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Impact of Chronic Alcoholism on the Aging Rat: Changes in Nutrition, Liver Composition, and Mortality

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The adverse effects of chronic alcohol consumption (mean 6.68 g/kg/d) were assessed in 150 male Sprague-Dawley rats over their life span (25 months). Evaluations were performed at 2, 3, 8, 13, 19, and 25 months of age for changes in nutrition status, biochemical tests for liver injury, compositional changes in liver, and hepatic regenerative capacity. In spite of nearly identical caloric intake, alcohol treatment was associated with nutritional levels 10–30% lower than controls.

Maximal changes were observed at the two extremes of ages (2–3 months and 19–25 months). Hence, a nutritional contribution to other adverse changes could not be excluded. Fatty compositional increases (triglycerides) occurred early (5-fold increases after 1 month of treatment) then declined to levels only slightly above controls. Biochemical tests on sera for liver injury (AST and total bilirubin) were consistently higher with alcohol treatment. Regenerative capacity measured by [^{3}H]thymidine uptake after partial hepatectomy was initially elevated in the alcoholic then rapidly declined beyond 7 months of age. In control animals, an age-related decline was also observed but occurred later beyond 12 months of age. Consistent with these adverse effects, ethanol diet survival was poorer than the pair-fed control groups by 15% (median survival for alcoholics, 17 months vs. 20 months in controls).

Key Words: Alcoholism, Aging, Nutrition, Liver Regeneration.

CHRONIC ALCOHOLISM has long been thought to promote premature aging. Most of the studies designed to characterize these changes have focused on mental and CNS alterations that characterize early senescence.[1–5] However, if the changes associated with aging are accelerated by alcoholism, numerous organ systems and physiologic processes should be involved. Several areas of change are well recognized as occurring in senescence, as well as representing targets for alcohol effects. One of these areas is nutritional status. During normal senescence, caloric requirements decrease[6] but the development of clinical protein calorie malnutrition (PCM) is not frequent. Chernoff and Lipschitz[7] report an incidence of 35% up to 65% in the elderly. This is associated with muscle wasting, weakness, and weight loss[8] with diminished subcutaneous fat stores.[9]

In the alcoholic, manifestations of PCM are also frequent and develop early.[10] In some it precedes clinical liver disease and may develop in the face of adequate caloric intake.[11] When combined with severe liver pathology the level of PCM has been shown to represent a significant prognostic risk factor.[12]

Another process known to be altered by both aging and alcoholism is the liver's regenerative capacity in response to injury. In liver tissue from older individuals, growth potential varies inversely with age such that liver cells[13] exhibit decreased cell division with increasing age of the donor. This is further confirmed by animal studies in which liver mass, DNA and RNA are reduced with age.[14] Similarly, alcoholism is also associated with altered liver regeneration after injury. Numerous studies indicate that DNA, RNA, and protein synthesis following partial hepatectomy are inhibited by chronic ethanol ingestion.[15–21]

In our previous studies,[22] we confirmed the diminished [^{3}H]thymidine incorporation into hepatic nuclear DNA at 24 hr. However, this was followed at 48 hr by a significantly increased uptake with an exaggerated burst of mitotic activity at 72 hr. The net result was that although the cell cycle was altered, over time liver cell mass and DNA synthesis was not significantly effected by chronic alcoholism. We did not, however, evaluate alcohol effects in conjunction with senescence.

This study describes our observations on age-related changes on both nutritional status, and hepatic regenerative capacity in response to chronic alcoholism in an animal model.

METHODS

Overall Design

One hundred and fifty male Sprague–Dawley rats (50–150 g) were studied starting at 1 month of age. All animals were divided into two groups of dietary treatment and given a liquid synthetic diet (Lieber–DeCarli type[23]). In one group ethanol diet (ED) supplied 35% of caloric intake. Animals in the other group [control diet (CD)] were fed an identical diet except that an isocaloric amount of dextrose replaced ethanol. Animals were pair-fed with the previous diet treatments for a maximum of 24 months. When possible, groups of 10 animals were studied after 1, 2, 7, 12, 18, and 24 months of treatment at ages 2, 3, 8, 13, 19, and 25 months, respectively. A comparison group was also studied who were maintained on Purina rodent chow 5001 (PD). For nutritional and biochemical analyses, 25 PD animals were killed in groups of 5 at identical ages to ED and CD. Survival data for PD animals were obtained on 205 subjects who were of comparable age and weight but were not killed as part of this study.

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Nutritional Status: Blood and Liver Compositional Assessments

Nutritional status was determined by the following parameters: (1) body weight expressed in hundreds of grams; (2) interscapular skin-fold thickness as a measure of fat stores, expressed in mm; (3) body length from tip of snout to base of tail as a measure of growth and expressed in tens of cm; (4) lean body mass estimated from 24-hr urinary creatinine[24] and expressed in hundreds of grams; and (5) serum albumin as a measure of visceral proteins expressed as g/dl. Using these units, each of the nutritional parameters was quantified, added together, and expressed as an overall mean nutrition score.

