Hepatic fine structure in young and aging rats treated with oxandrolone: a morphometric study

Douglas L. Schmucker and Albert L. Jones

Cell Biology Section, San Francisco Veterans Administration Hospital, and the Departments of Anatomy and Medicine, University of California, San Francisco, California 94121

Abstract Hepatic fine structural alterations induced by short-term administration of the hypolipidemic drug oxandrolone were evaluated using morphometric techniques. These changes are described in the livers of normolipidemic young adult and hyperlipidemic retired breeder male rats. Retired breeder rats, characterized by hyperlipidemia and a high incidence of arteriosclerosis, are thought to undergo premature aging. A previous morphometric study has shown that the hepatocytes of retired breeder rats are larger, contain a greater volume fraction of lysosomes, and have significantly less smooth-surfaced endoplasmic reticulum than those of young adult rats. However, after oxandrolone administration, the livers of these two animal groups were no longer distinguishable on the basis of these morphometric parameters. Unlike a number of other hypolipidemic drugs, oxandrolone does not induce a marked proliferation of hepatic microbodies. The effect of oxandrolone on the livers of prematurely aging rats suggests that the age-related fine structural changes are not the result of irreversible alterations in the genome or translation–transcription apparatus but may actually represent secondary reactions to extrahepatic and/or endocrine metabolic changes. The relationship between (*1*) aging and hyperlipidemia and (*2*) aging and the reduced hepatic capacity to metabolize drugs suggests a need to evaluate the effects of lipid-lowering drugs on the livers of old as well as young animal models.

Supplementary key words Anavar · quantitative electron microscopy · hepatic drug effects · membrane proliferation · endoplasmic reticulum · microbodies · aging

The relationship between hyperlipidemia and the increased risk of atherosclerotic heart disease (1, 2) has stimulated research on plasma lipid-lowering drugs (3). Oxandrolone (17-α-methyl-2-oxa-5-α-androstan-17β-ol-3-one, Anavar), a synthetic anabolic steroid (4), is effective in lowering the plasma triglyceride levels in patients with several types of hyperlipoproteinemia (5–7). Previous studies have demonstrated that oxandrolone lowers the plasma cholesterol levels in hyperlipidemic retired breeder male rats (8, 9). This steroid has been used extensively in Brazil for the treatment of hyperlipoproteinemia and is being used more frequently in this country for muscle development in athletes (10) and as a growth stimulant for stunted children (11). However, little is known regarding its mechanism of action or possible adverse side effects.

Quantitative data obtained from morphometric analysis of drug-induced ultrastructural changes can be meaningfully correlated with functional studies and are often more sensitive than biochemical methods. The rationale for investigating the effects of oxandrolone on hepatic fine structure is twofold: (*1*) the liver, a primary site of drug metabolism, may undergo toxic changes at the cellular level, and (*2*) because certain hypolipidemic drugs are thought to affect hepatic lipoprotein synthesis and/or secretion (12),[1] any pharmacological response may be indicative of the subcellular site or mechanism of drug action.

The hepatic fine structural changes associated with short-term oxandrolone administration have not been evaluated as thoroughly as those induced by clofibrate (13–18). Horvath et al. (19) reported a marked hypertrophy of the smooth-surfaced endoplasmic reticulum (SER) after acute oxandrolone administration, but no definitive quantitative studies, either biochemical or morphometric, have been published. Contrary to the findings of Horvath et al. (19), studies in this laboratory have been unable to demonstrate an oxandrolone-induced SER proliferation in rat liver based on qualitative electron microscopy and quantitative biochemical criteria, i.e., total microsomal protein.[2]

The incidence of hyperlipidemia increases with age in both rats (20, 21) and humans (22). Aging also results in a reduced hepatic capacity to metabolize drugs (23). The potential hepatotoxicity of lipid-lowering drugs in aged subjects should be evaluated prior to their therapeutic application.

The retired breeder rat has been suggested as a model of premature aging and is characterized by obesity (24),

Abbreviations: RB, retired breeder; YA, young adult; RER, rough-surfaced endoplasmic reticulum; SER, smooth-surfaced endoplasmic reticulum.

[1] Schmucker, D. L., R. Bills, and A. L. Jones. Unpublished observations.

[2] Mills, E. S., A. L. Jones, and B. M. Kriz. Unpublished observations.

hyperlipidemia (8, 25, 26), a high incidence of arteriosclerosis (27, 28), and a shorter life span than virgin rats (29). A recent morphometric study has shown that the hepatocytes of retired breeder rats are larger and they contain a greater volume fraction of lysosomes or dense bodies and have less SER surface area than those of young adult rats of the same strain (30). Mills, Kriz, and Jones (8) have demonstrated the advantages of employing the retired breeder rat as a physiological animal model for the study of hyperlipoproteinemia. Thus, the purpose of the present study is to quantitatively describe the fine structural changes induced by oxandrolone in the livers of normolipidemic young adult and hyperlipidemic aging rats, i.e., the retired breeder.

