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Liver and aging

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Introduction

Articles with similar titles have appeared in the past several years in different publications prepared by several authors including a very distinguished hepatologist[1-3]. In order to avoid repetitive discussions on the same topic, this review attempts to address only a limited number of subjects on the liver and aging which the author hopes to emphasize. Those who wish to be oriented more in detail to the general aspects of the aging liver are advised to refer to the literature cited in refereces 1-10. Some of these past articles are intended to be complementary[1,4] (rather than repetitive) to previous series of publications.

Does the liver age in man?

Several previous publications have raised the same question and attempted to answer it[1,4,11]. If the word "age" is defined as the deterioration of functions with time, the general conclusion drawn by Popper in his last article[1], that the liver does not age, may hold. This conclusion is important in interpreting many observations in experimental and clinical studies published in the past demonstrating a clear decline in liver functions with age. This conclusion is important in correctly interpreting data obtained from studies on rodents, in particular, on male rats which mostly have shown a considerable decline in many enzyme activities in the liver, while such activities were shown to be very stable with age in the female rat liver (for review, see refs. 4,12,13). The change with age in microsomal monooxygenase activities is only one example. Other detoxifying enzymes such as glutathione (GSH) S-transferases (GSTs)[14-17] and other phase II reactions[16,17], as well as alcohol detoxifying systems [18-20], and even some more fundamental enzymes such as superoxide dismutase (SOD) and catalase[21-23], all belong to this category of enzymes which show a general decline in activity in male, but not female, rat liver. This review attempts to perform a difficult task. First, the author attempts to eliminate as much as possible the past myth held by many clinicians as well as basic scientists that all organ functions, including the liver, decline with age. In the second part of this review, the author attempts to call the attention of clinicians, and hopefully basic scientists as well, to the fact that the apparent comparability of basal liver functions for young and old subjects does not indicate that the liver always functions similarly in young and elderly subjects. In many situations, in particular under stresses such as malnutrition or morbidity (infections in particular), liver functions in old subjects will become much less efficient than in the young. For this reason, the hypothesis of Popper that the liver does not age may be misleading. Many clinicians appear to have forgotten the once important question "Does the liver age in man?" after reading that most authoritative article of Popper. The present article attempts once again to attract their attention by concluding that liver functions can, in fact, be much lower in elderly patients than in younger subjects.

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General considerations

A thorough overview of all aspects of aging liver is beyond the scope of this review. As was emphasized, readers are advised to refer to several past artcles[1-5] as well as books[6-10] regarding these subjects. Here, only limited subjects important to clinicians will be briefly summarized.

How should routine liver function should be interpreted in elderly patients?

In the vast realm of tests known as liver function tests, most biochemical parameters such as serum enzyme activities may be evaluated in the elderly with the same criteria as are used for younger counterparts[24]. It is possible, though not clearly proven, that elderly subjects reveal slightly lower marker values for hepatocyte damage such as SGOT, GPT, and LDH, relative to healthy younger counterparts. The only exception which we will discuss is "serum albumin concentration". It is true that in many elderly patients without known liver (or kidney) diseases, the serum albumin level often tends to be lower[25]. Many clinicians in Japan, in particular geriatricians, still regard this as due to a natural aging process. It should be realized, however, that the fall in serum albumin in the elderly is a sign of morbidity rather than the result of natural aging, per se. Clinicians need be aware that as long as patients are healthy, the serum albumin level seldom falls below 4.0 g/dl for up to 90 years of human age. This does not mean, however, that elderly patients with low albumin levels have liver disorders or specific diseases. A fall in the serum albumin level can occur in the elderly without any obvious liver diseases. Rather, it is very often the result of general morbid conditions such as infections or malnutritions, or even immobility[26]. In other words, the serum albumin level is the simplest, most reliable marker for clinicians to judge whether an elderly subject is in good health or not. This was clearly demonstrated in a previous article which showed a highly significant correlation between the serum albumin level and a patient's mobility[26].

