PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 165, 178–187 (1980)

Age-Related Changes in Liver Drug Metabolism: Structure vs Function (40955)

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Abstract. Several definitive statements concerning the effect(s) of animal age on liver drug metabolism are made, and at the same time certain crucial questions that remain to be resolved are posed. Between 16 and 30 months of age, or maturity and senescence, the male Fischer rat exhibits a significant loss of hepatic smooth-surfaced endoplasmic reticulum (SER) which constitutes a real quantitative change. However, this does not exclude the possibility of qualitative age-related changes in this membrane, such as compositional alterations which may be reflected in its functional integrity. Second, there is a significant decline in the noninduced activities or amounts of enzymes and hemoproteins, respectively, of the microsomal mixed function oxidase system. With the exception of the loss of cytochrome *P*-450, which represents a quantitative change, the age-dependent alterations in the two enzymes may reflect quantitative and/or qualitative changes. Last, the reduced hepatic responsiveness to phenobarbital is manifested not only in the slower rates of induction and the lower induced activities or amounts of these microsomal constituents, but also in the age-related declines in the hepatomegalic response and the induction of microsomal protein. The exact relationship between the phenobarbital-induced proliferation of the SER and the induction of drug-metabolizing enzymes as a function of age remains unclear. A sequential stereological analysis of the SER in response to phenobarbital in young, mature, and senescent animals may permit uncoupling of SER proliferation from enzyme induction and evaluation of the effect(s) of aging on each response separately.

Background. Within the past 20–30 years, the number of elderly, or people over the age of 65 years, has undergone a marked increase, especially in the United States. Although the maximum life span has not increased significantly, more people are attaining the mean estimated life span than ever before. This phenomenon is demonstrated in data from life expectancy tables (1) and in Dr. Hayflick's presentation at this symposium (2). In addition, the elderly represent the most medicated segment of society. For example, approximately 25% of all prescription drugs in the United States is destined for this aged subpopulation (3, 4). There is considerable evidence, largely from clinical studies, which demonstrates an age-dependent decline in the rate of overall drug disposition (see (5) for a review). The incidence of adverse drug reactions increases in the elderly, particularly in cases of polypharmacy (6). Unfortunately, the amount of available information concerning specific effects of aging on drug disposition and the mechanism(s) involved is extremely limited. This is attributable, in large part, to the paucity of studies on drug metabolism or disposition in geriatric patients and the limited data from experimental studies in senescent animals.

There are a number of factors which probably contribute to the age-related decline in the rate of drug disposition and most are included under changes in (a) the intestinal absorption of drugs; (b) body composition; (c) hepatic drug metabolism; and (d) renal and hepatic clearance of drugs and their metabolites. The effect of aging on intestinal drug absorption has not been well studied and there is little definitive evidence which demonstrates an age-related change. The significant reduction in splanchnic blood flow, approximately 40–50% by age 70, may contribute to slower absorption rates of lipid-soluble compounds and drugs which exhibit first-pass kinetics, for example, propranolol (see (7, 8) for reviews).

The age-related increase in body fat mass, generally at the expense of muscle mass, may extend the retention times of lipid-soluble drugs. In addition, the re-

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ported 10–15% decrease in body water content may contribute to a reduction in the volume of distribution of certain drugs (see (9, 10) for reviews). Another factor which may affect the plasma levels of unbound or pharmacologically active drugs is the degree of plasma protein binding, primarily to albumin (11). There appears to be a direct correlation between the estimated 20% reduction in the serum albumin concentration and reduced plasma protein binding of drugs as a function of age. Age-related declines in the renal clearance of drugs or their metabolites is well documented (see (12) for a review). This probably results from a combination of factors, including (a) a reduction in renal blood flow which causes a decline in the glomerular filtration rate and (b) a gradual loss of functional nephrons.

While the information concerning the age-dependent changes in the hepatic clearance of drugs is limited, Kitani *et al.* (13) demonstrated a marked age-related reduction in the biliary secretion of ouabain, a drug which is eliminated in its native state. There is considerable evidence which suggests that much of the age-dependent decline in drug disposition may be attributable to a concomitant reduction in the hepatic capacity to metabolize drugs. A large number of drugs and other xenobiotics undergo mandatory biotransformation in the liver prior to their elimination. This requires the integrity of the microsomal mixed function oxidase system which includes the drug-metabolizing enzymes. Furthermore, there is a large amount of indirect evidence which suggests an age-dependent decline in liver drug metabolism. These data have been obtained primarily in clinical studies and consist of measurements of the plasma half-lives and/or plasma clearance rates of drugs which are metabolized in the liver (see (5, 8, 9, 14–16) for reviews). Many drugs which undergo hepatic biotransformation exhibit increased plasma half-lives or decreased rates of plasma clearance as a function of patient age, including such compounds as diazepam, lidocaine, amylobarbitone, aminopyrine, and propranolol. Unfortunately, there are no direct studies on the effects of aging on liver drug-metabolizing enzymes in humans.

