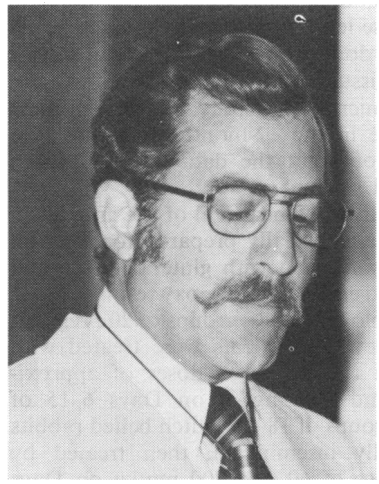
Toxicological Studies on Gemfibrozil

by Dr S M Kurtz,
Dr J E Fitzgerald, R A Fisken,
J L Schardein,
Dr T H Reutner
and J A Lucas
(*Parke, Davis Research Laboratories, Ann Arbor, Michigan, USA*)



Dr S M Kurtz

Animal tolerance studies with gemfibrozil began in 1970, at which time there were already several publications in the literature dealing with the morphology and hypothetical structural-functional relationships of the liver cell following treatment with clofibrate.

Paget (1963) had originally observed that clofibrate (Atromid) was very well tolerated by rats and monkeys for periods in excess of six months and that, even at high doses, few if any clinical, laboratory or pathological changes could be elicited. The one striking exception was the alteration in the structure of rat liver cells, which reached a maximum shortly after initiation of drug administration, but was not associated with indications of damaged liver function.

By light microscopy, this change was characterized as an intense granular eosinophilia of hepatocytes, evident earliest in the centrolobular region, then rapidly encompassing the lobule as a whole. By electron microscopy, Paget (1963) observed the concomitant accumulation of microbodies in the cytoplasm of these hepatocytes, which he mistakenly identified as lysosomes. Hess *et al.* (1965, 1967) confirmed Paget's results, properly identified the microbodies, and undertook a series of cell fractionation studies to understand better the relationship between the already established liver enzyme changes associated with clofibrate treatment, the striking accumulation of microbodies and the enzyme content of the microbodies.

This report on gemfibrozil describes the equally good toleration of laboratory animals to short- and long-term exposure, the presence of large numbers of microbodies in the rat liver without signs of degeneration, and the absence of teratogenic findings in two species of laboratory animals.

MATERIALS AND METHODS

The animals used in tolerance studies were young mature mice, Sprague-Dawley derived rats, beagle dogs, and rhesus monkeys. Those used in teratology studies were rats and rabbits.

In acute single-dose oral studies, 10–20 male mice and rats were used per dose level. The drug was administered as a 10% suspension in acacia and water because of its low solubility. Animals were observed carefully for clinical reactions and time of death. Survivors were observed for 14 days, then were killed and autopsied. LD_{50} values were calculated by the Miller-Tainter method.

Chronic tolerance studies were undertaken for two projected treatment periods, one for three months and one for 12 months. The regimens, observations and laboratory procedures were the same for both studies. Rats, dogs and monkeys were used in the former study, while rats and dogs only were used in the latter.

Twelve rats per sex were given approximately 30, 150 and 300 mg/kg per day of gemfibrozil admixed with their diet for the three-month study. For the 12-month study, 25 rats per sex were used at each dose level.

Several rats from each dose level were killed at intervals during the studies to monitor hæmatological, pathological and blood biochemical changes. At autopsy, all organs were examined grossly for abnormalities, and specimens of organs, tissues and all grossly observed lesions were prepared for microscopic evaluation. The data from the 12-month study have not yet been fully evaluated.

Groups of dogs and monkeys were dosed each day by capsules and gavage, respectively, at levels of 25, 150 and 300 mg/kg. In addition to periodic evaluation of their hæmatological and blood biochemical status, open surgical biopsies of liver, kidney and bone marrow were obtained under general anæsthesia from each animal at intervals during the studies. At the conclusion of the

three-month studies, the 3 dogs and 3 monkeys from each dose level were killed. Complete gross autopsy was done and portions of all major organs and tissues and all gross lesions were prepared for microscopic study. Autopsy of the 8 dogs per dose in the 12-month study has also been carried out, but the data have not been fully evaluated.

Samples of liver from several of the animals in each group were specially prepared for electron microscopy by fixation with gluteraldehyde and osmic acid, and embedded in epoxy resin.