Some liver loading tests such as sulfobromophthalein (BSP) or indocyanine green (ICG) tests as well as the galactose elimination capacity test are a few exceptions which can serve as an index for liver function, rather than for liver cell damages. The BSP (or ICG) retention value in serum in a routine dye retention test has been reported to increase with age[27]. A careful study on healthy volunteers, however, has revealed that the 30 min retention value is not sifnificantly different between young and elderly subjects[28]. This finding is important because if an elderly patient shows a value above the normal range, that patient should be regarded as unhealthy. Again, this does not definitely indicate that the patient has a liver disease. A number of mechanisms other than liver diseases appear to cause an abnormal dye retention value. This is very important information regarding the care of elderly patients, especially when patients are treated with drugs that are eliminated through the hepatobiliary system.

Wynne et al. examined ICG clearance in elderly subjects. They concluded that ICG clearance is a reliable index for blood flow of the liver in the elderly[29]. This premise is accurate as long as the subjects are healthy; however, in many elderly patients with no obvious liver disease, an abnormal increase in ICG retention value may be manifested. As emphasized above, this does not definitely indicate either the existence of liver disease or reduced blood flow in these patients. It is possible that in these patients the ratio of extraction of the dye by the liver (and possibly its biliary excretion) is reduced by mechanism(s) unrelated to specific liver disease. As in the case of serum albumin concentration, the ICG test is also useful when we need to evaluate the health of a patient's liver, since a value exceeding "the normal range" can be regarded as abnormal in the elderly also.

Possible susceptibility to liver diseases and drug-induced liver damage in the elderly

In hepatology textbooks, it is frequently stated that in the elderly, the clinical course of acute viral hepatitis may be more severe[30]. Similarly, drug-induced liver damage may lead to a more severe clinical course. There is insufficient evidence to prove the first issue to the knowledge of the author. At the same time, there are not enough

data to exclude such a possibility. This is an uncertainty that clinical hepatologists may solve in the future. Vulnerability to drug-induced liver damage in the elderly is another difficult issue to be concluded. The tentative conclusion, which James and his group[31] reached, was that the incidence of drug-induced liver damage does not increase with age. However, once liver damage is induced in the elderly, it may tend to follow a more severe course. The author agrees with this conjecture, but it is obvious that we need much more information in this regard to reach a more general conclusion. The question of whether or not, and if so, how, liver diseases in elderly patients differ from those in the young also awaits an answer in the future specific to each liver disease[31].

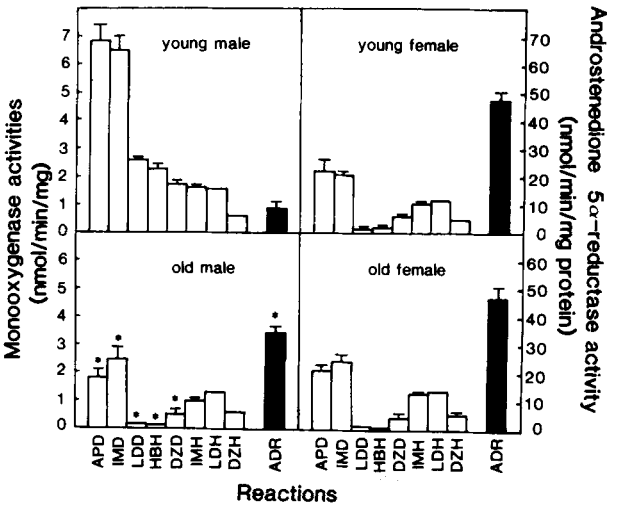

Fig. 1 Hepatic microsomal drug-metabolizing enzyme activities in young (6-month-old) and old (30-month-old) male and female Fischer-344 rats. *Significantly different from corresponding activities in young male rats. Abbreviations: APD, aminopyrine N-demethylase; IMD, imipramine N-demethylase; LDD, lidocaine N-deethylase; HBH, hexobarbital hydroxylase; DZD, diazepam demethylase; IMH, imipramine hydroxylase; LDH, lidocaine hydroxylase; DZH, diazepam hydroxylase, ADR, androstenedione 5 α-reductase. Decline with age for enzyme activities occurs only in male (but not in female) rat livers. In old age, enzyme activity patterns of male rat liver become identical to those in (young and old) female rat liver. (Reproduced with permission from the publisher and authors. Fujita S, et al.: Effect of senescence on the metabolism of drugs affecting the central nervous system in rats and mice, In: Kitani K, ed. Liver and aging–1986, Liver and brain, Amsterdam: Elsevier Science Publishers, 1986:103–114).