However, the livers of senescent rats exhibit a reduced capacity to respond to a variety of stimuli, including drugs (see (5) for a review). A classic series of studies by Kato and his associates (17–19) reported an inverse relationship between the chronological age of rats and the activities of their liver microsomal drug-metabolizing enzymes. Data from these studies, as well as those of Birnbaum and Baird (20) and Gold and Widnell (21), demonstrated age-related declines in the noninduced activities of certain liver drug-metabolizing enzymes. On the other hand, Adelman *et al.* (22, 23) did not observe an age-dependent loss in the noninduced specific activity of hepatic microsomal NADPH cytochrome *c* reductase.

Kato and Takanaka (17–19) also reported that the responses of hepatic drug-metabolizing enzymes to phenobarbital and other inducers were impaired in old animals. Even after the chronic administration of these agents, the activities or amounts of mixed function oxidase components in the livers of old rats were significantly lower than those in young animals. These data were not corroborated by Adelman *et al.* (22, 23) or Birnbaum and Baird (20) who reported similar maximally induced activities of NADPH cytochrome *c* reductase in young and old rats. Furthermore, Adelman *et al.* (22, 23) observed an age-dependent lag in the initiation of phenobarbital-induced synthesis of cytochrome *c* reductase, although this did not result in differences in the maximally induced levels between young and old rats.

Thus, there is considerable controversy surrounding the current understanding of the effects of aging on hepatic drug metabolism and its contribution to the age-dependent decline in the overall rate of drug disposition. If there is a reduction in the liver's capacity to metabolize drugs as a function of age, there is no indication as to the mechanism(s) responsible. Such an age-related decline may reflect qualitative changes, for example, increased synthesis of functionally impaired enzymes; quantitative changes, such as less functionally

competent enzymes; or a combination of both. A thorough evaluation of this phenomenon is important to our understanding of the effects of aging on drug disposition as well as the etiology of the aging process. The primary clinical value of such information is to provide an experimental and theoretical framework for interpreting observations in humans and, ultimately, for designing safe and effective drug regimens for the elderly.

Correlation of cell structure and function. The approach to the study of drug metabolism and other phenomena at the cellular level in this laboratory involves a correlated structural and functional analysis. Therefore, our interests include not only the functional capacity of the liver drug-metabolizing enzymes, but their structural correlate as well, the smooth-surfaced endoplasmic reticulum (SER). The hepatic SER is considered to be the primary intracellular site of the mixed function oxidase system. Hepatocytes typically contain two types of endoplasmic reticulum membranes: (a) a form which appears as parallel lamellae of membranes whose outer surfaces are studded with ribosomes, the rough-surfaced endoplasmic reticulum (RER); and (b) another type which appears as irregular profiles of smooth-surfaced membrane, i.e., devoid of ribosomes—the SER (Figs. 10 and 11). Jones and Fawcett (24), as well as others (25), illustrated the structural/functional relationship between the SER and the mixed function oxidase system by demonstrating that phenobarbital induces the activities of hepatic microsomal drug-metabolizing enzymes and causes a concomitant proliferation of the SER.

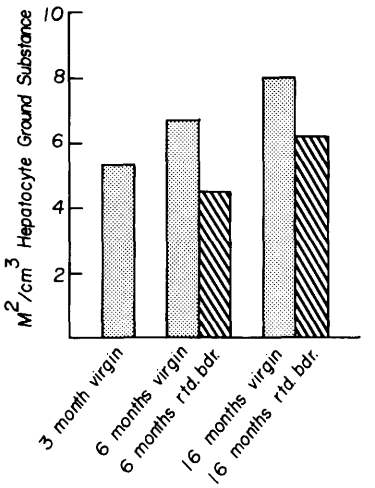

FIG. 1. The concentration of hepatic smooth-surfaced endoplasmic reticulum membrane (area per volume of hepatocyte ground substance) increases with age, at least up to 16 months, in both virgin and retired breeder male Sprague–Dawley rats. However, the virgin animals possess greater amounts of membrane in comparison to age-matched retired breeders. (Data from Schmucker, J. Gerontol. 31, 135, 1976.)