At the specified time of sacrifice, additional serum analysis included measurements of AST, bilirubin, and alkaline phosphatase. Livers were weighed and a portion taken for tissue histology. The remainder was homogenized and processed for quantitation of liver triglycerides,[25] protein,[26] and liver nuclear DNA.[27] The nuclear fraction of the liver homogenates was isolated by differential centrifugation and quantified by the diphenylamine method of Burton[27] with calf thymus DNA (Sigma) as standard. Because both liver weight and cellular protein content are known to increase as a result of alcohol consumption,[28] they are poor reference parameters assessing compositional changes in the liver. For that reason all hepatic compositional values are expressed in terms of the entire organ (i.e., mg or g in the liver), corrected for body weight (100 g body weight). This is especially necessary for the second set of evaluations measured during liver regeneration.

Hepatic Regenerative Capacity

The ability of the liver to regenerate after controlled injury was evaluated in 52 animals from the initial group of 130. Dietary treatments were identical to those previously described. Controlled injury was induced by partial hepatectomies under Nembutal anesthesia by the method of Higgins and Anderson.[29] Fluid loss at the time of surgery was replaced by administering 10 ml of sterile physiological saline intraperitoneally at the completion of surgery. For the first 24 hr, 20% (w/v) dextrose replaced the drinking water, in order to prevent hypoglycemia during the immediate postoperative period. Synthetic diets continued to be offered ad libitum. Liver regenerative capacity was evaluated by injecting 20 μCi [^{3}H]thymidine (methyl-^{3}H) (specific activity 6.0 Ci/mm; Schwartz/Mann) intraperitoneally 24 hr after surgery. All animals were killed 4 days later on the 5th day posthepatectomy. Radiotagged thymidine uptake into liver nuclear DNA was measured on a Beckman LS-230 liquid scintillation system; quench calibration was accomplished by the external channels ratio.

Data Analyses

Unless otherwise stated, results are expressed as mean ± SE. For comparison of groups (CD vs. ED) at specific times, data were assessed for normality and equality of variance and then analyzed using Student's *t* test. For differences between CD and ED over the entire aging period (time 2–25 months), a one-way analysis of variance was used (SAS Statistics).[30] Survival distributions were estimated by using the product limit life table method LIFETEST of the SAS computer programs.

RESULTS

Pair-fed male Sprague–Dawley rats over the course of the study consumed a mean caloric intake of 13.15 kcal/100 g body weight/day in the CD group and 13.16 kcal/100 g body weight/day in the ED group. In the ED animals, this represented a mean ethanol intake of 6.68 ± 1.61 g ethanol/kg body weight/day. Relative to body weight, ethanol intake was maximal in the initial first and second months of treatment (mean ± SD consumption 10.7 ± 0.7 and 9.9 ± 0.9 g/kg/day, respectively). As shown in Fig. 1, based on kg of body weight, the g of ethanol consumed appeared to decline. However, the absolute amount of ethanol consumed daily actually increased from a mean of 2.74 g/day in rats with mean weights of 0.26 kg (2.74/0.26 = 10.7 g/kg/day) up to a maximum at 12 months of treatment of 4.67 g/ethanol/day in rats with mean weights of 0.66 kg (4.67/0.66 = 7.08 g/kg/day). Beyond 12 months, ethanol intake declined slightly in absolute amounts, while body weight continued to increase resulting in a 24-month mean intake of 4.1 g ethanol/kg body weight/day. This level of dietary caloric intake was associated with an age-related increase in each of the five parameters used to assess nutrition status (i.e., body weight, skin-fold thickness, body length, lean body mass, and serum albumin). These data are shown in Table 1. The pattern of nutritional changes with the aging process was essentially identical for all groups. The nutrition score progressively increased but then declined with advancing age. Comparing the PD-fed animals to those consuming the CD diet, PD consistently produced a 10% higher nutrition score ($p < 0.05$) through the initial year of treatment. Beyond 1 year, however, CD was associated with the better level of nutrition ($p = 0.0009$ at 19 months). The nutrition score for the ED group compared with CD was persistently lower than the CD animals at all time periods with maximal differences at the two extremes of age (i.e., 2 and 3 months, 19 and 25 months). These differences, although small (1–13%), were statistically significant ($p < 0.001$; one-way analysis of variance).

Age-related compositional liver analyses consisted of protein, triglycerides, and liver nuclear DNA (Table 2). In the PD groups, liver triglyceride concentrations were low within a narrow range between a mean 3 mg/g up to 5 mg/g. CD-fed animals had moderate but significantly higher liver triglycerides relative to PD (range 7–11 mg/g). In contrast, the ED groups were extremely elevated (range 13–40 mg/g). Maximal increases were observed early during the first 3 months, with increases in excess of 500% of controls; $p < 0.0001$ for each age period. Beyond 3 months (7, 12, 18, and 24 months of treatment) the amount of hepatic triglyceride accumulation progressively declined in the ED group such that by 24 months there was only a 34% difference (Table 2). Hepatic protein concentrations were not consistently different between CD and ED treatments, although ED animals tended to have slightly higher levels in 5 of the 7 age groups studied.