METHODS

Animals

This study is based on a quantitative electron microscopic evaluation of the livers of 18 male Sprague-Dawley (Holtzman strain) rats. Nine retired breeder (RB) (500 g body wt) and nine young adult (YA) (350 g body wt) rats, obtained from the Holtzman Company, Madison, Wis., were maintained on a standard diet of Purina laboratory chow and water ad lib. Oxandrolone, an extremely insoluble steroid, was administered orally by mixing the drug (100 mg/kg body wt) with 250 mg of corn-oil margarine (Fleischmann). Mills et al. (8) have found this feeding procedure to be satisfactory in that the rats readily eat the desired drug dosage, it eliminates the need for oral intubation, and the ingestion of this amount of margarine does not affect the animals' plasma lipid levels. Five RB and five YA animals were fed the drug–margarine mixture, while four RB and four YA control animals received 250 mg of the vehicle per day for 21 days. The rats were fasted for 24 hr and the last drug dosage was administered 12 hr prior to killing the animals.

Microscopy and morphometry procedures

On the morning of the 22nd day, the animals were anesthetized with sodium pentobarbital (i.p.) and their livers were perfused via the hepatic portal vein with 2.7% glutaraldehyde–0.8% paraformaldehyde in 0.2 M sodium bicarbonate buffer (pH 7.4) according to the method of Wisse (31). Small pieces of perfused fixed liver were immersed in the same fixative for 2.5 hr, rinsed in buffer overnight, postfixed in bicarbonate-buffered osmium tetroxide, dehydrated, and embedded in Spurr's epoxy resin (32).

Five tissue blocks were randomly selected from each animal providing they contained at least one central vein in cross section as determined in sections 1 μm thick. The selected tissue blocks were retrimmed to a 10–12-cell radius around the central vein. This procedure was employed to ensure a certain degree of uniformity in tissue sampling. An analysis of Epon thick sections containing both central veins and portal triads revealed a mean hepatic lobular radius of 24 cells. According to the morphometric data of Loud (33), our sampling areas may contain some mid-lobular hepatocytes along with the centrolobular cells. However, Weibel et al. (34) have disregarded sublobular variation in their morphometric analysis of the normal rat liver on the basis of extensive homogeneity throughout the hepatic lobule.

Ultrathin sections (approximately 600 Å thick) were cut from each of five tissue blocks per animal and stained with uranyl acetate and lead citrate. Five micrographs per block were taken at each of two primary magnifications, 4600× and 13,800×, and enlarged to 13,800× and 30,900×, respectively. Micrographs were taken according to an unbiased, systematic sampling procedure that necessitated that the grid square be totally covered with tissue and that two sides of the photographic field be defined by grid bars, i.e., a corner of the grid square.

For determining the volume densities of the intralobular extrahepatocyte space, hepatocytes, hepatocyte cytoplasm, nuclei, mitochondria, microbodies, lysosomes or dense bodies, and biliary space, a coherent double-lattice test system was superimposed over the low-magnification micrographs (13,800×), and points were counted according to the general procedure described by Weibel et al. (34). Microbodies and lysosomes were classified on the basis of their characteristic fine structural appearances and electron densities. The lysosomes, because of their multiplicity of forms ranging from autophagic vacuoles to residual bodies, were grouped as dense bodies. In general, the criteria used to differentiate subcellular structures were similar to those employed by Loud (33), Weibel et al. (34), and others.

A coherent, multipurpose test system (34) was used to estimate the surface densities of the rough- and smooth-surfaced endoplasmic reticula. The surface area of the Golgi membranes was estimated according to the technique of Sturgess and de la Iglesia (35). The data are expressed as the volume of a particular component per cubic centimeter of intralobular liver tissue, hepatocyte, or hepatocyte cytoplasm. Wheatley (36) has shown that 27% of adult rat liver hepatocytes are binucleate and that this percentage increases steadily with age, and because a more direct correlation of biochemical and morphometric data is realized when the latter are expressed in geometrical rather than biological units, our data are expressed in relative unit volumes. The confidence level of sampling for all estimates of volume density exceeded 95% according to the method presented by Weibel (37) for isotropic systems. A total of 900 micrographs were analyzed. Statistical analysis included the mean value, standard deviation, standard error, and a probability analysis (Student's t test).

TABLE 1. Volume densities in livers of control vs. oxandrolone-treated young adult rats

Component	Volume Density: Control (20)[a]	Volume Density: Experimental (25)[a]	P
	cm^3/cm^3		
Extrahepatocyte space[b]	0.18 ± 0.02	0.15 ± 0.02	NS
Biliary space[c]	0.06 ± 0.02	0.05 ± 0.01	NS
Hepatocyte[b]	0.82 ± 0.02	0.85 ± 0.02	NS
Nuclei[d]	0.08 ± 0.01	0.08 ± 0.01	NS
Cytoplasm	0.74 ± 0.02	0.77 ± 0.02	NS
Mitochondria[e]	0.23 ± 0.01	0.20 ± 0.01	NS
Microbodies	0.014 ± 0.001	0.015 ± 0.001	NS
Lysosomes	0.004 ± 0.000	0.005 ± 0.001	NS

[a] Values are means ± SEM; number of animals in each group is in parentheses.
[b] Volume per unit volume of intralobular tissue.
[c] Volume per unit volume of extrahepatocyte space.
[d] Volume per unit volume of hepatocyte.
[e] Volume per unit volume of cytoplasm.