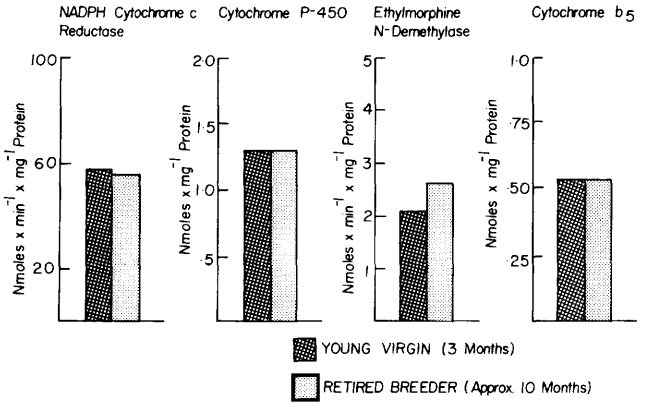

FIG. 2. Activities or amounts of several hepatic microsomal mixed function oxidase system enzymes and hemoproteins in virgin and retired breeder male Sprague–Dawley rats. There are no statistically significant differences in any of these parameters between these two groups of animals. (Data from Anthony and Jones, Biochem. Pharmacol. 25, 1549, 1976; Anthony *et al.*, J. Lipid Res. 19, 154, 1978.)

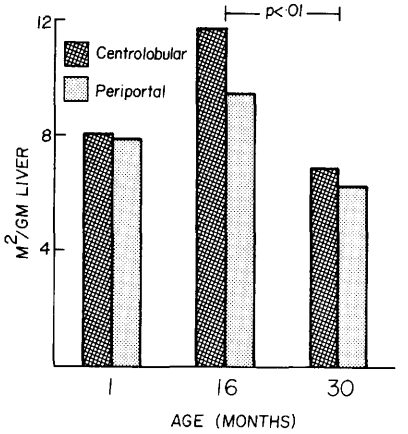

FIG. 3. The concentration of smooth-surfaced endoplasmic reticulum membrane increases in both centrolobular and periportal hepatocytes during development and maturation (1–16 months of age), but subsequently declines between maturity and senescence (16–30 months of age) in male Fischer rats. Since (1) these data were originally expressed as area per cm^3 of liver tissue (2) the specific density of rat liver tissue is approximately 1, the expression of the results as area per g of liver tissue facilitates the correlation of structural and functional data. (Data from Schmucker *et al.*, Science 197, 1005, 1977; J. Cell Biol. 78, 319, 1978.)

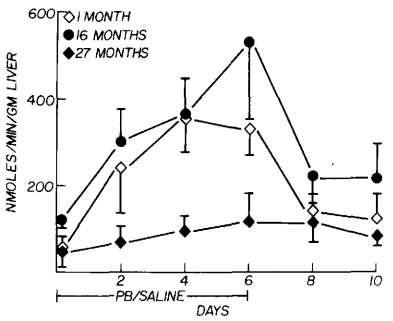

FIG. 5. Effect of animal age on phenobarbital-induced activity of liver microsomal NADPH cytochrome *c* reductase and its subsequent postinduction recovery. The rates of induction and the maximally induced levels of enzyme activity after 6 days of drug treatment are significantly greater in the young and mature rats vs the senescent animals. Similarly, the rates of postinduction recovery are faster in the two younger age groups. (Data from Schmucker and Wang, unpublished.)

Several years ago we employed this correlated structural and functional approach in studies on the effects of plasma lipid-lowering drugs on the liver. Some of these experiments included retired breeder rats as animal models. The particular strain employed (Sprague–Dawley) was characterized by hypercholesterolemia, an increased incidence of arteriosclerosis, and a shorter life span and longer narcosis time in comparison to virgin rats—suggesting that retired breeder rats may be undergoing premature aging.

An extensive stereological analysis of hepatic fine structure revealed that the concentration of the SER was significantly greater in the virgin rats vs retired breeder animals of the same age (Fig. 1) (26). This

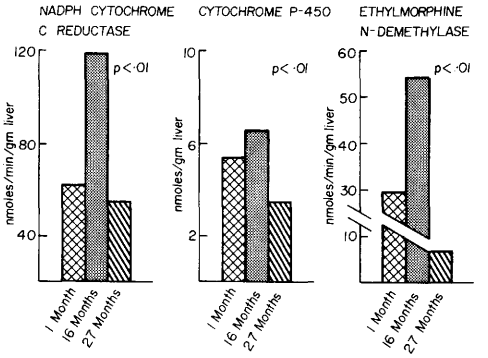

FIG. 4. Age-related changes in the noninduced activities or amounts of several components of the liver microsomal drug-metabolizing system. The activities of NADPH cytochrome *c* reductase and ethylmorphine *N*-demethylase exhibit marked increases and subsequent declines during development/maturation and senescence, respectively. Although the difference in the concentration of cytochrome *P*-450 between the young (1 month) and mature (16 month) animals is small, there is a significant loss of hemoprotein during senescence. (Data from Schmucker and Wang, unpublished.)