An attempt to eliminate the myth that liver ages in man

Many clinicians and basic scientists probably feel that, when they reach the fifth decade in their lives, the functions of their organs start to deteriorate. Thus, before they initiate research to determine whether organs in the elderly are functioning less efficiently than in the young, they may often have a bias that organ functions, including that of the liver, are lower in the elderly. When they obtain results showing that the elderly have lower parameters for organ functions, they (including refereees in many journals) accept it as a natural, reasonable, and not surprising fact. The opposite idea is more difficult to accept. Once, the author submitted a manuscript to a gerontology journal which showed no difference in liver functional parameters between young and old animals. The comment of one referee was "Why?". In my opinion, this question should not have been directed to the author of the manuscript, but to scientists in general. Before we try to answer such a question, we first need to accept the facts. It is surprising that liver functions, in particular enzyme activities, are maintained so steadily even very late in life (**Figs. 1 and 2**) in many animal species. It is true, as mentioned earlier, that if P-450 functions in the liver in male rats of different ages are determined, drastically lower enzyme activities for many reactions are observed in older rats (for review, see refs. 11–13) (**Figs. 1 and 2**). Unfortunately, many past reports demonstrating such a decline have concluded that a similar process may also occur in the human liver. As is evident in **Figures 1 and 2**, if we observe identical parameters in female rat liver, we do not see such a decline during the life of the animal. The decline in P-450 concentration as well as many P-450-dependent enzyme activities observed in male rat liver has been shown to be due to the feminization of the P-450 isozyme population of male rat liver with age[32,33] (**Fig. 2**). In contrast, practically nothing happens in female rat liver with aging in terms of either P-450 concentration or enzyme activities, since the P-450 isozyme proportion pattern in

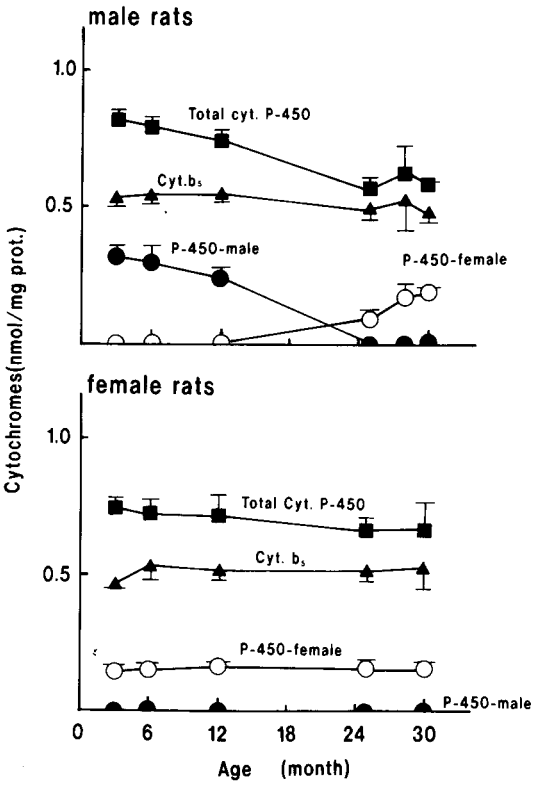

Fig. 2 Age-related changes in the contents of cytochrome (Cyt) P-450 and Cyt b5 in liver microsomes of male (A) and female (B) rats. Sex-specific P-450 isozyme pattern changes with age only in male (but not in female) rat livers. In old age, isozyme patterns are identical for male and female rat livers. (Reproduced with permission from the publisher and authors. Kamataki T, et al.: 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 1985;233(1):222-228).

female rat liver does not change with age (**Figs. 1 and 2**). Accordingly, the drastic decline in enzyme activities observed in male rat liver during aging should be regarded as a phenomenon specific to male rat liver and should not be extrapolated to humans. Thus, the primary question of whether P-450-dependent functions decline in human liver with age cannot be answered from these rodent data. Recent studies on liver from nonhuman primates[34,35] as well as humans[36,37] all agree that enzyme activites do not decline with age, although it must be mentioned that such data on human liver are too limited to draw a final conclusion on this issue.