RESULTS

Young adult rats

The short-term administration of oxandrolone reduced the unit volume of the extrahepatocyte space, i.e., the intralobular liver volume occupied by all components except hepatocytes, by approximately 3% in the YA animals. The average hepatocyte unit volume was increased by a similar amount, although neither of these differences was significant (**Table 1**). There was no difference between the volume density values of the biliary space, which was considered part of the total extrahepatocyte space. The small difference between the hepatocyte unit volumes was realized when the cytoplasmic volume densities were compared; the mean cytoplasmic volume fraction in the control rats (74%) was less than that found in the experimental livers (77%) (Table 1). There was no difference between the estimates of mean nuclear volumes in the two groups. The smaller nucleocytoplasmic ratio found in the drug-treated livers (0.103) compared with the control organs (0.112) supported the contention that the slight difference in the hepatocyte volume densities was attributable to a greater volume fraction of cytoplasm in the parenchymal cells of the experimental animals.

The subdivision of the hepatocyte cytoplasm into component or organelle volume fractions resulted in small differences between the experimental and control YA rats (Table 1). Although the volume densities of the mitochondria, microbodies, and lysosomes or dense bodies changed slightly, the differences did not approach the minimal level of significance ($P < 0.05$).

Changes in the SER were of interest because a variety of drugs, such as phenobarbital, cause an adaptive hepatic response expressed morphologically by a hypertrophy of this membrane system (38, 39). The mean surface density of the SER (per unit volume of cytoplasmic ground substance) increased by 35% after oxandrolone administration (**Table 2**). Similarly, the surface density of the rough-surfaced endoplasmic reticulum (RER) in the experimental animals was approximately 25% greater than the estimate for this parameter in the control rats. The surface density of the Golgi apparatus decreased by 19% after drug treatment.

TABLE 2. Surface densities in livers of control vs. oxandrolone-treated young adult rats

Component	Control (20)	Experimental (25)	P
	m^2/cm^3		
Smooth-surfaced endoplasmic reticulum	5.33 ± 0.14	7.19 ± 0.22	<0.001
Rough-surfaced endoplasmic reticulum	2.46 ± 0.17	3.08 ± 0.22	<0.05
Golgi	0.36 ± 0.05	0.30 ± 0.04	NS

Component values (means ± SEM) expressed per unit volume of hepatocyte ground substance (excludes mitochondria, microbodies, lysosomes). Number of animals in each group is in parentheses.

Retired breeder rats

A previous morphometric analysis of the hepatocytes of the YA and RB rats demonstrated several significant differences between these two age groups that may reflect the aging process (30) (compare control data in Tables 1–4). Oxandrolone does not significantly affect the volume densities of the extrahepatocyte space (14%) or the hepatocytes (86%) in comparison with the control RB rats, 13% and 87%, respectively (**Table 3**). Due to, as yet, unexplained variability, the reduction in the mean volume fraction of the biliary space in the drug-treated livers (26%) was not significant.

TABLE 3. Volume densities in livers of control vs. oxandrolone-treated retired breeder rats

Component	Control (20)[a]	Experimental (25)[a]	P
	cm^3/cm^3		
Extrahepatocyte space[b]	0.13 ± 0.01	0.14 ± 0.01	NS
Biliary space[c]	0.10 ± 0.02	0.07 ± 0.02	NS
Hepatocyte[b]	0.87 ± 0.01	0.86 ± 0.01	NS
Nuclei[d]	0.07 ± 0.01	0.08 ± 0.01	NS
Cytoplasm	0.80 ± 0.02	0.78 ± 0.01	NS
Mitochondria[e]	0.21 ± 0.01	0.23 ± 0.01	NS
Microbodies	0.014 ± 0.001	0.016 ± 0.001	<0.05
Lysosomes	0.006 ± 0.001	0.006 ± 0.001	NS

[a] Values are means ± SEM; number of animals in each group is in parentheses.
[b] Volume per unit volume of intralobular tissue.
[c] Volume per unit volume of extrahepatocyte space.
[d] Volume per unit volume of hepatocyte.
[e] Volume per unit volume of cytoplasm.

TABLE 4. Surface densities in livers of control vs. oxandrolone-treated retired breeder rats

Component	Control (20)	Experimental (25)	P
	m^2/cm^3		
Smooth-surfaced endoplasmic reticulum	4.55 ± 0.24	7.62 ± 0.31	<0.001
Rough-surfaced endoplasmic reticulum	2.75 ± 0.16	3.33 ± 0.24	<0.05
Golgi	0.26 ± 0.04	0.37 ± 0.04	<0.05

Component values (means ± SEM) expressed per unit volume of hepatocyte ground substance (excludes mitochondria, microbodies, lysosomes). Number of animals in each group is in parentheses.

