decline with age in male rat livers but stayed mostly unchanged with age in female rat livers. This tendency has been confirmed by a subsequent study by Carrillo et al. (1989) who also reported that GST activities barely decline with age in female mouse as well as in female rat livers (Carrillo et al., 1990) (Fig. 2). Relatively stable GST enzyme activities with age in female rat and mouse livers (Carrillo et al., 1989, 1990, 1991) may well be compatible with stable activities of this enzyme as well as other Phase II enzymes reported for human livers (for review, see Kitani, 1988).

The relative stability of GST enzyme activities with age in female rat and mouse livers does not mean, however, that nothing is happening during aging with these

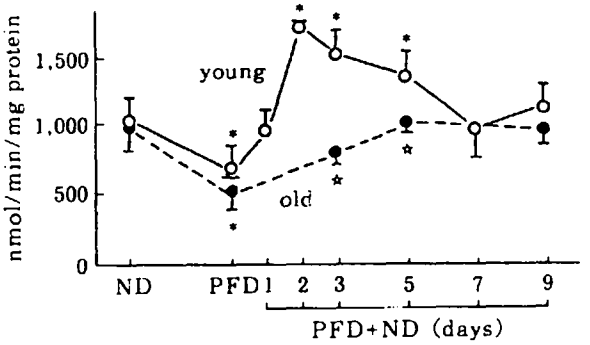

Fig. 2. Changes in activities of GSTs toward CDNB before and during diet manipulation in female C57 BL mice. *Significantly different from corresponding values in control mice on the ND only ($P < 0.05$). ☆ Significantly different from corresponding values in young mouse livers ($P < 0.05$). (Reproduced with the permission of the publisher from Carrillo, M.C., Kitani, K., Kanai, S., Sato, Y., Nokubo, M., Ohta, M. and Otsubo, K. (1989): Differences in the influence of diet on hepatic glutathione *S*-transferase activity and glutathione content between young and old C57 black female mice. Mech. Ageing Dev. 47, 1–15.

GST isozymes. The proportion of subunits for these isozymes changed drastically with aging not only in male but in female rat livers (Carrillo et al., 1991). In male rat livers, changes in subunit concentrations with age were primarily an increase in concentrations for subunit 2 and a decrease for subunit 4, leading to a subunit proportion very similar to that in young female rats. However, unlike *P*-450 isozymes, GST subunit patterns also changed drastically in female rat livers with aging, leading to a drastic decrease in the concentration of subunit 4 and thus making the pattern of subunit proportion in old female rat livers totally different from that of young female rats. We have also found that the elution pattern of subunits of GSTs through an affinity column chromatography is changed with age. The elution of all 4 subunits in males and subunit 1 in females were all accelerated in old rat livers compared with the young. This observation may suggest that the affinity of these subunits to *S*-hexyl GSH (used as an eluent), and possibly GSH, changes with age. The implication of this observation in terms of experimental gerontology awaits future studies.

The rather stable GST activities with age found for female rat and mouse livers do not indicate, however, that this detoxyfying system functions in old rodents as it does in young ones. When mice (Carrillo et al., 1989, 1992b) or rats (Carrillo et al., 1990) were exposed to a protein-free diet (PFD) for 1 week, activities of GSTs declined both in young and old animals, but the differences between the two age groups were not so conspicuous, although the decrement was somewhat greater in old animals. However, when animals started to eat a (protein-containing) normal diet (ND) after a 1-week PFD, GST enzyme activities showed definite differences between young and old rodent livers. In young animal livers, GST activities started to increase right after the refeeding of ND, overshooting the basal enzyme activities in 2 days and returning back to physiological levels in 1 week. In contrast, in old animal livers, this type of overshooting was never observed during the recovery phase for GSTs after ND refeeding, with enzyme activities slowly returning to basal levels (Fig. 2). Thus, despite comparable basal enzyme activities in young and old animal livers, large and significant differences could be demonstrated in values on day 2 of ND refeeding following 1 week of PFD. Generally speaking, observations made in rodents cannot be directly extrapolated to humans as is emphasized below in this review. However, in humans too, Phase II reactions are believed to be stable with age (for review, see Kitani, 1988a). Furthermore, a study reported by Wynne et al. (1990) has shown that only frail elderly (and not healthy elderly) have significantly lower clearance values for acetaminophen than young human subjects. These observations raise the possibility that hepatic detoxifying systems may be comparable for young and old subjects when they are in a healthy condition: however, when they are exposed to unfavourable conditions such as alcoholism, malnutrition, infection, morbidity in general, the detoxyfying system in the elderly becomes much less efficient than that in the young, leading to a higher incidence and possibly graver consequence of hepato- (as well as systemic) toxicity of drugs in the elderly.

