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Lifetime Prolongation in Voluntary Alcohol-Consuming Rats (SHR) Treated With Clofibrate

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SCHLICHT, I., S. FALK, A. KRÖSSIN, AND A. MOHNHAUPT. *Lifetime prolongation in voluntary alcohol-consuming rats treated with clofibrate.* ALCOHOL 9(2) 139–148, 1992.—Clofibrate affects lipid and alcohol as well as drug and eicosanoid metabolism. Spontaneous hypertensive rats (SHR) further increase their high voluntary alcohol consumption during clofibrate feeding. The interaction of alcohol and clofibrate was studied in two long-term trials. Seventy-nine male SHR (aged 27 weeks) were offered increasing concentrations of ethanol, up to 30% (tap water ad lib), and 3 months later 0.5% clofibrate-food. Four groups were established: N, normal controls; NA, standard diet + alcohol; C, clofibrate feeding; and CA, clofibrate feeding + alcohol. Food intake, alcohol consumption, body weight, and laboratory values were recorded continuously. Life duration (weeks) after the start of the trial was 63.3 ± 3.3 in N,73 ± 2.6 in NA, 77.7 ± 4.3 in C, and 90.3 ± 2.8 in CA. There were no alcohol-related liver findings in NA and CA. Most of the animals died of cardiac and renal failure. An increase of tumors in clofibrate-treated rats was not observed. Voluntary alcohol consumption or clofibrate feeding significantly lengthens lifetime, which is prolonged by 42% if ethanol is combined with clofibrate. This is obviously not mediated by the lipid lowering effect or an influence on body weight and blood pressure of either clofibrate or alcohol.

Lifetime prolongation Alcohol Ethanol metabolism Voluntary alcohol consumption Clofibrate Clofibrate analogues Alcoholic liver disease Drug metabolism Eicosanoids Prostaglandins Liver Rats Spontaneous hypertensive rats (SHR) Hypertension

CLOFIBRATE (ethyl-α-*p*-chlor-phenoxyisobutyrate) and analogues, for example, nafenopin, affect lipid, alcohol, drug, and eicosanoid metabolism when given orally in the diet: 0.25–0.77%, i.e., approximately 100–700 mg/kg. Within one to two weeks it causes enlargement of the liver in rats by inducing hypertrophy and hyperplasia of up to 100% and more (1, 6,8,18,24,35,39,48).

The increase in liver weight is accompanied by a marked rise of peroxisomes with a strikingly elevated catalase activity (24,35,38,41,42,48) even in primates under ciprofibrate treatment (57,59). Moreover, mitochondriae increase in number, size, and internal surface area, as does the amount of smooth endoplasmic reticulum (1,16,23,35).

Cytochrome oxidase and mitochondrial enzymes involved in the β-oxidation of fatty acids are highly increased (5,15, 18,30,39,41,47,48). In addition, enhancement of cytochrome P-450 and NADPH-cytochrome-C reductase has been reported (1,13,15,44,53). Furthermore, as clofibrate increases the O_2-dependent NAD reduction (41), the lactate/pyruvate ratio is diminished (6,29).

Clofibrate treatment accelerates the metabolism of ethylmorphine, aminopyrine, and aniline in rat liver microsomes (43). The sleeping time after pentobarbital or zoxazolamine administration is reduced (6).

Recent investigations demonstrate that various isoenzymes of cytochrome P-450 with relation to eicosanoid metabolism, e.g., P-450 IVA1 and IVA3 as well as P-452, are also induced by clofibrate (3,4,13,62).

CLOFIBRATE-ALCOHOL INTERACTION

Clofibrate increases ethanol oxidation and detoxification by enhanced alcohol dehydrogenase, acetaldehyde dehydrogenase, and catalase activity in male Sprague-Dawley rats (37,40). The alcohol disappearance rate in blood is increased if Wistar or Sprague-Dawley rats are pretreated with clofibrate (25,29). Moreover, the negative effect of ethanol on the redox

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state of the NAD/NADH couple in the perfused rat liver and in vivo is diminished (29).

Finally, clofibrate treatment reduces triglyceride accumulation after ethanol application for 24 days in rat liver by approximately 50% (66) respectively 60% after pretreatment even in female Sprague-Dawley rats (10).

Therefore, it is of interest to study the alcohol-clofibrate interaction in a lifetime trial specifically. Such a study addresses the question as to whether a high and prolonged voluntary alcohol consumption leads to severe alcoholic liver disease and whether the increased alcohol metabolism after clofibrate administration prevents or enhances it.

Spontaneous hypertensive rats (SHR) show a more accelerated alcohol disappearance rate from blood than Wistar rats when fed chronically with alcohol (28). Moreover, they voluntarily consume large amounts of alcohol which may be further increased by clofibrate (61). Therefore, this strain was chosen for the present study.

METHOD

Two long-term experiments were undertaken:

1. Evaluation of lifetime and autopsy including microscopic examination of organs after spontaneous death.
2. Evaluation of laboratory values in blood and relative organ weights at various intervals.

Male spontaneous hypertensive rats (SHR), specific pathogen-free SPF (Charles River Co., Germany) were housed in individual plastic cages and were maintained at a temperature of 23°C and a humidity of 75% on a 12-h light-dark cycle (8 a.m. to 8 p.m.) with music for several hours daily (experiments were carried out in the Zentrale Versuchstierlaboratorien der Freien Universität, Berlin). The rats at an age of 27 (trial 1) and 28 weeks (trial 2), respectively, were fed standard food (No. 1320, Altromin, Germany) with a given composition including 18% protein and caloric contents (complete composition of food is available on request from the authors). (Table 1).