An indirect estimate of enzyme activities in the liver is possible by using pharmacokinetic parameters such as systemic clearance values for drugs primarily metabolized by the liver. Many data have shown a decrease in clearance values with age, suggesting that enzyme activities may decline with age in human liver. However, many other data have shown no significant difference between young and old subjects for their clearance values (see refs. 11-13). This discrepancy in clinical pharmacokinetic data will discussed in greater detail in the next section, in particular, in relation to frailty and morbidity of elderly patients. It must be realized that there is no conclusive data that P-450 functions decline with age in the human liver.

It should be made clear that as long as we reseach rat liver for this type of aging study, we are unable to eliminate sex-related differences. The P-450 system is not the sole exception. A variety of phase II detoxifying systems in the liver such as glutathione S-transferase, glucuronosyl transferase, and sulfotransferase are also known to be very sex dependent in the rat in terms of age effects on these enzyme activities. Unlike the P-450 system which generally manifests higher enzyme activities in male than in female rat liver at a young age, these latter reactions often show higher enzyme activity values in female rat liver at a young age. The consequence of the feminization of male rat liver with age is that enzyme activities which are higher in young male than in females tend to decline, but those which are lower in young males tend to increase with age, all approaching the respective values of female rat liver, while in females very little change occurs duing aging[16,17]. However many important studies showing age-related changes in male rat liver in the past have misinterpreted them as an age-dependent changes. There are other examples. Enzymes handling different kinds of alcohol in the liver also decline with age in male rat liver but essentially stay unchanged with age in females[18-20]. A recent study by Rikans et al. further clearly revealed that age-dependent decline in activities of catalase in the liver is seen only in male but not in female rat liver[21]. Instead, the catalase activity

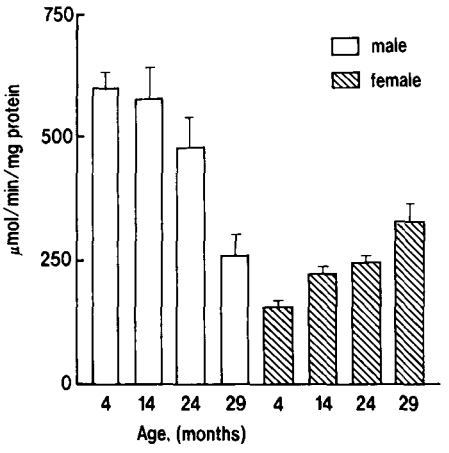

Fig. 3 Age-related changes in catalase activities in livers of Fischer 344 rats of both sexes. While activities in male rat liver tend to decline with age, in female rat liver the opposite tendency (i.e., an increase with age) is clearly oserved. (Reproduced with permission from the publisher and authors. Rikans LE, et al.: Sex-dependent differences in the effects of aging on antioxidant defense mechanisms of rat liver. Biochim Biophys Acta 1991;1074:195–200).

showed a gradual increase with age in female rat liver (**Fig. 3**). Our own recent study also confirmed this important fact (Kitani et al., unpublished observations).