5. Antioxidant enzymes and glutathione (GSH) in the liver

There is a growing argument that the deterioration of organ and cellular functions with age is causally related with oxygen free radical-induced tissue damages during

aging. If this thesis is true, intracellular antioxidant enzymes may work to counteract this type of tissue damage. If antioxidant enzyme activities are lowered during aging, it may further accelerate the aging processes. In fact, Reis and Gershon (1978) have shown that superoxide dismutase (SOD) activities in the liver significantly decline with age in rats. Richardson and coworkers also have shown that enzyme activities as well as mRNA levels of SOD and catalase (CAT) tended to decline with age in rats (Semsei et al., 1989; Rao et al., 1990). We have found, however, that SOD activities change little with age in F-344 rat livers of both sexes (Carrillo et al., 1992a). Further, CAT activities in male rat livers declined with age as Richardson's group reported; however, activities showed a definite increase with age in female rat livers (Carrillo et al., 1992a). These observations are supported by another independent study by Rikans et al. (1991), which also showed an age-dependent increase in CAT activities in female rat livers. A drastic increase (rather than decrease) in SOD activities in some selective brain regions was found in male (but not in female) rats (Carrillo et al., 1992a). We are of the opinion that antioxidant enzyme activities do not necessarily decline with age in general. It is possible, however, that when animals approach the terminal stage of their lives, these antioxidant machinaries are perturbed. Hazelton and Lang (1985) have shown that GSH peroxidase (Px) activities as well as GSH concentration in the liver become lower only in very old age. The present author, however, regards these observations as phenomena that already exceeded the physiological aging process, reaching pathological conditions, since in our studies, we seldom observed the decrease in GSH concentration in the natural aging process as is discussed below.

GSH is the substrate for conjugation reactions catalysed by GSTs. Furthermore, GSH itself in the reduced form is a powerful antioxidant. The concentration of GSH may be an important determinant for the aging process of the liver and eventually the organism. In the past, several reports are available demonstrating a drastic decline of GSH concentration in the liver during aging (e.g. Stohs et al., 1982). This, however, appears to be a phenomenon that occurs only in specific animal models. In our hands, GSH concentration in the liver was found to be very stable with age in F-344 female rat (Carrillo et al., 1990) as well as in C57BL female mouse (Carrillo et al., 1989; Rikans et al., 1992) livers. Rikans and Kosanke (1984) also reported that GSH concentration remained unchanged with age in livers of male F-344 rats. GSH concentration is known to have a marked diurnal rhythm. An age-associated change in GSH concentrations in the liver must be carefully examined keeping this fact in mind. We have recently found that the diurnal rhythm in GSH concentration has a sex difference in mouse liver (Rikans et al., 1992). Interestingly, aging caused a change in the diurnal rhythm in male mouse livers, leading to the feminization of male rhythm with aging, while in female mice, the diurnal rhythm as well as the concentration of GSH barely changed with age (Rikans et al., 1992). A more important question is, however, whether the supply (synthesis) of GSH by the liver is maintained during aging as it is in the young, especially when extraordinary GSH amounts are required. At the moment, no information is available on this very important issue. The only information in this regard unfortunately dealt with developing rats and the data were interpreted in terms of aging (Lauterburg et al., 1980).