Twenty-six and 14 rats from trials 1 and 2, respectively, were offered increasing concentrations of ethanol from 10% up to a 30% v/v watery solution within 12 weeks of accommodation. Both alcohol solution and tap water were available ad lib. This two-bottle system for experiments on voluntary alcohol consumption is similar to the methods in previous studies (50,61). The amount of alcohol in grams was obtained by multiplying its volume percent by a factor of 0.79. Alcohol, food consumption, and body weight were measured once weekly. The calculation in grams per kilogram per day were permissible, since four animals were frequently observed for 3 months at night and day. The pattern of alcohol and food intake was the same each day. C and CA groups were fed 0.5% clofibrate food. Alcohol was removed in trial 1 in both drinking groups NA and CA for 2 days after 40 weeks, and after 91 weeks in group CA for testing the alcohol-deprivation effect (63,64). The animals of trial 1 were autopsied after spontaneous death. In trial 2, one or two animals from each group were sacrificed at different time intervals. Blood samples were collected by heart puncture in CO_2 anesthesia, investigated by standard automated methods, and autopsy performed. Relative organ weights of heart, kidneys, and liver were recorded. The organs including pancreas, spleen, aorta, and skeletal muscles were fixed in formalin and embedded in paraffin; for the liver epoxy resin (Technovit, Germany) was used. Sections were stained with hematoxylin-eosin and hematoxylin-Sudan.

TABLE 1

Subdivision of the Groups	Trial 1(*n*)	Trial 2(*n*)
1. Normal controls (N)	12	7
2. Standard diet + alcohol (NA)	13	7
3. Clofibrate feeding (C)	13	7
4. Clofibrate feeding + alcohol (CA)	13	7

STATISTICAL EVALUATION

To analyze the survival time of the four rat groups, the product limit estimate of the cumulative survival curve was computed.

The test of the equality of the survival distributions of the different groups was performed by the generalized Savage (Mantel-Cox) as well as by the generalized Wilcoxon (Breslow) test. For assessment of the dependence of alcohol, food, calorie consumption, as well as body and organ weights on clofibrate and/or alcohol uptake, the Kruskal-Wallis one-way analysis of variance (ANOVA) and the Mann-Whitney *U* Rank-Sum test were employed.

The occurrence of tumors in the clofibrate and nonclofibrate groups was tested for its group dependence by a contingency frequency table with the Yate's corrected chi-square test of row and columns. Linear regression analysis was performed to establish the dependence of organ weights during lifetime in trial 2, weeks 27–43. For all statistical calculations, BMDP statistical software, 1985, for the DEC PDP 11 was used.

RESULTS

Lifetime (Fig. 1)

Trial 1. Mean survival was calculated after the start of the trial at age 27 weeks. Survival was shortest in the normal controls (N) at 63.3 ± 3.3 weeks (mean ± standard error). Lifetime of the control drinking group NA was 73 ± 2.6 weeks (an increase of 16%). Clofibrate feeding in the control group C led to a lifespan of 77.7 ± 3.4 weeks (+22%). Survival was enhanced by voluntary alcohol consumption with clofibrate treatment in CA to 90.3 ± 2.8 weeks (+42%). All differences in survival with the exception of NA/C, were sig-

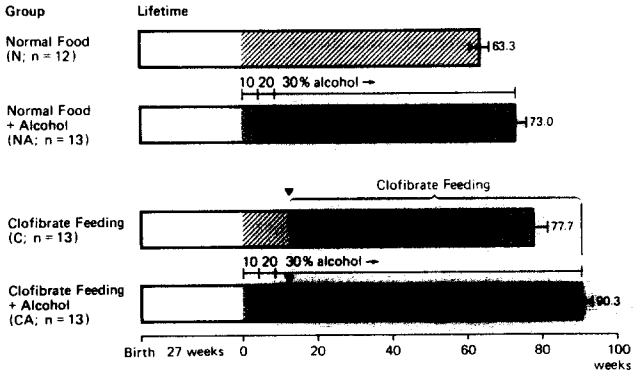

FIG. 1. Trial 1. Survival after the start of the trial at an age of 27 weeks. Start of clofibrate feeding in week 38 after birth (arrow).

nificant. [Generalized Wilcoxon (Breslow): N/NA, $p < 0.05$; N/C, $p < 0.05$; N/CA, $p < 0.001$; NA/C, $p < 0.1858$ (NS); NA/CA, $p < 0.001$; C/CA, $p < 0.05$. Generalized Savage (Mantel-Cox): N/NA, $p < 0.01$; N/C, $p < 0.005$; N/CA, $p < 0.001$; NA/C, $p < 0.05$; NA/CA, $p < 0.001$; C/CA, $p < 0.05$.]

Voluntary Alcohol Consumption (Fig. 2)

Trial 1. As reported previously (61), voluntary alcohol consumption in our experiments rose continuously and stepwise up to a mean of 4.7 ± 1.0 g/kg per d within the first 12 weeks. The rats consumed the alcohol solution over periods of about 1–2 minutes or more, with approximately 300 licks/min at night and day. This seems to correspond to the drinking behavior as described elsewhere (17). Within 24 hours, several periods of alcohol, water, and food intake, as well as sleeping and activity periods were observed.