The above fact is not well recognized even by many experimental gerontologists. For example, some experimental gerontologists have attempted to explain the decline in P-450 functions in terms of aging theory such as changes in membrane fluidity[38,39], lipid peroxidation and eventual production of malondialdehyde[40], or intracellular condensation due to an increase in potassium concentration and decrease in water content[41]. It is clear that since in female rat liver and most probably in human liver as well, such a decline does not occur with aging per se, the generation of hypotheses for underlying mechanism(s) of such a decline which occurs only in male rat liver does not bear a rationale in terms of an aging theory. A recent sophisticated study discussing the decline in the SOD activity in the liver by another group[23] by using a molecular biology technique should also be interpreted with great caution, since in female rat liver the SOD activity does not decrease with age[21]. For this particular and very important, enzyme activity, the results are quite contradictory, since in some studies the activity was shown to remain unchanged with age even in male rat liver[21]. As an experimental gerontologist who immigrated from his original field of hepatology, the author welcomes the increasing interest of hepatologists in aging research. However, it is the position of the author that the true aspect of aging liver is difficult to obtain from a simple comparison of parameters between young and old animal livers, and that the interpretation of the data obtained from a study using a single sex of a single strain needs to be carefully limited in order to avoid generalization.

Another important belief of the author is that the proper selection of age groups of rodents is of paramount importance for the interpretation of data. In general, up to 3 to 6 months of rat age should be regarded as the development period and up to 1 year should be regarded as the young adult stage. A simple comparison of 1-(or 2-) month-old animals with older animals focuses only on changes occurring during the development of animals (for details, see ref. 42). Many previous studies are difficult to interpret in terms of the effects of aging because of the improper selection of animal ages. If should be emphasized that many physiological parameters such as plasma volume, liver size, liver to body weight ratio, and liver blood flow, and biochemical parameters as well are changing rapidly during the first 6 months of rat life, but that thereafter we see surprising stabbility in these basal functions of the liver, as shown in **Figure 4** (for a review, see ref. 42).

It is clear from **Figure 4** that changes in the first six months in rodent life should be regarded in general as developmental changes. A good example is the change in GSH turnover with age. Several studies have shown a drastic (almost straight line) decrease in this parameter with age[43,44]. All of these studies[43,44] worked on relatively young rats up to 6 months, judging from their body weight changes. If these values are extrapolated, the turnover of GSH becomes zero before the animals become 1 year old, which is not at all likely. Indeed, our preliminary studies have

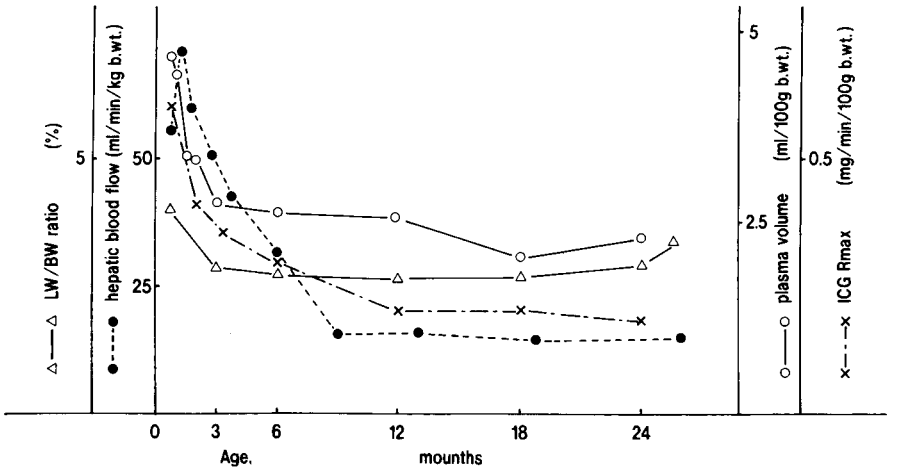

Fig. 4 A composite of age-related changes in several parameters related to liver functions in rats. O···O, plasma volume; △—△, liver to body weight ratio; ×···×, ICG maximal removal capacity; (from Kitani K, et al. No. 12): O—O, hepatic blood flow; (from Iwamoto K, Watanabe J, Araki K, et al.: J Pharm Pharmacol 1984;37:466). The figure was redrawn based on the data from above sources with permission of publishers and authors.