In the teratology studies, groups of 20 pregnant Sprague-Dawley derived rats were treated with gemfibrozil in their diets at doses of approximately 100 and 300 mg/kg on Days 6–15 of pregnancy. Groups of 18–26 Dutch belted rabbits were artificially inseminated, then treated by gavage at doses of 60 and 200 mg/kg on Days 6–18 of pregnancy. Similarly sized control groups of rats and rabbits were handled in a similar fashion, except that gemfibrozil was withheld. One day before anticipated parturition, the dams were killed and the pups were removed surgically. Pertinent data concerning number, sex, deaths, implantation sites, and corpora lutea were recorded. The pups were examined grossly and by dissection for abnormalities and the carcasses were then prepared for skeletal examination.

RESULTS

Acute Oral Toxicity

The toxicity of gemfibrozil was low, yielding LD_{50} values of 3162 ± 199 mg/kg and 4786 ± 130 mg/kg for mice and rats respectively. The clinical signs of intolerance were similar for both species and included incoordination, depression, flaccid prostration, and dyspnœa. Almost all deaths occurred within 24 hours of dosing. Autopsy of those dying disclosed no effect that was clearly related to dosing with the drug. Those survivors killed 14 days after dosing showed only slight hepatocellular enlargement in histological sections.

Chronic Oral Tolerance

Rats: In rats, there was little or no detectable clinical response at any dose level of gemfibrozil, except for slight to moderate dose-related depression in weight gain among female rats, the maximum reaching 25% at the high dose. A normal weight gain pattern was not re-established in the latter group during the reversal period. Food intake in both sexes was unaffected.

Variations were present among the blood biochemical and hæmatological values obtained at the various sampling periods, but these were not related to dose level and they tended to remain within acceptable limits. Alkaline phosphatase and cholesterol values, while not excessively altered, were more consistently disturbed than other biochemical values, perhaps attesting to the altered metabolism of the liver. At the 42-day and 91-day sampling periods, the liver weights of both males and females were significantly increased. After two weeks on standard rations these values had returned to normal in the females, while there was still slight enlargement in the males. In addition to the enlargement, the livers had a greenish-grey discoloration. Other pathological findings were limited to a few incidental lesions not related to treatment.

Drug-related microscopic changes were confined to the liver and consisted principally of hepatocellular hypertrophy, with pale eosinophilic cytoplasm, diminished or absent basophilic substance and enlarged nuclei (Fig 1). While present in all treated animals, this alteration was especially prominent in the livers of male rats and more marked at the high doses. These changes had reverted towards the normal after two weeks on standard rations, with the females on the former low dose having the greatest recovery.

Preliminary results from the 12-month study indicated that gemfibrozil was well tolerated over the dosing period, and the only clinical evidence of intolerance was a dose-related suppression of weight gain, a feature observed in the three-month study. Similarly, there were fluctuations in blood biochemical and hæmatological values, but these were not consistent nor clearly dose-related. Gross autopsy examination disclosed only liver enlargement, a feature previously noted in the three-month study.

Dogs: There was no clinical response discernible during the three-month study except for a slight but not dose-related weight loss in some dogs. Yellow crystals, presumed to be unabsorbed drug, were seen regularly in the fæces of dogs on the higher doses.

A variety of minor departures from pre-test hæmatological and blood biochemical values occurred at the various sampling intervals, but these remained generally within acceptable previously established limits. Biochemical values which are generally affected by impaired liver function or liver injury, such as SGP- and SGO-transaminase, alkaline phosphatase and LDH, were mildly and sporadically elevated, particularly in the early part of the dosing period. Bone marrow and urine analysis likewise yielded few significant abnormalities. The animals were all killed following their final doses of gemfibrozil, and gross autopsy disclosed no effects related to the drug regimen. One dog from the mid-dose group was found to have early degeneration of