After the start of clofibrate feeding, ethanol consumption in the CA group rose quickly up to a mean of 8.2 ± 2.1 g/kg per d during week 20. In the NA group ,it rose only to a maximum of 5.9 ± 2.2 g/kg per day. The further course was characterized by a continuous increase in alcohol intake until spontaneous death with a maximum of 7.7 ± 2.6 g/kg per day in NA rats (week 57) and of 12.5 ± 2.5 g in CA rats (week 90). A very high voluntary alcohol consumption was noted during the final weeks: 12.9 ± 0.6 g/kg per day in NA rats (n = 3, week 81) and 15.8 ± 3.2 g/kg per day in CA rats (n = 4, week 97). Alcohol intake in individual cases increased up to 14.4 g/kg per day in one NA rat and to 18.3 g/kg per day in one CA rat; after deprivation, the intake reached 25.6 g/kg in one rat. Alcohol consumption during the whole trial was significantly higher in CA than in NA rats ($p <$ 0.001). This was calculated from weeks 14–65, since this period was a time of relatively stable, though increasing drinking behavior. Thereafter, illnesses as well as death, occurred with increasing frequency.

Food consumption in trial 1 (Fig. 3) also followed a multiphasic course. The ethanol drinking groups consumed significantly less food than the nondrinkers. After the start of clofibrate feeding in weeks 12–20 in groups C and CA a pronounced increase of food intake was recorded. After that period, a relatively stable time interval on a lower level followed

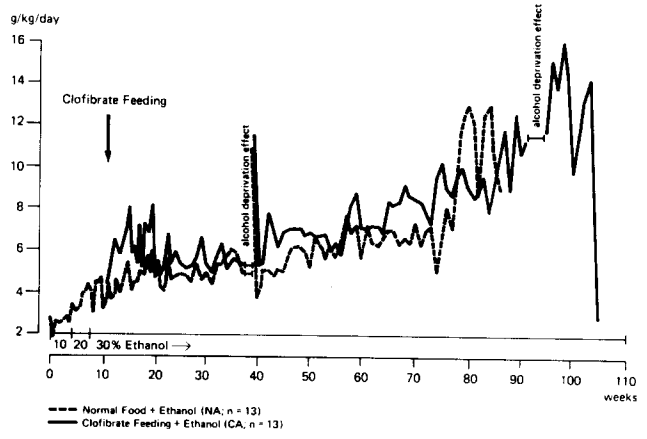

FIG. 2. Trial 1. Mean voluntary alcohol consumption. Alcohol accommodation period with 10%, 20%, and 30 % v/v solution for 12 weeks. Start of clofibrate feeding at week 12 of the trial.

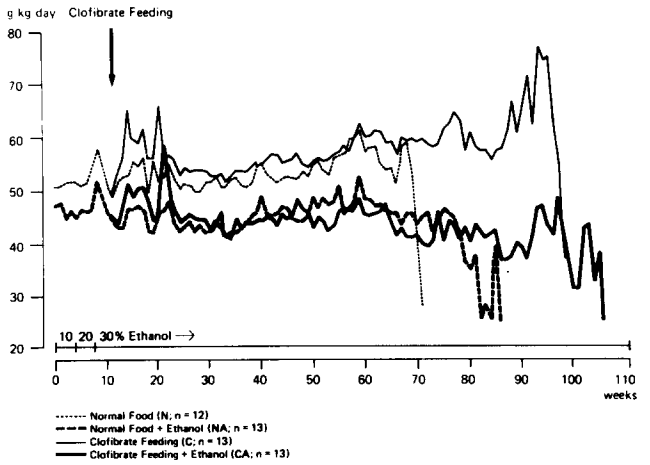

FIG. 3. Trial 1. Mean food consumption: Note the markedly lower food intake in the alcohol consuming groups NA and CA.

(weeks 20–50). Statistical evaluation of food consumption (weeks 14–65) for N/NA, C/CA, N/CA, C/NA, and N/C in all comparisons was $p < 0.001$. NA/CA was nonsignificant (p = 0.446). Since the food contained 0.5% clofibrate, the daily intake of drug in C and CA rats was nearly 200–300 mg/kg per day.

Total caloric intake (calories from food and alcohol, trial 1, Fig. 4) intake in all groups ranged from 115 kcal/kg per day to 270 kcal/kg per day. The lowest mean caloric intake was recorded in control group N, whereas the control group C consumed significantly more food ($p < 0.001$). Total caloric intake from group CA, as compared to group C, was also significantly higher ($p < 0.001$). In NA and CA rats, caloric intake was higher than in nondrinking groups N and C ($p <$ 0.005).