The 2% reduction in the volume fraction of the cytoplasm in the experimental livers was below the accepted level of significance. However, the smaller nucleocytoplasmic ratio in the control livers (0.093) supports the morphometric data that suggest that these cells contain a greater volume of cytoplasm than those in the experimental livers (0.100). The differences in the mitochondrial and lysosomal or dense body volume densities between the experimental and control groups were not significant (Table 3). However, there was a significant change in the volume fraction of microbodies in the oxandrolone-treated livers.

Although the hepatocytes in the RB control rats contained significantly less SER than those in the YA control animals (30), oxandrolone increased the surface density of the SER in the older animals to a level 68% above the control value (**Table 4**). As a result, there was no significant difference in the SER surface densities between the RB and YA experimental livers. Oxandrolone also resulted in an increase in the surface density of the RER in the RB animals (21%) similar to that observed in the YA rats. The surface density of the Golgi apparatus increased by 41% after 3 wk of drug treatment. This increase was contrary to the drug's effect on this organelle in the young animals.

DISCUSSION

A number of hypolipidemic drugs, such as clofibrate, nafenopin, and bis-(hydroxy-ethyl-thio)1-10 decane (LL 1558), cause hepatomegaly in the rat (40–42). Previous studies have been unable to demonstrate a significant increase in the liver weight/body weight ratios in either YA or RB rats after 3 wk of oxandrolone administration.[2] Kaneko et al. (14) and Dalton et al. (43) have reported that the hepatomegalic effect of clofibrate is due to an increase in the total intracellular membrane content of the hepatocytes. Although oxandrolone increased the surface densities of both the RER and SER significantly, this change was not reflected in concomitant increases in the cytoplasmic volume fractions. The relatively small changes in the volume densities of the hepatocytes and their cytoplasm observed in the drug-treated livers demonstrates that oxandrolone does not cause significant hepatomegaly in either the YA or RB rats (Tables 1 and 3). After oxandrolone administration, the YA and RB rat livers could no longer be differentiated on the basis of their relative hepatocyte or cytoplasm volume fractions. This resulted, most likely, from a combination of the small differences between the control values for these parameters and the different, although insignificant, effects of oxandrolone on the relative volume fractions in the two experimental groups.

Several lipid-lowering drugs appear to have a marked effect on hepatic ultrastructure. However, the extent of fine structural alterations varies considerably. Clofibrate has been reported to cause no changes, transient elongation and swelling, and severe alterations, including the loss of cristae (44) in rat liver mitochondria. Horvath et al. (19) reported that oxandrolone caused variations in mitochondrial size and profile. In the present study, distinct changes in the fine structural appearance of hepatic mitochondria were not apparent in either the YA or RB drug-treated animals (**Fig. 1**). In addition, the absence of any significant differences in mitochondrial volume fractions between the control and experimental animals suggests that oxandrolone has little effect on this organelle.

Few studies have noted changes in hepatic lysosomes or dense bodies resulting from hypolipidemic drugs (14). Our qualitative and quantitative electron microscopic data demonstrate that the short-term administration of oxandrolone does not alter the distribution or volume fraction of these organelles in the livers of aging rats. Although the volume density of these bodies increased in the experimental YA livers, this increase was below the accepted level of significance. A possible explanation assumes that upon maximal SER proliferation, an adaptive increase in lysosomal enzymes occurs in order to promote optimal membrane turnover. A similar situation is thought to occur during phenobarbital-induced hypertrophy of the SER (45). Because RB livers contain a greater volume fraction of lysosomes or dense bodies than YA livers (30) and because biochemical evidence suggests that the turnover rate of these organelles is lower in the livers of aging rats (46, 47), the RB livers may already contain the maximal volume fraction of lysosomes.

Several studies with hypolipidemic drugs, including clofibrate (48–50), S-8527 (44), and nafenopin (41, 51), have demonstrated a marked proliferation of hepatic microbodies and, in most cases, a concomitant increase in catalase synthesis. Morphometric data from the present study clearly demonstrate that oxandrolone does not induce a proliferation of microbodies in the livers of YA rats. The small increase in the volume fraction of microbodies observed in the experimental RB animals (15%) has not