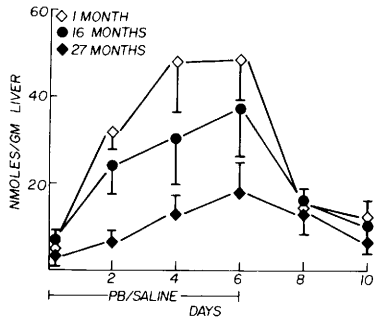

FIG. 6. Effect of animal age on the concentration of hepatic microsomal cytochrome *P*-450 during and after phenobarbital induction. The rates of induction and postinduction recovery, as well as the maximal concentrations achieved after 6 days of drug administration, were greatest in the younger age groups in comparison to the 27-month-old rats. (Data from Schmucker and Wang, unpublished.)

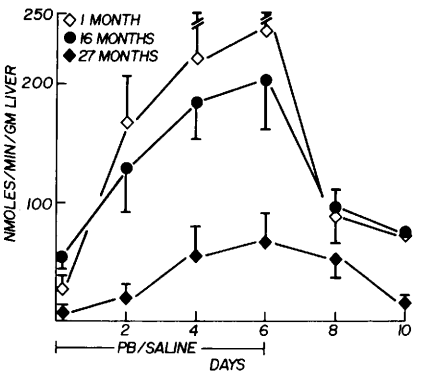

FIG. 7. Effect of animal age on the activity of liver microsomal ethylmorphine *N*-demethylase during and following chronic phenobarbital administration. Phenobarbital causes a rapid induction of this enzyme in both young and mature rats, but not in senescent animals. Although the peak induced activities are significantly higher in the younger age groups vs the 27-month-old animals, the former return to near noninduced values within 2 days of the cessation of drug treatment. (Data from Schmucker and Wang, unpublished.)

observation was of considerable interest in view of the reported age-related decline in liver drug metabolism and, therefore, permitted us to speculate that this might represent a structural correlate of the loss of functional capacity. However, subsequent studies compared the noninduced levels of several components of the microsomal mixed function oxidase system in 3-month-old virgin and ~10-month-old retired breeder rats (27). These results demonstrated that there were no statistically significant age-related differences in the specific activities of the enzymes or in the amounts of hemoproteins (Fig. 2). These data did not suggest any correlation of cell structure and function with regard to age-dependent changes in the amount of hepatic SER and the capacity of the liver microsomal drug-metabolizing system. In view of the uncertainties associated with the use of retired breeder rats in aging studies and their unresolved status as a model of premature aging, we extended our studies to a well-characterized animal model widely employed in experimental gerontology—

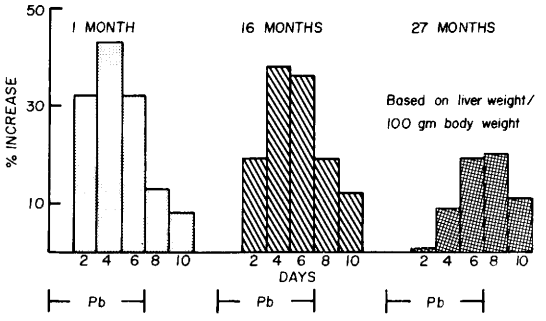

FIG. 8. Effect of animal age on the phenobarbital-induced hepatomegalic response and subsequent recovery in male Fischer rats. The increases in liver weight are similar in the young and mature animals, although the 1-month-old rats responded more rapidly during the first 2 days of phenobarbital administration. The net increase, the rate of increase, and the rate of postinduction recovery were all considerably greater in the younger age groups in comparison to the senescent rats. (Data from Schmucker and Wang, unpublished.)

male Fischer 344 rats from the aging colony maintained by the National Institute on Aging.

Stereological analysis of the livers of these animals demonstrated a significant age-dependent decline in the concentration of SER between maturity (16 months) and senescence (30 months) (Fig. 3). The increase in the amount of this membrane between 1 and 16 months of age corroborates a similar finding in the virgin and retired breeder Sprague–Dawley rats (Fig. 1) (28). In order to facilitate any comparisons between the stereological results for the Fischer rats and subsequent data on the specific activities and concentrations of microsomal enzymes and hemoproteins, respectively, both sets of data are expressed per gram of liver tissue.