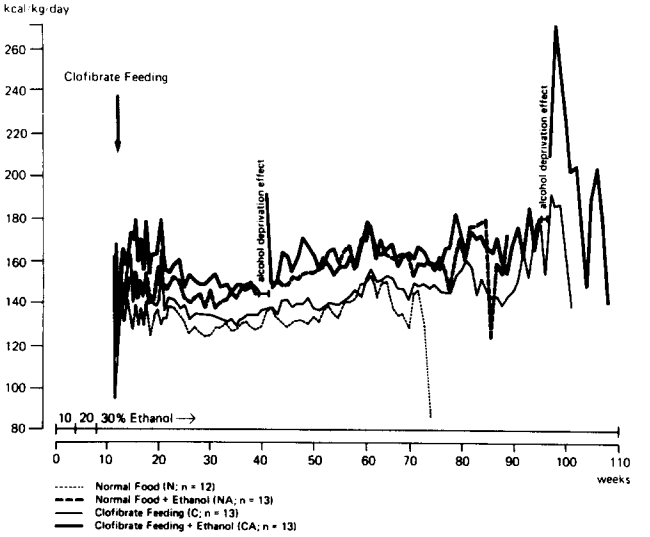

FIG. 4. Trial 1. Mean total caloric intake, calculated after start of clofibrate feeding. Note the markedly higher intake in alcohol consuming rats.

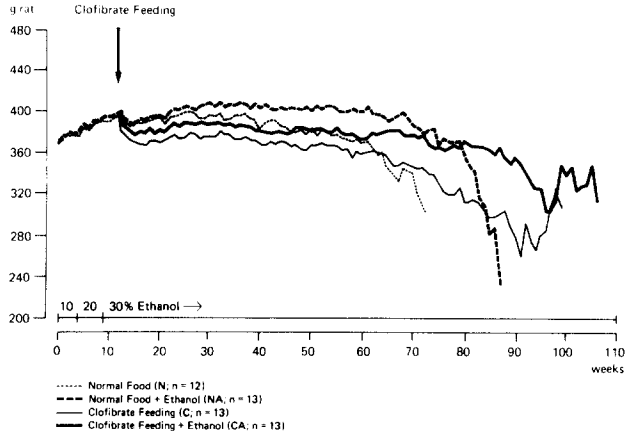

FIG. 5. Trial 1. Mean body weight: Note the lower one in comparison C/N and CA/NA despite of higher caloric intake of C and CA.

Percentage of Calories from Alcohol

Trial 1. The mean maximum caloric intake from alcohol ranged from only 33% in the NA group during week 77 (n = 11) to 48% in the CA group during week 91 (n = 7). However, a caloric intake from ethanol of 59% and 60% was achieved in the prefinal stages in one NA and CA rat, respectively. The NA group reached the highest body weight, the lowest was recorded in group C course of body weight (Fig. 5). Control group C exhibited significantly lower body weight than control group N, as well as NA rats ($p < 0.001$ in all comparisons) in spite of a higher total caloric intake. Moreover, the body weight of the CA group was lower as compared to group NA. In some animals, an increase in the last weeks of life due to fluid retention in C and CA rats appeared. Statistical evaluation of body weight (weeks 14–65) for N/C, NA/CA, C/CA, NA/C, and N/NA was $p < 0.001$. Between N/CA rats there was no significant difference since the N rats lost weight because of earlier aging ($p = 0.379$). Fig. 5 clearly demonstrates that lifetime prolongation in NA, C, and CA is not dependent on body weight, i.e., it may not be interpreted as a result of low body weight.

Trial 2. The course of alcohol consumption food intake and total caloric intake, as well as body weight, corresponded to that of trial 1.

Pathology

Relative organ weights (Table 2) were calculated in trial 1 after subtraction of pleural effusions, ascites volume, and tumor weight. In trial 2, the mean of relative organ weight of all animals in each group was calculated, despite the different age of the animals at the time of sacrifice. This is justified because the regression lines of relative organ weights over the whole period between weeks 27 and 43 did not indicate a definite change.

The relative liver weight of old animals dying spontaneously (trial 1), as compared to adult healthy rats of trial 2, increased to 34.2% in group N, 34.6% in group NA, 35.9% in group C, and 30.7% in group CA. There were no statistical differences within the groups of trial 1.