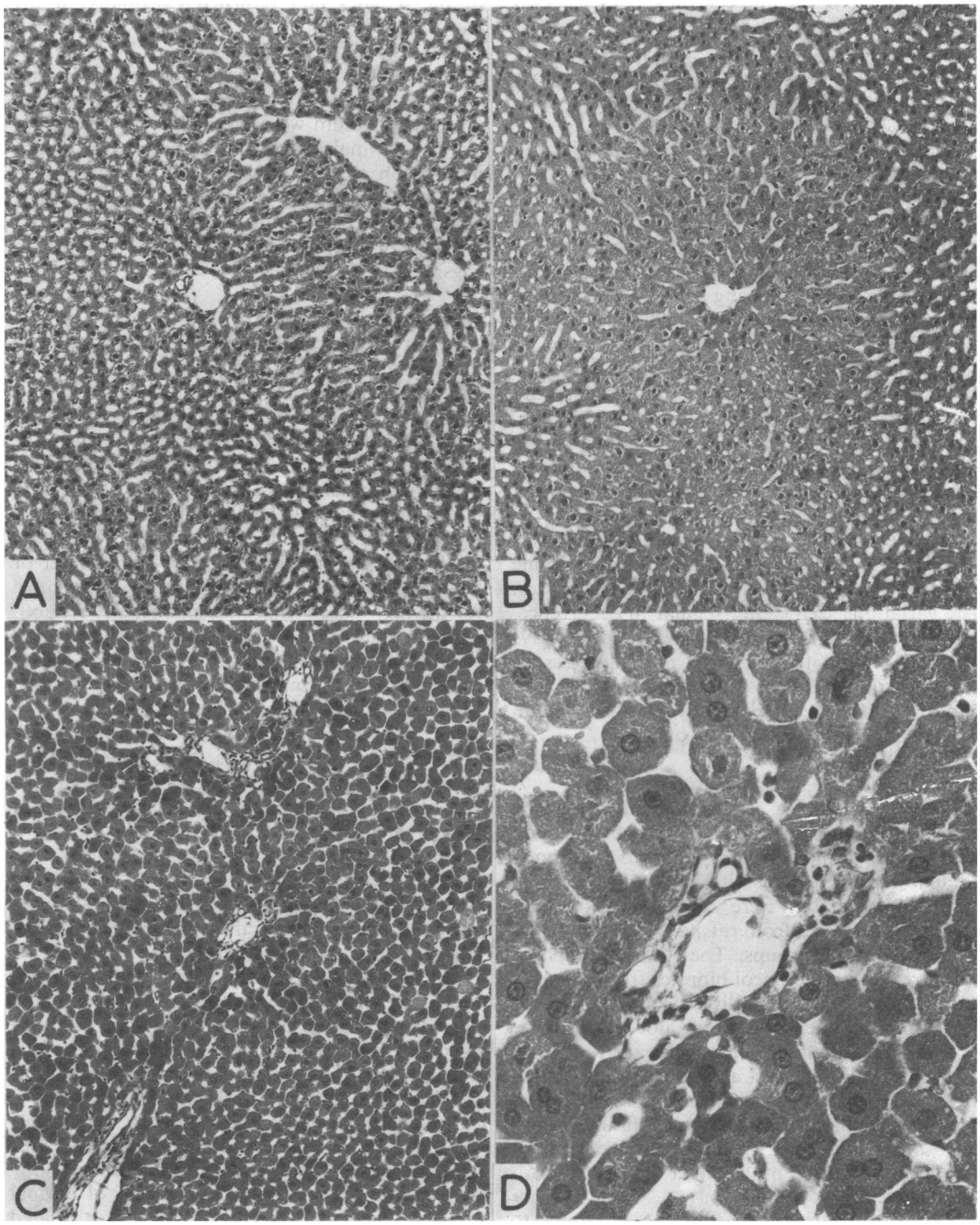


Fig 1A, *liver, control male rat. The microscopic structure illustrated is similar for male and female. H & E.* × *33.* B, *liver, female rat, treated with gemfibrozil. Observe the centrolobular hepatocyte hypertrophy, characteristic for the female rat. H & E.* × *33.* C, *liver, male rat, treated with gemfibrozil. Hepatocytes are hypertrophied uniformly through the liver lobules. H & E.* × *33.* D, *liver, male rat, treated with gemfibrozil. Higher magnification permits appreciation of the granular dense cytoplasm of the hepatocytes. H & E.* × *130*

centrolobular hepatocytes in microscopic section, with areas of mononuclear leukocytes concentrated in some portal and central vein areas. Two dogs from the high dose regimen had equivocal hepatocellular changes, but 1 had frank atrophy of centrolobular liver cords. The only other abnormalities noted microscopically were considered to be incidental.

The dogs in the 12-month study tolerated gemfibrozil equally as well as those in the three-month study. Blood biochemical and hæmatological values obtained at the various sampling intervals fluctuated, but without consistency or relationship to dose or duration of treatment. Gross examination during scheduled autopsy disclosed no drug-related abnormalities.