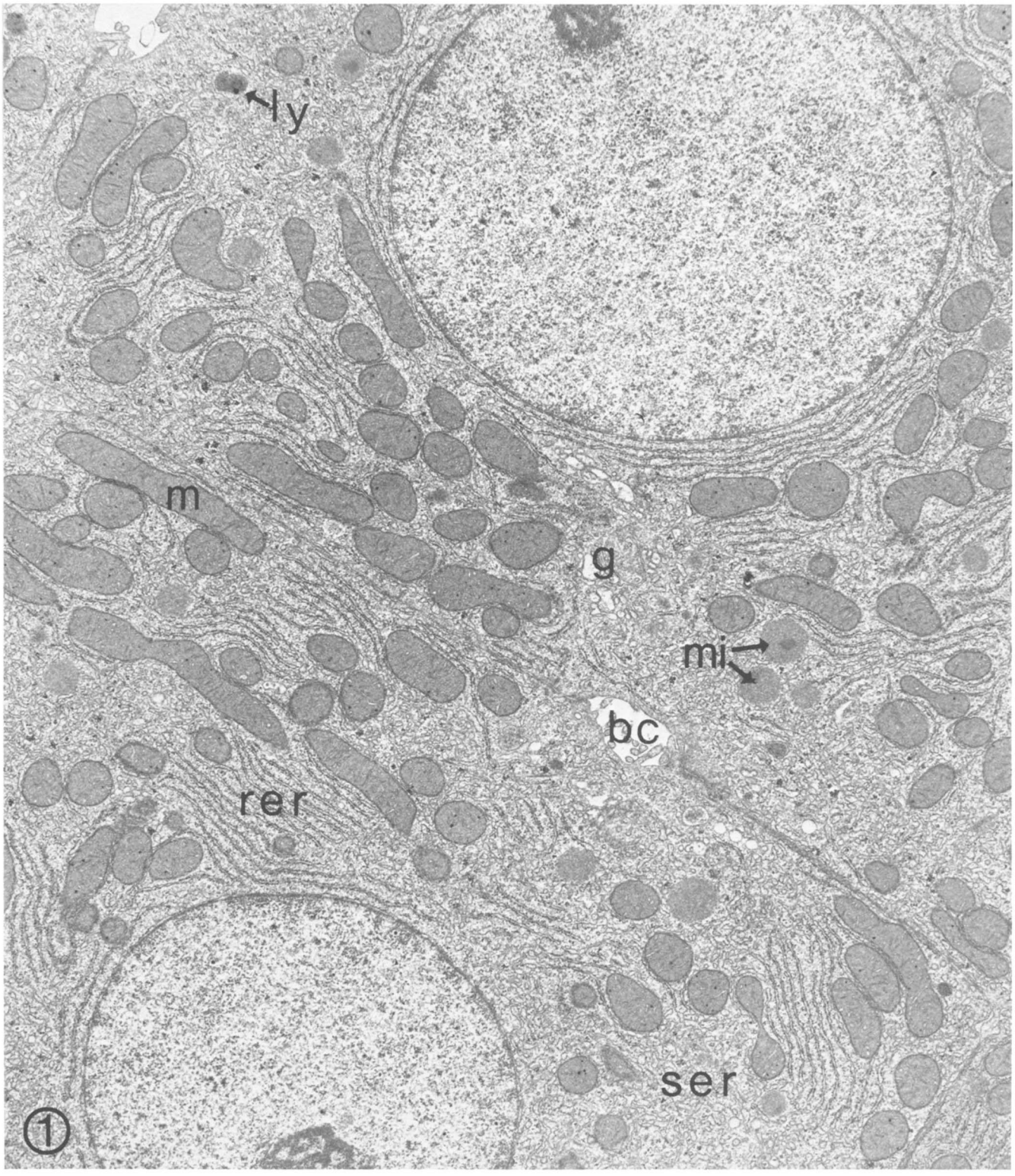

Fig. 1. Hepatocytes of an RB rat after 3 wk of oxandrolone administration. The overall cell fine structure appears quite normal. Both RER and SER membranes are extensive. Abbreviations: m, mitochondria; bc, bile canaliculus; mi, microbodies; ly, lysosomes; g, Golgi apparatus. × 12,200.

been explained. However, this increase is considerably lower than the only available estimate of clofibrate-induced microbody proliferation (approximately 76%) (49).

Svoboda and Azarnoff (40) and others have suggested that the increased number of microbodies can be correlated with the hypocholesterolemic effect of clofibrate. Other

studies, however, have indicated that this particular hepatic response to clofibrate is independent of its hypolipidemic action (48). Our morphometric data, coupled with biochemical evidence demonstrating a reduction in the plasma cholesterol levels of both YA and RB animals (8, 9), suggest that increased catalase synthesis may not be essential to the hypocholesterolemic effect of oxandrolone.

Hypertrophy of the SER membranes is a typical hepatic response to a variety of stimuli, including steroid hormones (52) and drugs (38, 39). Although several biochemical and qualitative electron microscopic studies have reported that clofibrate (15, 18, 43) and oxandrolone (19) induce SER proliferation in the rat hepatocyte, our morphometric data provide the first *quantitative* evidence that a hypolipidemic drug increases the surface density of this membrane system. Unlike the control groups, the hepatocytes of the RB and YA experimental rats could not be differentiated on the basis of their respective SER surface densities (Tables 2 and 4). The low value for the SER surface density observed in the RB control livers may represent a morphological manifestation of the reduced hepatic capacity to metabolize drugs with age (53). The period of oxandrolone administration may have been of sufficient length (3 wk) to permit maximal proliferation of SER membranes and associated enzymes in the aging RB livers.