6. Protein turnover and protease inhibitor model of aging

Although the mechanism(s) of aging at the cellular and molecular levels is poorly understood, one attractive theory of cellular aging appears to have come from a study on brain cells. Ivy et al. (1984) infused leupeptin, a thiol protease inhibitor, into a lateral ventricle in young rats and demonstrated an accumulation of electron-dense, autofluorescent bodies which, by several criteria, resembled age pigments, or lipofuscin. From these and other observations, Ivy elaborated a theory of a cellular mechanism of aging suggesting that slowed proteolysis is one of the primary mechanisms for cellular aging processes (for review, see Ivy, 1992). In accord with this thesis, several past studies have indicated that a perturbation of protein turnover is a cellular process accompanying aging (for review, see Ivy et al., 1986). The perturbation of proteolysis appears to be not only an important mechanism for normal, but also for pathological, aging processes. We have found that the intraventricular infusion of leupeptin causes not only the accumulation of lipofuscin-like granules but also the abnormal build-up of immunoreactivities to antibodies directed against the abnormally phosphorylated carboxyl terminal of tau proteins and to ubiquitin, both of which can also be found in brain cells of aging rats (Ivy et al., 1989). We attempted to expand these observations to cell types other than brain cells, including

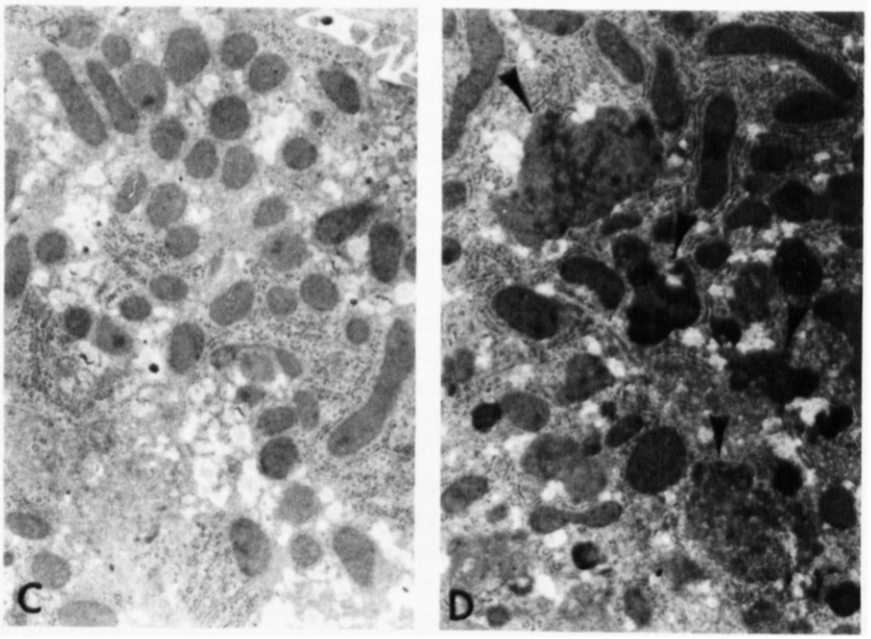

Fig. 3. Electron micrographs of hepatocytes from a saline treated rat (c) and a leupeptin-treated rat (20 mg/100 g per day for 2 weeks) (d). Note the abnormal dense amorphous bodies in (d) (arrows). Magnification is 13 700× (c and d). (Reproduced with the permission of the publisher from Ivy, G.O., Kanai, S., Ohta, M., Sato, Y., Otsubo, K. and Kitani, K. (1991b): Leupeptin causes an accumulation of lipofuscin-like substances in liver cells of young rats. Mech. Ageing Dev., 57, 213–231.)