Monkeys: The monkeys on the high-dose regimen (300 mg/kg) had sporadic anorexia and occasional salivation and vomiting. However, there was no weight loss, and the animals were all in good condition at the time of autopsy. There were no clinical reactions in animals given the middle and low doses. Consonant with the paucity of clinical response was the lack of significant alteration of hæmatological, bone marrow, blood biochemical or urinalysis values. The variations seen were irregular in occurrence, not clearly related to dosage, and almost always remained within the range of pretest values or previous controls. Gross and microscopic examination of tissues removed at autopsy disclosed no drug-related lesions, regardless of dose.

Electron Microscopic Observations

In view of the increased liver weight in the rats and the known association of this phenomenon with a marked increase in hepatocyte microbody content in rats dosed with clofibrate, electron microscopic studies were carried out on liver specimens removed from rats, dogs and monkeys in the high-dose groups. These were compared with pretreatment control biopsies from the dogs and monkeys and with liver from non-treated rats. Since the phenomenon has not proved to be of any clinical significance with regard to the use of clofibrate in humans, a similar finding associated with the enlarged livers observed after gemfibrozil administration would diminish concern over the hepatocellular enlargement. It would suggest an adaptive, rather than a degenerative change.

The rats, in particular the males, were found to have undergone this modification of hepatocyte cytoplasmic organelles. There was a great abundance of microbodies throughout the cytoplasm, associated with a decrease in rough endoplasmic reticulum and a hypertrophy and dilatation of smooth reticulum. These changes tended to revert only partially in males during the two-week reversal period, while the hepatocytes of the females had returned essentially to the control condition.

Increase in microbodies was not a prominent feature in the hepatocytes of treated dogs and monkeys. Hypertrophy and dilatation of the smooth reticulum were present in both, particularly in the males, again with some concomitant diminution of the rough reticulum. There were also a few subtle changes in mitochondria associated with the treatment regimen.

Some of these liver changes are shown in Figs 2–5.

Teratological Studies

Rats: In the 2 treated groups of rats, actual drug intakes of 81 and 28 mg/kg were achieved. Clinically significant reactions consisted of a moderate to severe fall in food intake, and depression of weight gain at the higher dose level, but only slight depression at the lower dose level during the actual dosing period.

Examination of the fetuses removed from dams one day before the anticipated date of parturition disclosed no significant effects on either litter or fetal parameters. The number of corpora lutea for the high-dose group was significantly reduced compared to controls. However, the overall calculated pre-implantation losses were higher for the control group, owing to a lower rate of implantation of the ova shed at ovulation. All other indices of both treatment groups were comparable to the controls. No significant malformations were encountered among the almost 400 offspring examined from 36 litters obtained from treated dams.

Rabbits: Aspiration following intubation error and three unexplained deaths left only 12 pregnant does at the 200 mg/kg level for final analysis. Among these survivors, there was no evidence of clinical intolerance to gemfibrozil.

Autopsy and gross examination of the remaining pregnant does 1 day before the anticipated date of parturition disclosed few, if any, adverse effects of gemfibrozil on the fetuses. Litter parameters, including size of litters, number of corpora lutea, and pre-implantation loss of both treated groups were comparable to the vehicle controls. There was abortion in 3 treated dams (1 high dose, 2 low dose), but viable young delivered in the remaining litters were of normal size, had a normal sex ratio, and the calculated post-implantation loss in both treated groups was comparable to the controls. Most importantly, examination of almost 100 fetuses, representing 22 litters, produced by dams undergoing gemfibrozil treatment, revealed no significant drug-related malformations.