The noninduced levels of three components of the microsomal mixed function oxidase system in young, mature, and senescent Fischer rats—NADPH cytochrome *c* reductase, cytochrome *P*-450, and ethylmorphine *N*-demethylase—exhibited very significant age-related declines between maturity and senescence (Fig. 4). Interestingly, the noninduced activity of NADPH cytochrome *c* reductase is similar in the young (1 month) and senescent (27 months) animals, perhaps confirming the earlier observation of Adelman *et al.* (22, 23). Furthermore, these data clearly demonstrate a critical problem inherent in two-point aging studies—if only very young and old age groups are compared, the real age-related change(s) may go unnoticed.

There is a reasonably good correlation between these structural and functional data which may be indicative of an age-related change in the quantity of hepatic SER and, thus, in its constituent enzymes and hemoproteins. Subsequent studies were designed to evaluate the effect(s) of animal age on the hepatic response to a drug stimulus. Male Fischer 344 rats at 1, 16, and 27 months of age were treated with sodium phenobarbital or saline for up to 6 days, at which time the drug was withdrawn and the animals permitted to recover for an additional 4 days. Phenobarbital (60 mg/kg body wt/day) caused a greater and more rapid induction of microsomal NADPH cytochrome C reductase activity in young and mature rats in comparison to senescent animals (Fig. 5). Even after 6 days of phenobarbital administration, the level of activity in the old rats did not approach that measured in either of the younger age groups. However, as early as 2 days post-induction, there were no significant age-related differences in the specific activity of this enzyme. Similar patterns were found for the rates of induction and postinduction recovery of the hemoprotein cytochrome *P*-450 (Fig. 6) and the enzyme ethylmorphine *N*-demethylase (Fig. 7).

These data demonstrate that there is an

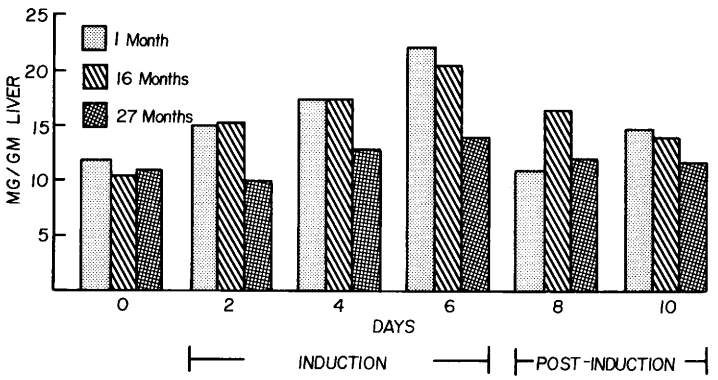

FIG. 9. The concentration of hepatic microsomal protein in response to phenobarbital administration and subsequent withdrawal in young, mature, and senescent male Fischer rats. Phenobarbital causes a more rapid and marked increase in microsomal protein in the 1- and 16-month-old animals vs the 27-month-old rats. (Data from Schmucker and Wang, unpublished.)