hepatocytes. An i.p. infusion of leupeptin for 2 weeks caused an accumulation of yellow-fluorescent pigments in hepatocytes which resemble lipofuscin from light and electron microscopic criteria lipofuscin (Ivy et al., 1991b) (Fig. 3). Further, a rapid build-up of immunoreactivities to ubiquitin was also observed in leupeptin treated young rat livers which looked quite similar to hepatocytes in old rats (Ivy et al., 1991a). These observations suggest that a decrease in proteolytic enzyme activities or an increase in protease inhibitors (or both) are taking place in aging cells. Goto and coworkers in Japan attempted to compare degradation rates of different species of foreign proteins that were introduced into hepatocytes of young and old animals. They have demonstrated quite clearly that the degradation of proteins is slowed down in old animal hepatocytes (see the article of Goto et al. in this issue). Whether this type of change happens for intrinsic proteins in hepatocytes remains to be demonstrated in the future. However, these in vitro experiments appear to strongly support the forementioned hypothesis of the protease inhibitor model of aging.

7. Membrane alterations: FRAP studies on liver smears

Many investigators believe that physical–chemical alterations of different kinds occur in the cellular membranes during aging. Qualities of the surface membranes of hepatocytes, if altered with aging, may affect cellular functions considerably. Indeed, several earlier studies suggested that the membrane quality, and consequently its functions, may be altered with aging. All of these past works have been performed by conventional methods utilizing electron spin resonance (ESR) or fluorescence anisotropy method using a probe which perturbs the lipid domain of membranes. The effect of aging on hepatocyte surface membranes in the past literature, however, is not conclusive, yielding discrepant results depending on different methodologies, sexes of animals and different reports (for review, see Nokubo, 1985).

There is another methodology which can determine the lateral diffusion constant of proteins (and lipids) of cell surface membranes which is called the 'fluorescence recovery after photobleaching (FRAP) technique'. This technique was applied to liver smears to measure the lateral diffusion constant of surface membrane proteins of hepatocytes. A standard method for this technique requires a labeling of a fluorescent probe to membrane proteins. However, we have found that hepatocyte surface membrane proteins are extremely labile to these external probes reducing the diffusion constant of proteins with increasing concentration of available probes (Lustyik et al., 1987). Further, the fractional recovery of fluorescence intensity after photobleaching was also lowered by the labeling process (Lustyik et al., 1987). We, however, have succeeded in measuring the protein mobility of hepatocyte membranes by using the autofluorescence of membranes which was enhanced in intensity by incubating liver smears with H_2O_2 (Zs.-Nagy et al., 1986). The source of this yellow-greenish fluorescence was shown to be from oxidized riboflavin which is bound to surface membrane proteins (Nokubo et al., 1988, 1989). Using this unique technique we have found the following. (1) Aging causes an almost linear decline in the protein diffusion constant of hepatocyte surface membranes in rats and mice of both sexes, although the slope values differed among different animal models (Zs.-

Nagy et al., 1986, 1989a). (2) The protein diffusion constant can be up-regulated by dietary restriction (DR) (Zs.-Nagy et al., 1993b); however, this occurs only to certain upper limits which can be achieved by 3-month DR, while a continued DR (longer than 3 months) can exert no additional effect on the diffusion constant. (3) Long-lived rodents (*Peromyscus leucopus*) have a much higher diffusion constant than short-lived ones (*Mus musculus*) (Zs.-Nagy et al., 1993a). (4) Finally, treatments with several drugs such as idebenone (Zs.-Nagy et al., 1990), centrophenoxine (Zs.-Nagy et al., 1989b) and perfringolysin, a membrane toxin (Zs.-Nagy et al., 1988), and spironolactone (SP) (Kitani et al., 1988) can all significantly increase the protein diffusion constant. Interestingly, two previous studies have reported a decrease in lipid fluidity of hepatocyte surface membranes by treatment with SP (Miner et al., 1983; Smith and Gordon, 1988). These studies used a fluorescence anisotropy method using diphenylhexatriene as a probe. Very recently, we examined the effect of the same SP treatment on the lipid lateral diffusion constant using NBD-PE as a label for lipids and found that a lipid diffusion constant is significantly elevated by SP treatment (Zs.-Nagy and Kitani, unpublished data).