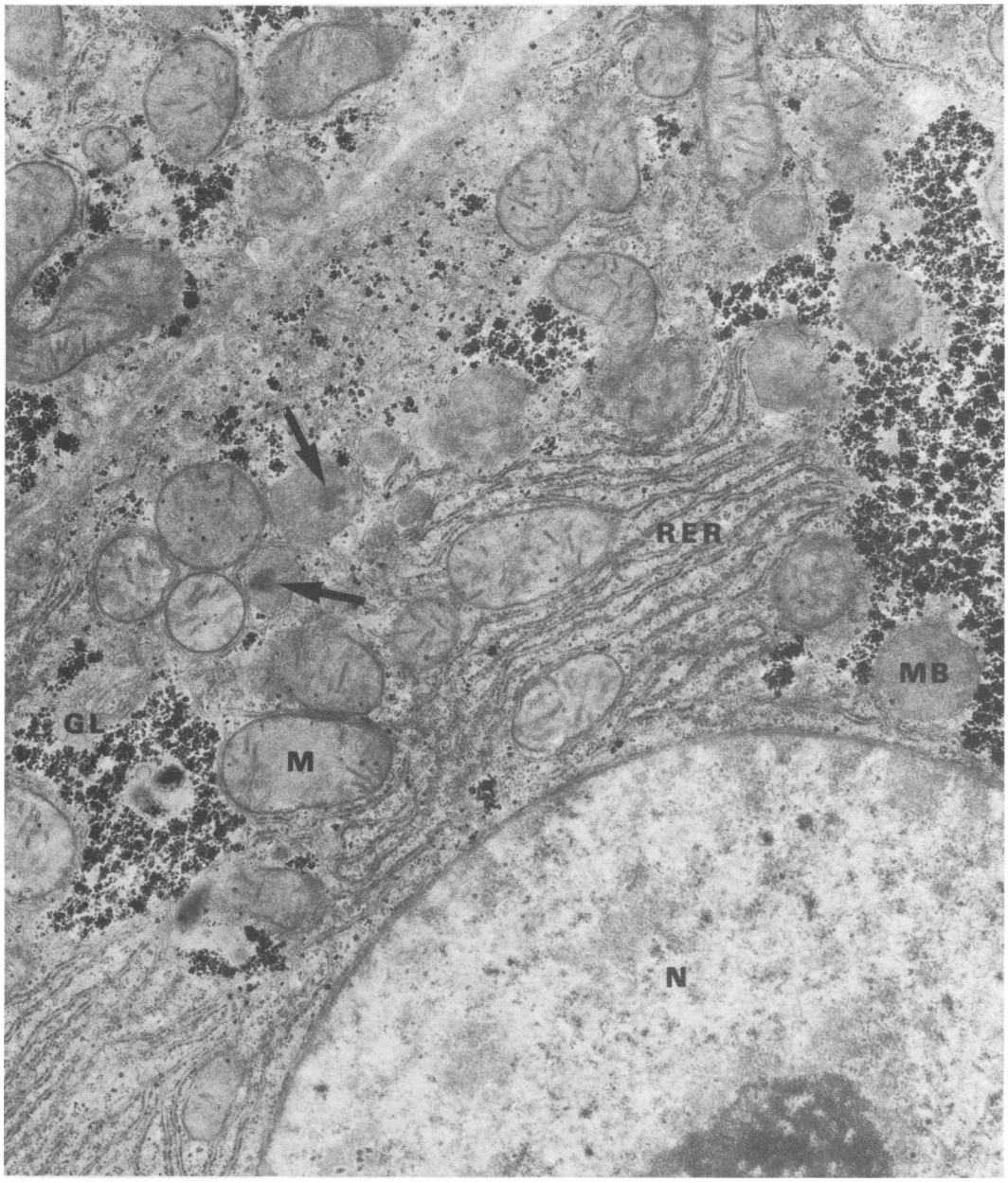


Fig 2 *Liver, normal control male rat. Electron micrograph of typical hepatocyte.* **N,** *nucleus.* **M,** *mitochondria.* **MB,** *microbody.* **RER,** *rough endoplasmic reticulum.* **GL,** *glycogen. Uricase indicated by arrow.* × *15 000*