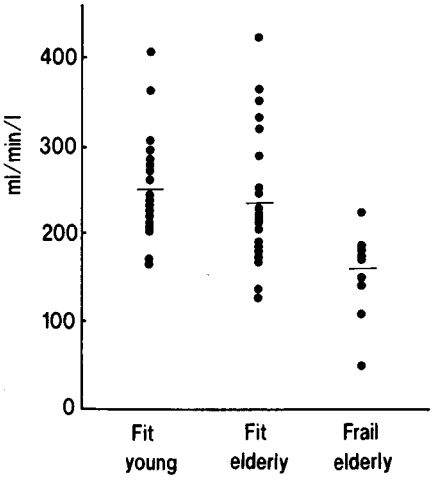

Fig. 5 Paracetamol clearance per unit volume of liver in three groups ($P<0.01$ ANOVA) (Reproduced with permission from the publisher and authors. Wynne, HA et al.: The association of age and frailty with paracetamol conjugation in man. Age and Ageing 1990; 19:419–424).

shown that GSH turnover is very stable after 6 months up to 30 months of rodent lives (Rikans et al., unpublished data).

A recent study on hepatic regeneration using a sophisticated molecular biology technique[45] is another unfortunate example. As has been discussed above, 1 year of rat age should be regarded as a "young adult stage". Because of the selection of 6-week-old rats as a young group in this study, the interpretation of the data has to be limited to only the developmental aspects of rats and nothing should be discussed in terms of aging, since "aging" is clearly considered separately from development. It is the belief of the author that essentially the same results as those reported in this study can be obtained if researchers compare animals of 1 months and 6 months, instead of 12 months of age. If a comparison were made between 6-month-old and 24-month-old (or older) animals, which is a more rational selection of rat ages for the purpose of obtaining insight into the effects of aging on liver functions, the author would not expect such striking differences as were reported in this study[45]. It is partly for this reason that the general myth that the liver ages with age still remains in an academic society.

An attempt to eliminate another myth that liver functions are the same in the elderly as in the young

In the previous section, it was emphasized that basal liver functions barely decline with aging in rodents or even in primates. While this remains basically true, it does not mean that the liver in the elderly always functions as efficiently as in the young. The liver will function even in old age as efficiently as in youth as long as the subject keeps his (or her) health, especially interms of basal (physiological) liver functions. However, once a subject is exposed to unfavorable conditions such

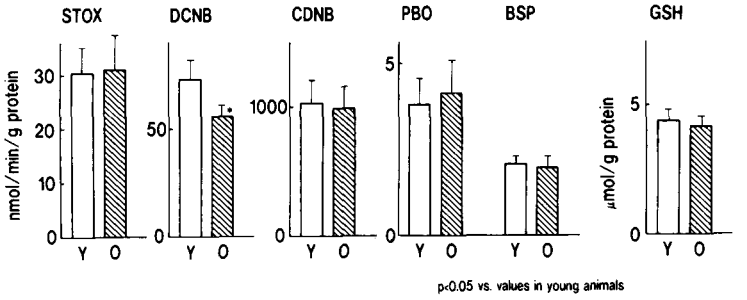

Fig. 6 Enzyme activities of GSTs toward five different substrates in liver cytosols obtained from young (8-month-old) and old (27-month-old) female C57BL mice. *Significantly different from the corresponding value in young mouse liver. Abbreviations: BSP, sulfobromophthalein; PBO, benzalacetone; STOX, Styrene oxide; DCNB, 1-2-dichloro-4-nitrobenzene; CDNB, 1-chloro-2,4-dinitrobenzene; GSH, glutathione. The figure was drawn based on the data reported previously (Carrillo MC, et al.: Difference 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 1989;47:1–15) with permission from the publisher and authors.