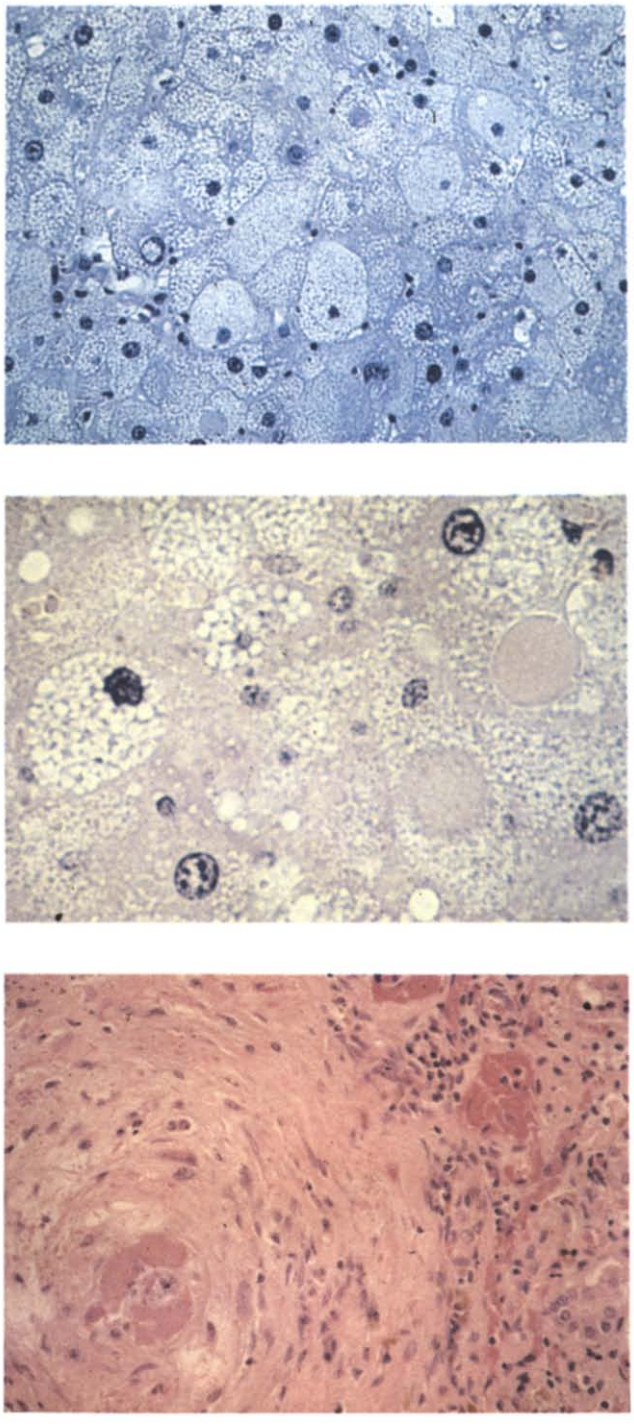

FIG. 6. Histology, trial 1. Staining Hematoxylin/Eosin; A and B: Malignant fatty liver in an NA rat. Steatosis with formation of very fine fat droplets, hydropic degeneration of hepatocytes (ballooning). Hyaline eosinophilic droplets within the cytoplasma. C: Angiopathy in kidney vessel in an CA rat. Enormous fibromuscular thickening of the media as well as hyaline thickening of the intima.

TABLE 2
RELATIVE ORGAN WEIGHT (g/100 g OF RAT)

	Liver	Heart	Kidneys
Trial 1			
N	5.46 ± 0.38	0.79 ± 0.06	1.00 ± 0.05
NA	5.48 ± 0.23	0.82 ± 0.06	1.08 ± 0.07
C	5.53 ± 0.18	0.84 ± 0.05	1.22 ± 0.06
CA	5.32 ± 0.35	1.05 ± 0.06	1.21 ± 0.04
Trial 2			
N	4.07 ± 0.14	0.46 ± 0.02	0.81 ± 0.01
NA	3.91 ± 0.13	0.44 ± 0.01	0.82 ± 0.01
C	5.57 ± 0.11	0.44 ± 0.02	0.96 ± 0.02
CA	5.40 ± 0.13	0.42 ± 0.01	0.92 ± 0.02

Trial 1: After spontaneous death (n = 51). Trial 2: Week 27 until week 43 after start of trial (n = 28).

Relative liver weight in trial 2 showed a marked increase in the clofibrate-treated groups (see Table 1). The differences between groups were: N/C, +37%; NA/CA, +38%; NA/C, +42%; and N/CA, +33%. (Statistical evaluation for N/C, N/CA, C/NA, and NA/CA was $p < 0.002$; for N/NA and C/CA it was nonsignificant.)

In trial 1, the relative heart weight as a long-term indicator for blood pressure was significantly elevated in the CA group as compared to groups N, C, and NA (N/CA, $p < 0.005$; C/CA, $p < 0.005$; NA/CA, $p < 0.05$). Relative heart weight of trial 2 was significantly lower in these relatively young animals when compared to the old rats dying spontaneously in trial 1 ($p < 0.005$ for all four groups of trial 1 and 2). In trial 2, all comparisons within the four groups did not yield any significant differences.

Moreover, in trial 1, a statistical difference of *kidney weight* between group N and CA ($p < 0.02$), as well as between group N and CA ($p < 0.05$), was evident. In all clofibrate-treated groups of trial 2, kidney weight was significantly higher than in animals not treated with the drug (N/C, N/CA, C/NA, and NA/CA: $p < 0.002$).

Autopsy Findings (Table 3)

There were three main causes of death in the animals.

1. Angiopathy with heart and/or renal failure. In only two cases cerebral hemorrhages were present.
2. Malignant tumors, mainly sarcomas.
3. Malignant fatty liver.

Macroscopic Findings

Trial 1. Usually, there were serous and sometimes hemorrhagic pleural effusions up to 20 ml and ascites up to 30 ml, as well as considerable retroperitoneal edema. The increased *heart* weight in all groups was mainly due to hypertrophy (Table 3) of the left ventricle. In addition, most of the animals exhibited dilated left and right atria with organizing thrombi. The *lungs* showed partly atelectatic regions and congestion. The *liver* was smooth and normal in color, but in 10 animals (of all groups), in the main branch of the vein, fresh or organized thrombi with a diameter of 3–5 mm could be seen as an obvious consequence of heart failure. This was accompanied by congestion of the liver and, in some cases, by ascites. The *liver* in four animals of group N and in four animals of group NA was considerably enlarged and yellow in color because of heavy steatosis. Macroscopically, *kidneys*, adrenal glands, spleen, testes, and gastrointestinal and Harderian glands were inconspicuous. In the *brain*, cerebral hemorrhage was present in only one rat of the C and CA groups (private communication, Dr. Stoltenburg, Institut of Neuropathology, Freie Universität Berlin).