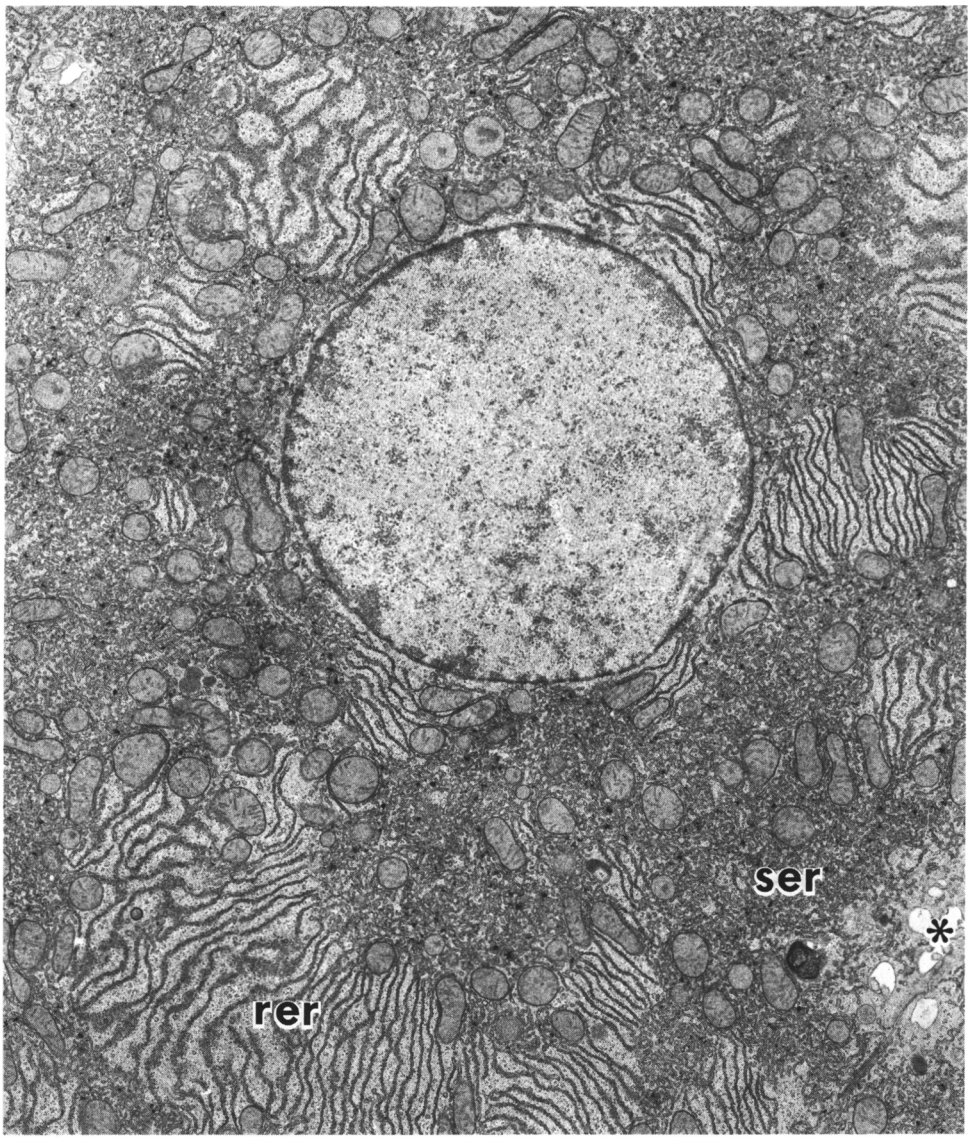

FIG. 10. Portion of a hepatocyte from a 16-month-old male Fischer rat after 6 days of phenobarbital administration (60 mg/kg body wt/day). The cytoplasm contains large aggregates of smooth-surfaced endoplasmic reticulum membrane profiles (ser). The amount of rough-surfaced endoplasmic reticulum (rer), characterized by parallel lamellae of membranes whose outer surfaces (extracisternal) are studded with ribosomes, does not appear to be diminished by this treatment. *, Bile canaliculus. ~5000×.

age-dependent decline in the responsiveness of the hepatic microsomal drug-metabolizing system and that this does not disappear even after chronic drug administration. Although the reduction in the concentration of cytochrome *P*-450 is indicative of a loss of hemoprotein, interpretation of the changes in the enzyme specific activities is less clear. For example, are these declines the result of a lower rate of synthesis of these enzymes or are they functionally impaired? The possibility also exists that the age-dependent decline in the inducibility of these microsomal components may be related to and, in fact, may reflect a concomitant reduction in the drug-induced

proliferative capacity of the hepatic SER. Studies currently in progress are attempting to isolate and purify these microsomal enzymes and to determine whether or not they undergo an age-related change in quality, i.e., absolute activity.

Phenobarbital, as well as numerous other compounds, causes hepatomegaly or an increase in liver weight and size. This hepatic response to drugs exhibits an age-dependent pattern similar to that of the enzymes—the extent of hepatomegaly is similar in the young and mature animals and this increase is significantly greater than in the senescent rats (Fig. 8). Some of this hepatomegalic response may be attributed to the increase in the concentration of microsomal protein which is also greater in