as malnutrition or various kinds of morbid conditions (infections in particular), a potential difference in liver function (especially in relation to its reserve capacity) between young and old subjects will become manifest, and the liver function in the elderly will no longer be as efficient as in young subjects. Clinical evidence for this premise can be confirmed by data in **Figure 5**. When systemic clearance values of acetaminophene (paracetamol), were compared between young and old nonfrail and frail subjects, clearance values corrected for liver size did not differ between young and old nonfrail subjects. Only frail elderly subjects showed significantly lower clearance values than both young and old healthy subjects[46]. This study has important implications. First, from the clinical point of view, it is important for clinicians to realize that although many pharmacokinetic studies have shown comparable clearance values for young and old (healthy) subjects, we cannot rely on such data in our daily patient care, since most subjects who are prescribed drugs are *unfit patients* rather than fit elderly. Accordingly, we must always bear in mind that the pharmacokinetics related to liver functions in our *old patients* may differ considerably from those of young patients. An exact comparison of the effects of frailty in young and old subjects is difficult to perform. Furthermore, it is not clear what kinds of factor(s) related to frailty really affect the kinetics leading to lowered clearance values in the frail elderly. **Figures 6 to 8** may compensate for at least in part, the lack of such clinical information. When we examined glutathione S-transferase (GST) activities toward 5 different substrates between young (9 months) and old (27 months) female C57Bl mice, enzyme values were almost identical for 4 substrates out of 5[14]. Only for 1,2-dichloro, 4-nitrobezene (DCNB) were the values statistically different between the two age groups. Thus, the first premise that basal liver functions are maintained well up to old age has been confirmed. However, when animals were fed a protein-free diet (PFD) for one week, all values declined significantly in comparison to respective control (basal) values and the decrement was generally greater in old animals[14]. More importantly, when animals were refed a normal diet (ND) after one week of PFD, in young mouse liver all enzyme activities rapidly increased and on day 2 or 3 of ND refeeding, all values exceeded respective basal values showing "an overshoot phenomenon". After 7 to 9 days of ND refeeding, they decreased to basal levels. In contrast, in old animal liver, there was no such overshoot and values tended to slowly return to their respective basal levels (**Fig. 7**). Thus, the biggest difference between young and old animals was observed in the early recovery period with ND[14]. We have recently repeated the study in female rats[15], and observed essentially similar data. However, in this case, the intervals for achieving the peak (due to overshoot)

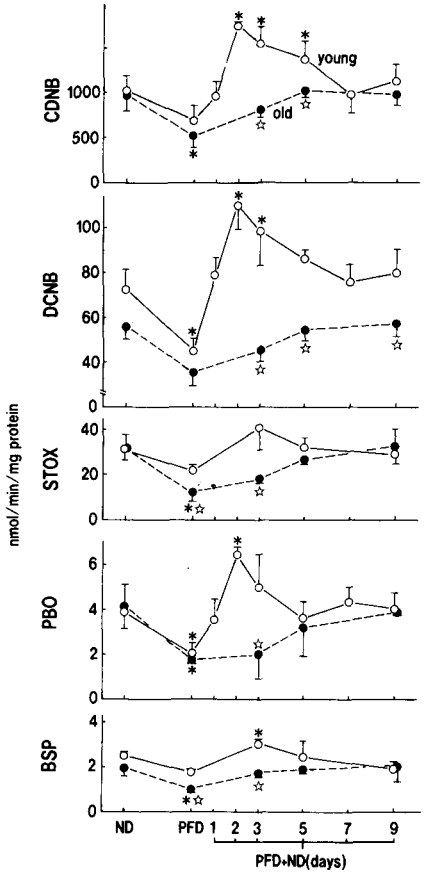

Fig. 7 Changes in activities of GSTs toward five different substrates before and during diet manipulation in female C57BL mice. * Significantly different from corresponding values in control mice with normal diet (ND) only ($P<0.05$). ☆ Significantly different from corresponding values in young mouse liver ($P<0.05$). Abbreviations and symbols are the same as in figure 6. (Reproduced with permission from the publisher and authors. Same article as Figure 6.)