In six animals, *malignant tumors* occurred. In one of the animals, there was a localized subcutaneous leiomyosarcoma

TABLE 3
AUTOPSY RESULTS

Trial 1	Angiopathy With Heart/Renal Failure Cerebral Hemorrhage (n)	Liver Vein Thrombosis (n)	Tumors (n)	Malignant Fatty Liver (n)
N	6	3	2 Leiomyosarcoma hemangioendothelioma	4
NA	7 (Cerebral hemorrhage: 1)	1	2 Osteosarcoma pancreatic-carcinoma	4
C	12 (Cerebral hemorrhage: 1)	3	1 Fibrosarcoma	0
CA	12	4	1 Myeloid leucemia	0

Note: No infections were observed.

in the back with a weight of 94 g (group N). In group NA, one animal died with massive hemorrhage in both pleural cavities. This rat revealed an osteosarcoma in the right femur (weight 48 g) with widespread metastasis difference between C + CA and N + NA is ns. For further details including histology see Table 3.

Trial 2. After sacrifice, macroscopic investigations could not detect pathologic findings.

Microscopic Studies (Trial 1)

Liver. In animals dying spontaneously, there were signs of liver atrophy mainly in the center of the lobules accompanied by an increase of binucleated liver cells and enlarged polyploid nuclei, physiologically similar to the aging process. Moreover, there was acute and sometimes chronic congestion. In the congested central areas, coagulation necrosis was often observed, especially in animals with hepatic vein thrombosis. Erythrophagocytosis by hepatocytes also was a frequent finding.

Four animals of both the N and NA group exhibited heavy steatosis with remarkable formation of very small fat droplets within the cytoplasm and diffusely within the lobules. The hepatocytes also sometimes contained very small basophilic inclusions. Moreover, there were single hepatocytes as well as groups and sheets of partly enormously enlarged necrobiotic liver cells, i.e., hydropic degeneration (ballooning). In several of the liver cells which were mostly loaded with fat, there were homogenous eosinophilic cytoplasmic droplets of varying size, so called hyaline or inclusion bodies. In the sinusoids only a slight inflammatory reaction (very few leukocytes and some proliferating Kupffer cells) was seen (Fig. 6a and b). These alterations resembled alcoholic liver disease in humans, but the type of fat distribution within the hepatocytes was reminiscent of Reye's syndrome, an often fatal pediatric disorder with encephalopathy and fatty degeneration of the liver (9,60). None of the animals in the clofibrate-treated groups showed this particular type of fatty liver. We call this finding *malignant fatty liver degeneration*, because it leads to a shortened lifetime. In contrast to findings in a long-term trial reported elsewhere (12), uncomplicated steatosis was not detected in the liver of all rats in trial 1 and 2.

Appendix to lifetime prolongation. Lifetimes of the four rats of group N exhibiting malignant fatty liver was 57.0 ± 8.9 weeks in contrast to 66.5 ± 2.25 weeks in the rats without this disorder ($p = 0.33$). However, the animals of NA group with malignant fatty liver survived 66.25 ± 4.6 weeks only, whereas the NA rats without fatty liver had a lifetime of 76 ± 2.5 weeks ($p < 0.05$, Breslow and Mantel-Cox). Statistic comparisons of groups N ($n = 8$) and NA ($n = 9$), excluding animals with malignant fatty liver, still showed a significant lifetime prolongation by alcohol and/or clofibrate feeding. [Generalized Wilcoxon (Breslow): N/NA, $p < 0.02$; N/C, $p < 0.06$; NA/CA, $p < 0.005$; N/CA, $p < 0.001$. Generalized Savage (Mantel-Cox): N/NA, $p < 0.01$; N/C, $p < 0.01$; NA/CA, $p < 0.002$; N/CA, $p < 0.001$).

In trial 2, microscopic examination of the rats liver did not show any remarkable changes, and malignant fatty liver degeneration did not appear.

Further histological findings of trial 1 showed mild *pancreatic* interstitial fibrosis in some cases; terminal necrosis of pancreatic parenchyma and adjacent adipose tissue was present in animals of all groups.

Cardiovascular system. The vast majority of cases showed focal areas of myocardial fibrosis; in some animals, hydropic degeneration of cardiomyocytes was observed; necroses were very infrequent. The coronary arteries exhibited thickening of the media, leading to a narrowing of the lumen in several instances. The thrombi in left and right atria and ventricle were organized, and sometimes contained, areas of calcification and/or metaplastic bone formation.

The aorta and organ arteries, especially in the kidneys, also showed a fibromuscular thickening of the media. In many cases, preferentially in renal arteries, a proliferation of endothelial cells led to changes consistent with an obliterative arteriopathy (Fig. 6c). Typical atherosclerotic changes, i.e., foam cells, xanthomatous plaques, or calcifications, were not detected. There were no differences between the four groups.

The *kidneys* exhibited varying degrees of arteriopathy with more or less pronounced narrowing of vessel lumina. Hyaline thickening of the intima was also seen (Fig. 6c). The vasculopathic changes of the kidneys were present in all groups; a semiquantitative grading showed them to be correlated to the survival time after the start of the trial. The arteriopathy also affected glomerular capillaries, many of which showed sclerosis. In several cases in each group, the glomeruli appeared enlarged; a hypercellular mesangium was frequently seen. Terminal changes included edema and/or necrosis of tubule epithelium, severe congestion of the blood vessels, and, in some cases, fibrinoid necrosis of glomerular loops.