Thus, the apparent discrepancy between our observations on protein lateral diffusion and the previously reported lipid fluidity in terms of the effect of SP does not appear to be due to the difference between proteins and lipids. The physiological significance of an age-dependent decrease in protein diffusion and its up-regulation by drugs remain unresolved. However, we have demonstrated significant correlations between protein diffusion constants and hepatic uptake rates for ouabain and taurocholic acid (Ohta et al., 1988; Ohta and Kitani, 1990; Kitani, 1991b) in terms of effects of aging and SP pretreatment, suggesting that the physical–chemical characteristics of surface membranes can act as a regulatory factor for membrane functions (Fig. 4). This technique has recently been applied to cultured striated muscle cells, a cell line of rat skeletal muscles (Zs.-Nagy et al., unpublished data).

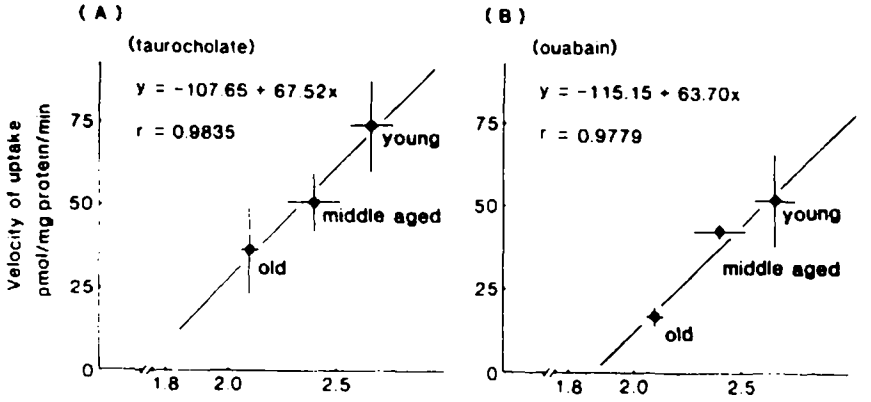

Fig. 4. The relationship between taurocholic acid uptake velocity at 1 μM and the diffusion constant of hepatocyte surface membrane proteins, as previously determined by our FRAP method in three different age groups (A) and a similar relationship for ouabain uptake rats (B). (Reproduced with the permission of the publisher from Ohta, M. and Kitani, K. (1990): Age-dependent decrease in the hepatic uptake of taurocholic acid resembles that for ouabain. Biochem. Pharmacol., 39, 1223–1228.)

8. Conclusions and future perspectives

Studies in the last three decades have revealed that alterations of rodent liver functions by aging are quite variable depending on species, strain and sex in particular. The present author has repeatedly cautioned against a simple extrapolation of data from rodents to the human situation. Likewise we should refrain from generating a theory of aging based on data obtained from a limited number of animal models. Despite these limitations, information on aging liver has accumulated steadily in the past. Based on these past data, it is suggested that liver functions in general barely decline in the natural aging process both in rodents and in humans. Many theories based on the assumption that enzyme activities in the liver decline with aging therefore require serious reconsideration. However, in old rodents, and possibly in humans, also, livers may function much less efficiently, once they are exposed to stresses, such as malnutrition, infection and other morbidities. More importantly, the recovery of lowered liver functions may be slower and less efficient in the elderly. The recognition of this fact is quite important in clinical wards, since subjects under medical treatment are all patients whose liver functions may be much lower than not only young counterparts, but also the healthy elderly. An elaboration of a new theory of a cellular mechanism of aging must also be based on the fact that enzyme activities in the liver do not decline with natural aging, at least in rodents. The elucidation of the mechanism(s) for the declining recovery response of liver functions with aging may lead to a new theory of aging. It is the belief of the author that this is the most fruitful direction in which the study of the aging liver should go.

Acknowledgements

The author expresses his deepest appreciation to all of his coworkers who are in the list of references in this chapter for their expert collaborations. Thanks are also due to Dr G.O. Ivy for her careful reading of the manuscript and Ms T. Ohara who typed the manuscript.

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