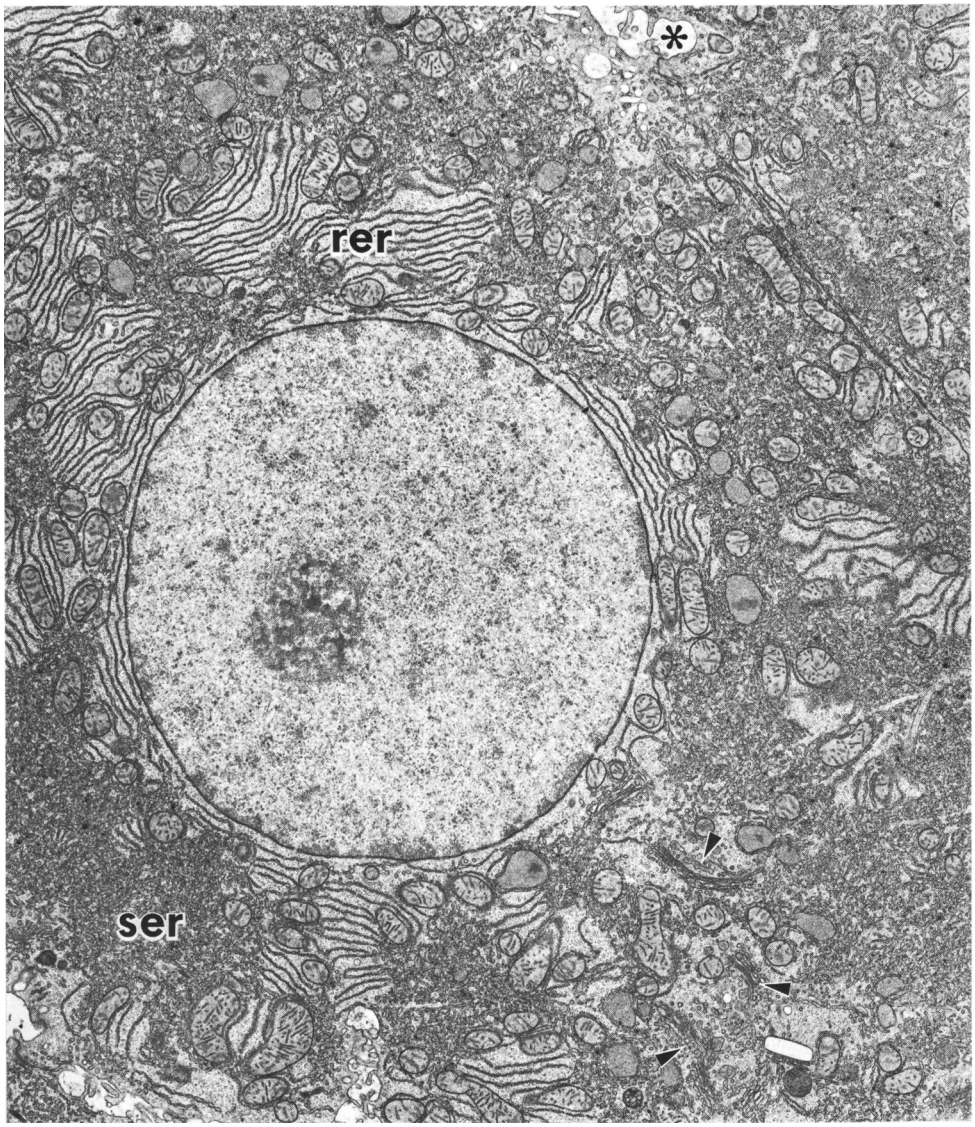

FIG. 11. Hepatocyte from a 27-month-old male Fischer rat following phenobarbital administration for 6 days. The cytoplasm is packed with profiles of smooth-surfaced endoplasmic reticulum (ser) suggesting that there is no apparent age-related decline in drug-induced membrane proliferation. rer, Rough-surfaced endoplasmic reticulum; *, bile canaliculus; arrowheads, Golgi complexes. ~5000×.

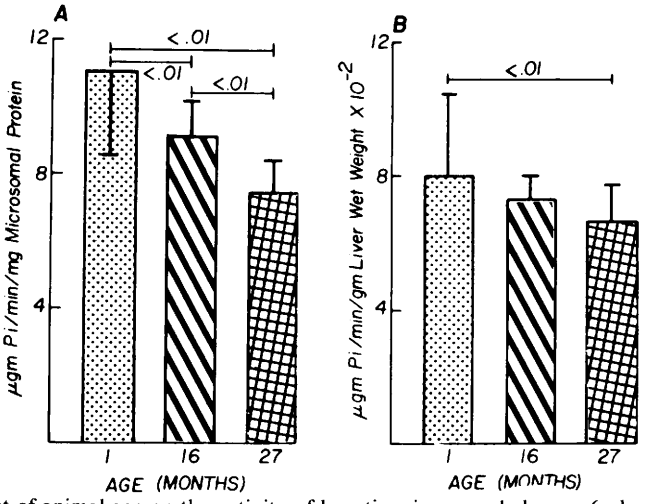

FIG. 12. Effect of animal age on the activity of hepatic microsomal glucose-6-phosphatase. Unlike the marked declines in microsomal drug-metabolizing components, glucose-6-phosphatase exhibits a gradual age-related decline in its specific activity whether expressed per mg of microsomal protein (A) or per g of liver tissue (B). (Data from Schmucker and Wang, Exp. Gerontol., **15**, 7 (1980)).

the 1- and 16-month-old rats vs the 27-month-old animals (Fig. 9). This may be indicative of an age-related decline in the phenobarbital-induced proliferation of hepatic SER. However, we do not, as yet, have any definitive stereological data to support this assumption and a preliminary qualitative electron microscopic examination of these livers did not reveal any noticeable differences in cell fine structure (Figs. 10 and 11). The cytoplasm of hepatocytes from 16-month-old (Fig. 10) and 27-month-old (Fig. 11) Fischer rats treated with phenobarbital for 6 days contains large aggregates of SER.