In all groups, the *lungs* showed acute congestion and edema as well as arteriopathy. Skeletal muscle and the *Harderian glands* were unremarkable (histologic and electron microscopic changes in the rat brains will be investigated in a separate study). Infections could not be detected.

Laboratory values, trial 2 (Table 4), after 14, 27, 31, 39, and 43 weeks showed significant decreases in cholesterol levels in the clofibrate-treated groups (C and CA) which offered proof of the permanent clofibrate effect (N/C, N/CA, and NA/CA: $p < 0.005$). Serum albumin levels in clofibrate-treated N/C and NA/CA rats were elevated significantly ($p < 0.05$). An increase of GPT in the clofibrate-treated groups was remarkable (N/C, N/CA, NA/C, and NA/CA: $p < 0.01$). GOT was elevated in C and CA groups. This was significant only when comparing NA and CA ($p < 0.01$). AP was significantly increased in the two clofibrate-treated groups ($p < 0.05$). A significant decrease of urea in NA, in contrast to N rats, was evident. No significant changes in triglycerides, creatinine, and total protein were noted.

DISCUSSION

It is surprising that alcohol consumption, especially by simultaneous treatment with clofibrate, prolongs lifetime significantly, although it should be pointed out that moderate regular ethanol consumption in humans also leads to prolonged lifetime and diminished morbidity (55). The primary prerequisite for the longer survival of SHR rats consuming large amounts of alcohol voluntarily is the resistance of these animals to the toxic effect of ethanol. Therefore, SHR are a very suitable model to investigate the benefits of alcohol consumption.

It is interesting that voluntary alcohol intake in the hypertension prone Dahl rats is relatively high as well. Evidence that angiotensin II could modulate voluntary alcohol drinking has been presented (20,21).

The primary cause of death in the present study is angiopathy with coronary heart disease and cardiac failure as well as renal failure as a typical consequence of hypertension. This is in accordance with previous reports on SHR (19,51) and in most other rat strains, especially in males (34). However, in

TABLE 4
LABORATORY VALUES IN SERUM (TRIAL 2)

	N (n = 7)	NA (n = 6)	C (n = 7)	CA (n = 7)
Cholesterol (mg/dl)	130 ± 11	122 ± 5	69 ± 6	69 ± 8
Albumin (g/dl)	3.0 ± 0.1	2.8 ± 0.1	3.3 ± 0	3.2 ± 0
Urea (mg/dl)	39 ± 0.5	35 ± 1.4	42 ± 1.1	40.4 ± 2.4
GOT (U/l)	109 ± 18	83 ± 8	126 ± 15	167 ± 36
GPT (U/l)	64 ± 12	49 ± 2	154 ± 22	172 ± 47
AP (U/l)	286 ± 15 (n = 4)	322 ± 2 (n = 3)	755 ± 53 (n = 4)	839 ± 74 (n = 4)

our investigations, the terminal disorders and morphologic alterations obviously appear later in NA, C, and CA group rats than in N (normal) animals. The results of the postmortem analysis (trial 1) in the experimental animals cannot readily explain the lifetime prolongation in groups N, C, and CA with the exception of malignant fatty liver degeneration of unknown origin (see below).

The incidence of malignant tumors, i.e., mainly sarcoma, was not increased in the clofibrate-treated animals. Therefore, in SHR, we cannot confirm previous results (58,68) of an increased incidence of various malignant tumors, e.g., hepatocellular, pancreatic, and other carcinomas in F344 rats treated with clofibrate or analogues. Moreover, there are no reports of an increase of malignant tumors in humans treated with clofibrate for many years.

Malignant fatty liver degeneration of unknown origin which occurred in only N and NA rats was microscopically similar to alcoholic liver disease in humans. It remains to be resolved whether malignant fatty liver degeneration is caused by an insufficient amino acid composition or protein deficiency in the food, specifically of cystine or methionine, because similar changes have been reported previously (26,33). In any case, it was not possible to produce alcoholic liver disease in SHR despite very long and high voluntary alcohol consumption. The increase of GOT and GPT (trial 2) may be related to an increase of liver weight because liver damage could not be detected histologically. However, an alteration of skeletal muscle as a clofibrate side effect cannot be excluded. The elevated AP may be the result of a hepatic enzyme induction by clofibrate.

Lifetime prolongation in groups C and CA cannot just be related to the beneficial effects of clofibrate treatment in rats with malignant fatty liver degeneration. This is demonstrated by the statistical calculation excluding the animals with this disease. Above all it is important to note that lifetime prolongation in NA, C, and CA group rats is not a consequence of lower body weight: NA group rats with the highest body weight have a longer lifetime than controls N, and heavier CA animals remain alive longer than C controls with a lower body weight (Fig. 6). In our trial, higher caloric intake in C as well as in CA rats is associated with a lower body weight and longer survival. In contrast to these results, life expectancy in other rat strains was increased by caloric restriction and low body weight (2).

Moreover, lifetime prolongation in clofibrate-treated groups cannot be related to alterations in lipid metabolism, i.e., prevention of lipidosis of the arteries, since arterial lipid deposits could not be detected in any of the groups.

There were significant changes in relative organ weights. The increase of relative liver weight (RLW) in trial 1 was related to various factors in terminal stages, e.g., congestion, edema, and malignant fatty liver degeneration; on the other hand, it is influenced by diminished food/clofibrate intake and age-related atrophy of hepatocytes. However, the increase of RLW in clofibrate-treated groups C and CA in trial 2 in the absence of pathological findings is a proof of a persistent clofibrate effect—as also demonstrated by the significant decrease of cholesterol in serum.