The proliferation of the SER membranes is of particular interest because this organelle has been implicated in hepatic cholesterol (54) and lipoprotein (55) synthesis. However, preliminary studies have demonstrated that oxandrolone significantly reduces the amount of lipoprotein triglyceride separated by isolated perfused rat livers[1] and the plasma cholesterol levels of YA and RB rats. On the basis of these data, the SER proliferation observed in the present study may best be interpreted as a nonspecific pharmacological response rather than a specific induction related to the hypolipidemic effect of oxandrolone.

Horvath et al. (19) reported that the RER of rat hepatocytes became dilated and less conspicuous after acute oxandrolone administration (3 days). Electron microscopic observations in the present study failed to note any specific alterations in the appearance of this organelle. A significant increase in the surface density of the hepatic RER after hypolipidemic drug treatment has not been reported previously. Morphological observations on drug-induced SER proliferation (for a review see Ref. 50) and biochemical studies (56, 57) have provided evidence that suggests that the SER may be derived, in part, from the RER. This is one possible explanation for the increased surface densities of RER found in the experimental livers. The different responses of the Golgi membranes to oxandrolone in the RB and YA animals has not been resolved. Although the surface density of this membrane system increases in the RB rat livers, this difference is barely significant.

Quantitative analysis of the fine structural changes in the livers of young and prematurely aging rats has demonstrated that there are few age-related differences in the hepatic response to oxandrolone. A previous report of SER proliferation has been confirmed, and a significant increase in the RER surface density has been noted.

Data on intracellular membrane proliferation based on qualitative electron microscopic observations may be misleading. We would like to emphasize that morphometric techniques are an excellent means for assessing drug-induced changes in the amount or distribution of organelles, or both. Because alterations in organelle abundance and/or distribution often accompany changes in function, and because specific fine structural changes often precede demonstrable functional alterations, the application of morphometrics to the study of lipid-lowering drugs may reveal adaptive and/or toxic subcellular responses that may not be detected by routine biochemical procedures.

Finally, certain hepatic fine structural alterations thought to be associated with the aging process may not be the result of irreversible changes in the organism's genome. On the contrary, these differences may represent the structural correlates of functional changes at the organ level or higher.

The authors wish to thank Dr. Elinor S. Mills for many helpful discussions during the course of this project. The technical assistance of Mr. Jack T. Quan and Ms. Joan A. Hahn is gratefully acknowledged.

This research was supported by USPHS grant HL 16363-01 and the Research Section, San Francisco Veterans Administration Hospital.

Oxandrolone was kindly supplied by G. D. Searle Company, Chicago, Ill.

Manuscript received 3 June 1974 and in revised form 26 August 1974; accepted 12 December 1974.

REFERENCES

1. Kannel, W. B., W. P. Castelli, J. Gordon, and P. McNamara. 1971. Serum cholesterol, lipoproteins and the risk of coronary heart disease. *Ann. Intern. Med.* **74:** 1–12.
2. Albrink, M. S., and E. B. Mann. 1959. Serum triglycerides in coronary artery disease. *Arch. Intern. Med.* **103:** 4–8.
3. Kritchevsky, D. 1974. New drugs affecting lipid metabolism *Lipids.* **9:** 97–102.
4. Lennon, H. D., and F. J. Saunders. 1962. Biological activity of a new anabolic agent, SC-11585. G. D. Searle Biological Bulletin.
5. Glueck, C. S. 1971. Effects of oxandrolone on plasma triglycerides and postheparin lipolytic activity in patients with types III, IV and V familial hyperlipoproteinemia. *Metabolism.* **20:** 691–702.
6. Glueck, C. S., D. Scheel, J. Fishback, and D. Steiner. 1972. Progestagens, anabolic–androgenic compounds, estrogens: effects on triglycerides and postheparin lipolytic enzymes. *Lipids.* **7:** 110–113.