While there appears to be some correlation between these structural and functional data, the results are complicated and not easily interpreted. For example, consider another liver microsomal enzyme which resides primarily on the SER and is unrelated to the mixed function oxidase system, i.e., glucose-6-phosphatase. This enzyme exhibits a gradual decline in activity throughout the entire life span of the Fischer rat, rather than between maturity and senescence, and does not reflect the age-dependent decline in the concentration of hepatic SER (Fig. 12) (29).

The research reported here was supported by the Veterans Administration, NIH Grants AG 00357 and AM 25878, and Liver Center P50 AM 18520. The authors thank Ms. Jill S. Mooney for assisting in the electron microscopy analyses.

1. Comfort, A., "Ageing: The Biology of Senescence." Holt, Rinehart & Winston, San Francisco (1964).
2. Hayflick, L., Proc. Soc. Exp. Biol. Med. **165**, 206 (1980).
3. Butler, R., "Why Survive: Being Old in America." Harper & Row, New York (1975).
4. Rabin, D., Publ. HSM 73-3012, Reprint Series. USPHS, DHEW, Washington, D.C. (1972).
5. Schmucker, D., Pharmacol. Rev. **30**, 445 (1979).
6. Krupka, L., and Verer, A., Gerontologist **19**, 90 (1979).
7. Richey, D., *in* "The Physiology and Pathology of Human Aging" (R. Goldman and M. Rockstein, eds.), p. 59. Academic Press, New York (1975).
8. Richey, D., and Bender, A., Annu. Rev. Pharmacol. Toxicol. **17**, 49 (1977).
9. Crooks, J., O'Malley, K., and Stevenson, I., Clin. Pharmacokinet. **1**, 280 (1976).
10. Lee, P., Alcoholism Clin. Exp. Res. **2**, 39 (1978).
11. Bender, A., Post, A., Meier, J., Higson, J., and Richard, G., J. Pharm. Sci. **64**, 1711 (1975).
12. Gorrod, J., Gerontol. Clin. **16**, 30 (1974).
13. Kitani, K., Kanai, S., Miura, P., Morita, N., and Kisahara, M., Exp. Gerontol. **13**, 9 (1978).
14. O'Malley, K., Crooks, J., Duke, E., and Stevenson, I., Brit. Med. J. **3**, 607 (1971).
15. Triggs, E., and Nation, R., J. Pharmacokinet. Biopharm. **3**, 387 (1975).
16. Triggs, E., Nation, R., Long, A., and Ashley, J., Eur. J. Clin. Pharmacol. **8**, 55 (1975).

17. Kato, R., Vassarelli, P., Frontino, G., and Chiesara, E., Biochem. Pharmacol. **13**, 1037 (1964).
18. Kato, R. and Takanaka, A., J. Biochem (Tokyo) **63**, 406 (1968).
19. Kato, R. and Takanaka, A., Japan. J. Pharmacol. **18**, 389 (1968).
20. Birnbaum, L., and Baird, M., Exp. Gerontol. **13**, 299 (1978).
21. Gold, G., and Widnell, C., Biochim. Biophys. Acta **334**, 75 (1974).
22. Adelman, R., Freeman, C., and Cohen, B., Advan. Enzyme Regul. **10**, 365 (1972).
23. Adelman, R., Fed. Proc. **54**, 179 (1975).
24. Jones, A., and Fawcett, D., J. Histochem. Cytochem. **14**, 215 (1966).
25. Orrenius, S., Ericsson, J., and Ernster, L., J. Cell Biol. **25**, 627 (1965).
26. Schmucker, D., J. Gerontol. **31**, 135 (1976).
27. Anthony, L., Schmucker, D., Mooney, J., and Jones, A., J. Lipid Res. **19**, 154 (1978).
28. Schmucker, D., Mooney, J., and Jones, A., J. Cell Biol. **78**, 319 (1978).
29. Schmucker, D., and Wang, R., Exp. Gerontol., in press (1980).

Received June 15, 1980. P.S.E.B.M. 1980, Vol. 165.