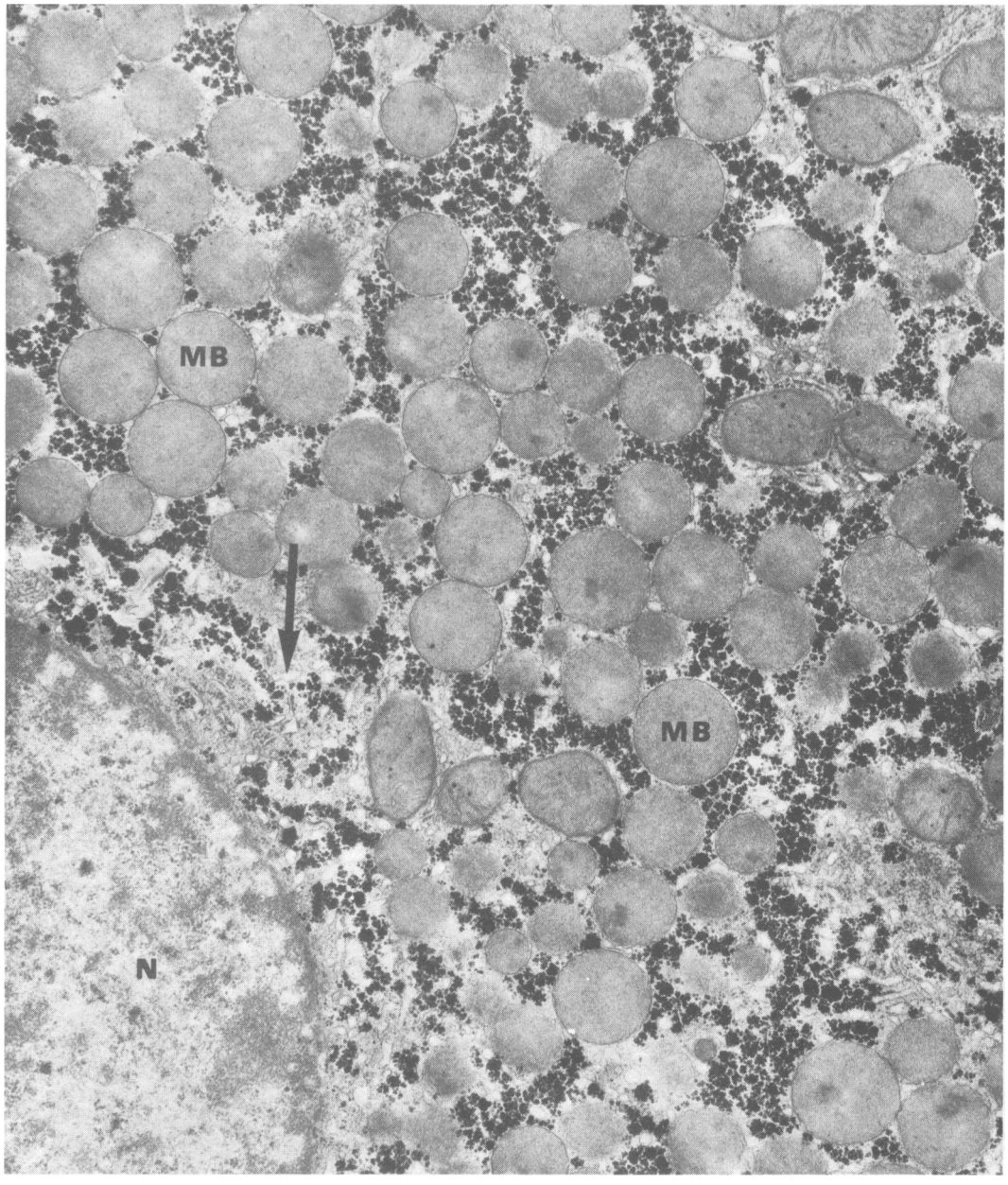


Fig 3 *Liver, male rat treated with gemfibrozil. Electron micrograph of characteristic changes in the hepatocyte. Observe the profusion of microbodies* (MB) *in the cytoplasm, most of which are free of uricase. Arrow points to area containing abundant smooth endoplasmic reticulum. Glycogen content obscures most of the smooth endoplasmic reticulum in the cytoplasm.* N, *nucleus.* × *15 000*