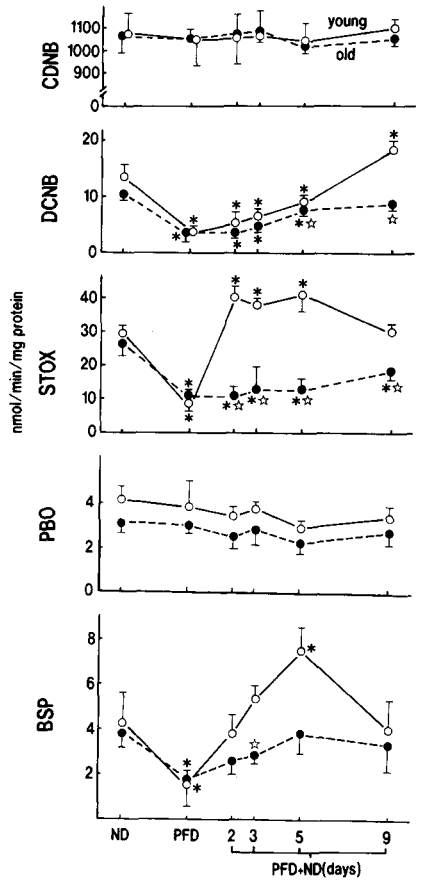

Fig. 8 Changes in activities of GSTs toward 5 different substrates before and during diet manipulation in female F-344 rats. * Significantly different from corresponding values in control rats with ND only ($P<0.05$). ☆ Significantly different from corresponding values in young rat liver ($P<0.05$). (Reproduced with permission from the publisher and authors. Carrillo MC, et al.: Effect of protein-free diet on activities and subunits of glutathione S-transferase in livers of young and aged female rats. Mech Ageing Dev 1990;56:237–251).

activities by ND refeeding in young animal liver differed depending on the substrates used, showing a more complex adaptation mechanism for GST to diet manipulation. However, a clear difference between young and old rat livers could be observed only in the presence or absence of the overshoot after ND refeeding (**Fig. 8**). Although direct extrapolation from animal data is not allowed, it is conceivable that in many clinical situations, diet conditions (in particular, protein intake) in old patients are not optimal, thus causing a decline in enzyme activities in old patients.

In contrast with GST activities which are known to be sensitive to diet (in particular, low protein). P-450 functions are known to be impaired by infections (viral infection in particular)[47,48]. Although no hard data exist, we must also consider the possibility that elderly liver is more vulnerable to these interventions and that recovery is not as efficient as in young liver.

As stated earlier, it is true that there are many data showing a significant difference between young and old subjects in terms of clearance values metabolized by the liver[14]. While there is

no way to exclude the possibility that at least some drug metabolism in the liver declines with age, available data so far are insufficient to support this theory. A more likely possibility is that most data showing lower clearance values in the elderly are due to reduced liver volume with age and/or are derived from elderly subjects with minor frailty which has not been excluded rigorously in these past studies. For example, the initial study of O'Malley et al. showed 40% longer T 1/2 values of antipyrine in geriatric patients than in young patients[49]. However, a later study by Vestal et al.[50], that rigorously excluded morbidity of subjects showed only a 0.5% decline per year on the average[50], which is well within the range explainable by the gradual decrease in liver size with age[28].

Conclusions

In conclusion, available data are not sufficient to conclude that age *per se* compromises liver functions in general as long as subjects maintain their health. This does not mean, however, that livers in the elderly can always function as efficiently as those in the young. In many situations, where the physical condition of the subjects is impaired, the liver in the elderly will respond to these stresses more sensitively, its function may decline more drastically and the recovery of reduced function may also be slower. In other words, if clinicians find evidence of liver malfunction in the elderly with no obvious liver diseases, (e.g., lower albumin, increase in ICG test, etc.), they should suspect latent morbidity in the patient and be ready for the possible impairment for other liver functions such as drug metabolism and detoxification. In other words, the aging of the liver is not a disease, and the aging liver maintains its sound functions. However, aged liver can be a factor in the reduction of liver functions, if unfavorable conditions like morbidities accompany it. This can occur in any patient with malnutrition and/or infection and, therefore, aging can be regarded as a latent liver disease factor in countries or communities where malnutrition and infections are prevalent. In this sense, the aging liver must be regarded as a potentially diseased liver, with no specific nomenclature.

The skillful secretarial work of Ms. K. Tagami is greatly appreciated.

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