7. Faergeman, O., and F. Damgaard-Pedersen. 1973. Increase of postheparin lipase activity by oxandrolone in familial hyperchylomicronemia. *Scand. J. Clin. Lab. Invest.* **31:** 27–31.
8. Mills, E. S., B. M. Kriz, and A. L. Jones. 1973. Reduction of plasma cholesterol levels in male retired breeder rats by an anabolic steroid, oxandrolone. *Proc. Soc. Exp. Biol. Med.* **144:** 861–863.
9. Mills, E. S., B. M. Kriz, D. L. Schmucker, and J. Mayerle. 1974. Effect of aging on lipid metabolism. *Anal. Rec.* **178:** 419. (Abstr.)
10. Rosenbloom, A. L., and J. L. Frias. 1973. Oxandrolone for growth promotion in Turner syndrome. *Amer. J. Dis. Child.* **125:** 385–387.
11. Limbeck, G. A., R. Ruvalcaba, C. P. Mahoney, and V. C. Kelley. 1971. Studies on anabolic steroids. IV. The effects of oxandrolone on height and skeletal maturation in uncomplicated growth retardation. *Clin. Pharmacol. Ther.* **12:** 798–805.
12. Azarnoff, D. L., D. R. Tucker, and G. A. Barr. 1965. Studies with ethyl chlorophenoxyisobutyrate (clofibrate). *Metabolism.* **14:** 959–965.
13. Hess, R., W. Riess, and W. Staubli. 1967. Hepatic actions of hypolipidemic drugs: effect of ethyl chlorophenoxyisobutyrate (CPIB). *Progr. Biochem. Pharmacol.* **2:** 325–336.
14. Kaneko, A., S. Sakamoto, M. Morita, and T. Onoe. 1969. Morphological and biochemical changes in rat liver during the early stages of ethyl chlorophenoxyisobutyrate administration. *Tohoku J. Exp. Med.* **99:** 81–101.
15. Svoboda, D. J., and D. L. Azarnoff. 1971. Effects of selected hypolipidemic drugs on cell ultrastructure. *Federation Proc.* **30:** 841–847.
16. Reddy, J. K., and D. J. Svoboda. 1971. Ethyl-α-ρ-chlorophenoxyisobutyrate induced hepatic microbody proliferation in rat liver and ubiquinone concentration. *Experientia.* **27:** 1059–1061.
17. Reddy, J. K. 1973. Possible properties of microbodies (peroxisomes). Microbody proliferation and hypolipidemic drugs. *J. Histochem. Cytochem.* **21:** 967–971.
18. Hruban, Z., M. Gotoh, A. Slesers, and S. Chow. 1974. Structure of hepatic microbodies in rats treated with acetylsalicylic acid, clofibrate and dimethrin. *Lab. Invest.* **30:** 64–75.
19. Horvath, E., K. Kovacs, J. A. Blascheck, and A. Somogyi. 1971. Ultrastructural changes induced in the livers of rats by various steroid compounds. *Virchows Arch. B.* **7:** 348–355.
20. Hruza, Z. 1971. Effect of endocrine factors on cholesterol turnover in young and old rats. *Exp. Gerontol.* **6:** 199–204.
21. Hruza, Z., and V. Zbuzkova. 1973. Decrease of excretion of cholesterol during aging. *Exp. Gerontol.* **8:** 29–37.
22. Keys, A., O. Mickelson, E. Miller, E. Hayes, and R. Todd. 1950. The concentration of cholesterol in the blood serum of normal man and its relation to age. *J. Clin. Invest.* **29:** 1347–1353.
23. Conney, A. H. 1967. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* **19:** 317–366.
24. Wexler, B. C., C. D. Antony, and G. W. Kittinger. 1964. Serum lipoprotein and lipid changes in arteriosclerotic breeder rats. *J. Atheroscler. Res.* **4:** 131–143.
25. Wexler, B. C., and G. W. Kittinger. 1965. Adrenocortical function in arteriosclerotic female breeder rats. *J. Atheroscler. Res.* **5:** 317–329.
26. Wexler, B. C., and R. F. Lutmer. 1972. Comparison of aortic and adrenal glandular lipids between non-arteriosclerotic and arteriosclerotic rats. *Brit. J. Exp. Pathol.* **53:** 625–637.
27. Wexler, B. C. 1964. Spontaneous arteriosclerosis in repeatedly bred male and female rats. *J. Atheroscler. Res.* **4:** 57–80.
28. Wexler, B. C. 1964. Spontaneous arteriosclerosis of the mesenteric, renal and peripheral arteries of repeatedly bred rats. *Circ. Res.* **15:** 485–496.
29. Saroff, J. and B. C. Wexler. 1969. Metabolic clearance and production rates of corticosterone in male and female virgin and breeder rats. *Acta Endocrinol.* **62:** 411–424.
30. Schmucker, D. L., A. L. Jones, and E. S. Mills. 1974. Morphometric analysis of the ultrastructural changes in the liver of aging rats. *J. Gerontol.* **29:** 506–513.
31. Wisse, E. 1970. An electron microscopic study of fenestrated endothelial lining of rat liver sinusoids. *J. Ultrastruct. Res.* **31:** 125–150.
32. Spurr, A. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26:** 31–43.
33. Loud, A. V. 1968. A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells. *J. Cell Biol.* **37:** 27–46.
34. Weibel, E. R., W. Stäubli, H. R. Gnagi, and F. A. Hess. 1969. Correlated morphometric and biochemical studies of the liver cell. I. Morphometric model, stereologic methods and normal morphometric data for rat liver. *J. Cell Biol.* **42:** 68–91.
35. Sturgess, J. M., and F. A. de la Iglesia. 1972. Morphometry of the Golgi apparatus in developing liver. *J. Cell Biol.* **55:** 524–528.
36. Wheatley, D. N. 1972. Binucleation in mammalian liver. *Exp. Cell Res.* **74:** 455–465.
37. Weibel, E. R. 1974. Stereological techniques for electron microscopic morphometry. *In* Principles and Techniques of Electron Microscopy. Vol. 3. M. A. Hayat, editor. Van Nostrand Reinhold, New York. 239–296.
38. Jones, A. L., and D. W. Fawcett. 1966. Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by phenobarbital (with a review of the function of this organelle in the liver). *J. Histochem. Cytochem.* **14:** 215–232.
39. Stäubli, W., R. Hess, and E. R. Weibel. 1969. Correlated morphometric and biochemical studies of the liver cell. II. Effects of phenobarbital on rat hepatocytes. *J. Cell Biol.* **42:** 92–112.
40. Svoboda, D. J., and D. L. Azarnoff. 1966. Responses of hepatic microbodies to a hypolipidemic agent, ethyl chlorophenoxyisobutyrate (CPIB). *J. Cell Biol.* **30:** 442–450.
41. Reddy, J., D. J. Svoboda, and D. L. Azarnoff. 1973. Microbody proliferation in liver induced by nafenopin, a new hypolipidemic drug: comparison with CPIB. *Biochem. Biophys. Res. Commun.* **52:** 537–543.
42. Martin, E., and G. Feldmann. 1974. Étude histologique et ultrastructurale du foie chez le rat apres administration subaigue d'un nouvel agent hypolipidemiant, le bis(hydroxy)-ethyl-thio)1-10 decane. *Pathol. Biol.* **22:** 179–188.
43. Dalton, C., W. C. Hope, H. R. Hope, and G. Sheppard. 1974. Relationship of serum triglyceride lowering to changes in hepatic composition induced by different classes of drugs. *Biochem. Pharmacol.* **23:** 685–696.
44. Sakamoto, S., K. Yamada, T. Anzai, and T. Wada. 1973. Morphological changes in the liver of rats treated with a new hypolipidemic agent, S-8527. *Atherosclerosis.* **18:** 109–116.
45. Bolender, R. P., and E. R. Weibel. 1973. A morphometric