The fact that the relative heart weight as a long-term indicator of blood pressure in trial 2 did not show significant differences in the four groups indicates that neither alcohol nor clofibrate influence the blood pressure. The increased relative heart weight in trial 1 was certainly related to cardiac hypertrophy in all groups as a consequence of increased blood pressure in the aged rats, especially in the CA group with the longest survival.

In male SHR a decreased blood pressure and lower body weight, as well as an increase of circulating antidiuretic hormone (AVP), was recorded during chronic alcohol consumption (27). However, these findings are not comparable to the results of our experiments, since voluntary alcohol consumption led to an increase of body weight and water intake (61). In contrast, in the above-mentioned experiments (27), the rats were forced to consume enormous amounts of ethanol, i.e., approximately 12–17 g/kg per day in a 20% solution. Aversion to alcohol reduced water intake by approximately 50% compared to the controls which, in contrast to the test group, had free access to tap water. This led to dehydration and increasing circulating antidiuretic hormone levels as an adaptative phenomenon. The pathologic conditions produced by the experimental set-up explain the decreased blood pressure and diminished increase of body weight because of dehydration and, probably, intoxication.

In conclusion, lifetime prolongation cannot be explained by an influence of clofibrate and/or alcohol on body weight, blood pressure, or lipid metabolism, i.e., development of atherosclerosis.

It is rather improbable that lifetime prolongation in CA rats is related to faster detoxification of ethanol by clofibrate because NA controls themselves have a longer lifetime than normal rats (N), and they are completely resistant to the toxic effects of alcohol. What could then be the reason for lifetime prolongation? Possibly, it is due to increased functional activity of the liver, which is permanently induced by alcohol and clofibrate. Morphologically, this is reflected by hypertrophy of the organ. Biochemically, several mechanisms, notably detoxifications, eicosanoid and prostaglandin metabolism are involved.

Eicosanoids are unsaturated lipids derived from arachidonic acid. Arachidonic acid itself is a precursor of prosta-

glandins (7,65). A significant influence of clofibrate via induction of various cytochromes and oxidizing enzymes, including in vivo conversion of eicosatrienoic and arachidonic acid, has been shown (31,32).

Clofibrate and its analogs, clobuzarit, bezafibrate, ciprofibrate, and others, induce cytochrome P-452 and P-450, respectively, as well as P-450 IVA1 and IVA3 which metabolize various prostaglandins. An eight-fold stimulation of omega- and omega-1-oxidation of fatty acids including arachidonic acid has been reported to occur under clofibrate (11). Therefore, the reason for lifetime prolongation of clofibrate-treated groups C and CA may be related to the influence of the drug on the eicosanoid system by enhanced prostaglandin synthesis or effects. The vasodilatory influence of certain prostaglandins on coronary arteries and kidney vessels may thus be increased by clofibrate (70). This could delay the onset of coronary heart disease and of renal arteriopathy as demonstrated by the striking autopsy findings in the present trial.

The lifetime prolongation due to ethanol consumption in NA and CA rats could be related to an additional influence of alcohol on eicosanoid metabolism. Ethanol-induced fluidization of membranes increases arachidonic acid substrate for the formation of eicosanoids, especially of prostacyclin (14), which is also a potent vasodilator and platelet antiaggregant agent. Moreover, acetaldehyde is a stimulant of prostacyclin production (44).

Furthermore, ethanol enhances various detoxification and toxification processes of xenobiotics, drugs, toxins, and carcinogens, especially by induction of cytochrome P-450 and isoenzymes such as P-450IIE1 and P-450LM3a (36,45,46, 49,69). This phenomenon represents an adaptative protective mechanism if related to detoxifications.

Therefore, the prolonged lifetime of clofibrate- and alcohol-consuming rats could also be due to an acceleration of detoxification processes. The toxins involved may be endogenous injurious substances, e.g., from the intestine or xenobiotics from food or other unknown environmental chemicals – which are possibly responsible for lifetime abbreviation in normal rats.

The present study demonstrates that the interaction of clofibrate and alcohol does not cause harmful effects in rats. However, an unexpected result was that a fatal liver disease, which is morphologically similar to alcoholic liver disease but of unknown nonalcoholic origin, could obviously be prevented by clofibrate. Moreover, clofibrate reverses orotic acid-induced fatty liver (52) and reduces triglyceride accumulation after ethanol application in the rat liver (10,66). The beneficial influence of clofibrate on the NAD/NADH and on the lactate/pyruvate ratio is significant. Finally, it has been found that H_2O_2 is generated from fatty acid metabolism at rates sufficient to support high rates of catalase-dependent ethanol oxidation (22). This could further accelerate ethanol oxidation and lead to H_2O_2 detoxification. H_2O_2 is a precursor of hydroxyl free radicals which, in addition to acetaldehyde, may be responsible for ethanol-induced hepatotoxity (56). The clofibrate-induced peroxisome proliferation accompanied by a highly increased catalase activity could, therefore, reduce free-radical generation.

In conclusion, the beneficial effects of clofibrate and its analogs on the liver justify further study and a treatment trial in human alcoholics who are resistant to psychiatric treatment.

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