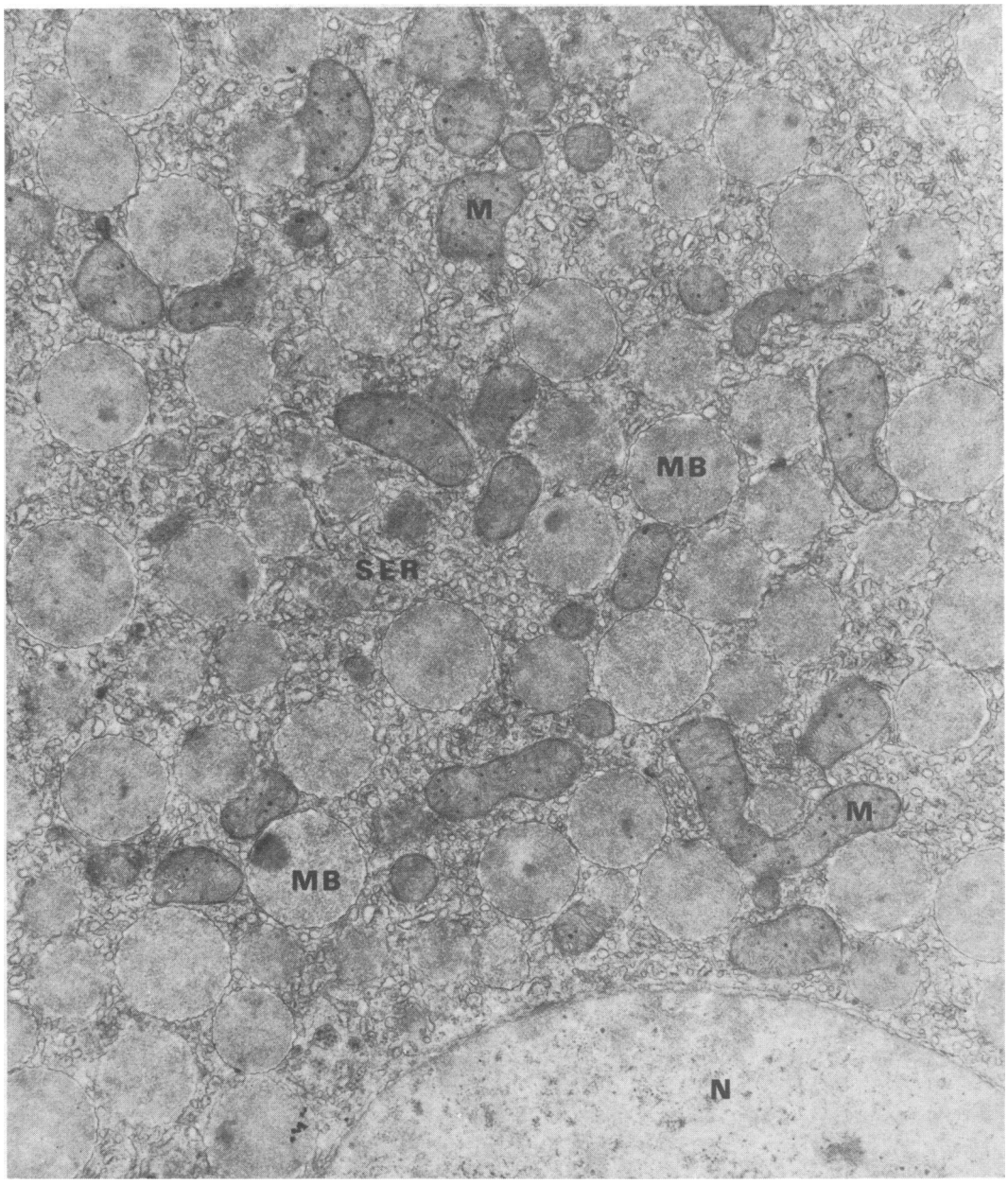


Fig 4 *Liver, male rat treated with gemfibrozil. Electron micrograph illustrating hypertrophied dilated smooth endoplasmic reticulum* (SER) *in a cell with reduced glycogen content.* **N,** *nucleus.* **M,** *mitochondria.* **MB,** *microbody.* × *15 000*