study of the removal of phenobarbital-induced membranes from hepatocytes after cessation of treatment. *J. Cell Biol.* **56:** 756–761.

46. Comolli, R., M. E. Ferioli, and S. Azzola. 1972. Protein turnover of the lysosomal and mitochondrial fractions of rat liver during aging. *Exp. Gerontol.* **7:** 369–376.
47. Platt, D., and H. Pauli. 1972. Age dependent determinations of lysosomal enzymes in the livers of spironolactone and aldosterone pretreated rats. *Exp. Gerontol.* **7:** 1–7.
48. Reddy, J., M. Chiga, S. Bunyaratvej, and D. J. Svoboda. 1970. Microbodies in experimentally altered cells. VII. CPIB-induced hepatic microbody proliferation in the absence of significant catalase synthesis. *J. Cell Biol.* **44:** 226–234.
49. Legg, P. G., and R. L. Wood. 1970. New observations on microbodies. A cytochemical study on CPIB-treated rat liver. *J. Cell Biol.* **45:** 118–129.
50. Jones, A. L., and E. S. Mills. 1973. Ultrastructural contributions to molecular pharmacology. *In* Modern Pharmacology: A Guide to Molecular Pharmacology–Toxicology. Vol. 1, Part 1. R. M. Featherstone, editor. Marcel Dekker, New York. 83–146.
51. Reddy, J., D. L. Azarnoff, D. L. Svoboda, and J. D. Prasad. 1974. Nafenopin induced hepatic microbody (peroxisome) proliferation and catalase synthesis in rats and mice. Absence of a sex difference in response. *J. Cell Biol.* **61:** 344–358.
52. Jones, A. L. and J. B. Emans. 1968. Hypertrophy of liver cell smooth surfaced reticulum following progesterone administration. *J. Histochem. Cytochem.* **16:** 561–571.
53. Fouts, J. R. 1971. Liver smooth endoplasmic reticulum "microsomal" drug metabolizing enzyme system. *Methods Pharmacol.* **1:** 287–325.
54. Jones, A. L., and D. T. Armstrong. 1965. Increased cholesterol biosynthesis following phenobarbital-induced hypertrophy of endoplasmic reticulum in liver. *Proc. Soc. Exp. Biol. Med.* **119:** 1136–1139.
55. Jones, A. L., N. B. Ruderman, and M. G. Herrera. 1967. Electron microscopic and biochemical study of lipoprotein synthesis in the isolated perfused rat liver. *J. Lipid Res.* **8:** 429–446.
56. Dallner, G., P. Siekevitz, and G. E. Palade. 1966. Biogenesis of endoplasmic reticulum membranes. I. Structural and chemical differentiation in developing rat hepatocyte. *J. Cell Biol.* **30:** 73–96.
57. Dallner, G., P. Siekevitz, and G. E. Palade. 1966. Biogenesis of endoplasmic reticulum membranes. II. Synthesis of constitutive microsomal enzymes in developing rat hepatocyte. *J. Cell Biol.* **30:** 97–117.