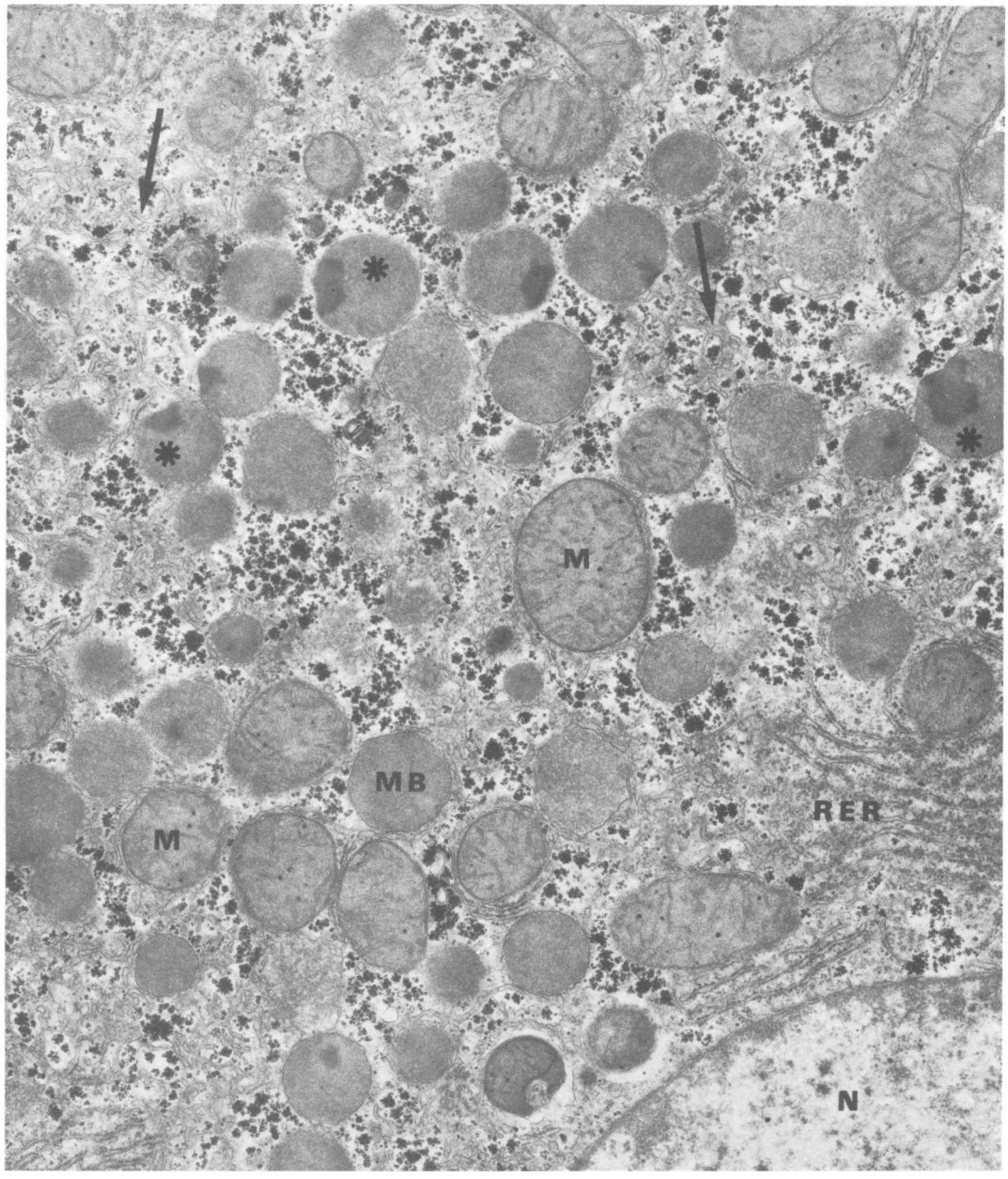


Fig 5 *Liver, male rat treated with gemfibrozil, then normal rations for two weeks. Electron micrograph illustrating marked decrease in number of microbodies, many of which (★) now contain uricase. The smooth endoplasmic reticulum (arrows) is still abundant, but not particularly dilated.* N, *nucleus.* M, *mitochondria.* MB, *microbody.* RER, *rough endoplasmic reticulum.* × *15 000*

DISCUSSION

The results of these experiments indicate that gemfibrozil is well tolerated clinically over the short term by rats, dogs and monkeys and over the long term by rats and dogs at doses of about 15 times the average effective human dose. Furthermore, the acute toxicity studies would indicate that there should be little hazard from acute overdose.

Autopsy studies disclosed variable hepatic centrolobular atrophy in 1 dog from the mid-dose and 1 from the high-dose group at three months, with no pathological findings in monkeys, and hepatocellular hypertrophy in rats. Preliminary but incomplete evaluation of rats and dogs from the 12-month study indicated similar if not identical findings. Thus, in rats, the morphological and functional changes appearing early in the liver following relatively short-term administration of gemfibrozil do not seem to evolve into frank degenerative changes.

Electron microscopy indicated that microbody formation in the rat liver cells conformed to that observed by Paget (1963) following treatment with clofibrate and that, while microbodies appeared in the hepatocytes of the dog and monkey, they were not prominent. Some mitochondria were remarkably enlarged with variable distortion of their normal cristæ. Smooth endoplasmic reticulum was also found to be hypertrophied in the hepatocytes of all three species. Characteristically, this latter phenomenon is associated with stimulation of microsomal enzymes and microbody formation, and therefore represents a feature of adaptive rather than pathological alteration.

Platt & Cockrill (1967) were the first to point out that the enlargement of the liver that accompanied treatment with cholesterol-lowering agents did not represent a manifestation of toxicity. The increased liver weights were attributed by Platt & Thorp (1966) and Hess *et al.* (1965) to the increased amount of protein associated with the increase in number of microbodies and increased enzymes present in other parts of the liver cell cytoplasm. Hess *et al.* (1967) concluded that the prominent increase in microbody content was not related to the blood lipid lowering effect of clofibrate, since the enzymes, predominantly catalase, found in microbodies were not involved in its biochemical mechanism of action. Thus, abundant microbody formation would seem to be a harmless manifestation of altered metabolism of the liver occurring in an as yet unresolved relationship to the principal activity of lipid lowering agents of the clofibrate class.

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