The ratio of hepatic nuclear DNA to body weight in both the CD and ED groups very gradually decreased with age (i.e., from 15 mg/kg body weight in CD animals at 2 months down to 13 mg/kg body weight at 19 months, and from 22 mg/kg body weight in ED animals down to 9.5 mg/kg body weight. CD and ED were not significantly different. Conversely, the absolute amount of DNA showed a different pattern of change. Total hepatic nuclear DNA concentrations in the CD animals gradually increased with age from 4.10 mg in the total liver at 2

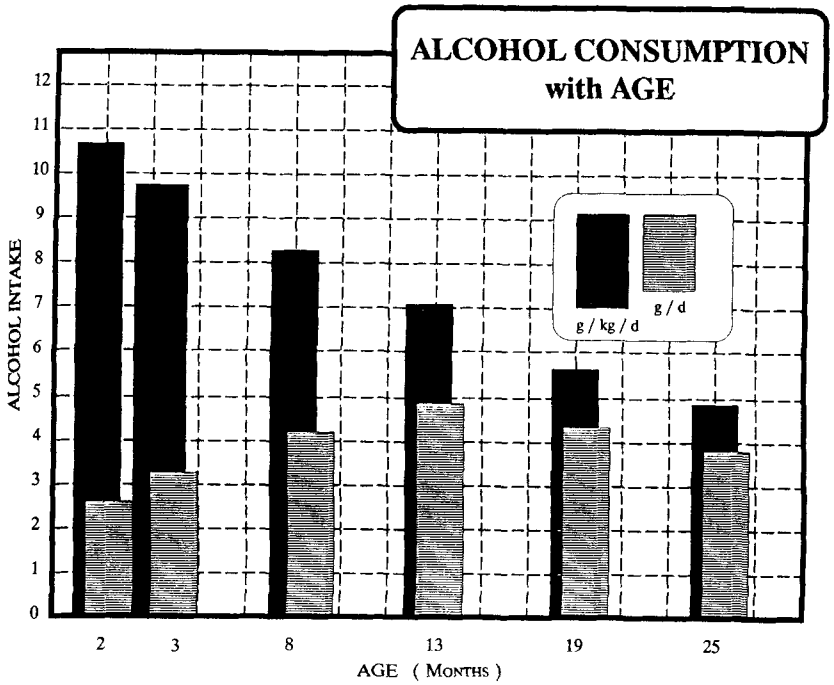

Fig. 1. Alcohol intake (ad libitum) provided as 35% of caloric intake increased in absolute amounts (g/day) with increasing age. However, body weight increased more rapidly so that intake based on body weight (g/kg/day) appeared to decline.

Table 1. Nutritional Evaluation

Duration of treatment (no.): / Age (mo.):		1 2	2 3	7 8	12 13	18 19	24 25
Treatment groups	Nutrition parameters						
		(n = 11)	(n = 11)	(n = 4)	(n = 5)	(n = 10)	(n = 3)
Controls (CD)	BW	2.67 ± 0.04	3.66 ± 0.16	5.96 ± 0.24	6.64 ± 0.25	8.37 ± 0.39	6.81 ± 0.91
	SFT	2.18 ± 0.03	2.39 ± 0.10	3.88 ± 0.21	4.20 ± 0.09	6.58 ± 0.60	4.42 ± 0.22
	BL	2.24 ± 0.02	2.44 ± 0.02	2.71 ± 0.05	2.80 ± 0.02	2.85 ± 0.01	2.77 ± 0.05
	LBM	2.32 ± 0.03	3.03 ± 0.13	4.93 ± 0.13	5.34 ± 0.18	6.45 ± 0.25	5.40 ± 0.71
	Alb.	2.84 ± 0.03	2.85 ± 0.05	3.22 ± 0.10	3.01 ± 0.06	2.67 ± 0.03	2.80 ± 0.10
	N.S.	2.45 ± 0.03	2.87 ± 0.05	5.14 ± 0.09	4.40 ± 0.08	5.38 ± 0.12	4.44 ± 0.37
		(n = 11)	(n = 10)	(n = 4)	(n = 5)	(n = 10)	(n = 1)
Alcoholics (ED)	BW	2.19 ± 0.09*	3.04 ± 0.11*	5.49 ± 0.33	6.01 ± 0.47	8.23 ± 0.32	5.11
	SFT	1.86 ± 0.07*	2.33 ± 0.08	3.81 ± 0.07	3.55 ± 0.18*	6.63 ± 0.41	3.50
	BL	2.07 ± 0.04*	2.32 ± 0.03*	2.70 ± 0.06	2.79 ± 0.06	2.76 ± 0.02*	2.60
	LBM	1.93 ± 0.07*	2.57 ± 0.09*	4.72 ± 0.25	4.95 ± 0.34	6.21 ± 0.22	4.18
	Alb.	2.82 ± 0.02	2.61 ± 0.05*	3.30 ± 0.08	3.00 ± 0.09	2.86 ± 0.08*	2.50
	N.S.	2.17 ± 0.05*	2.57 ± 0.05*	4.00 ± 0.12	4.06 ± 0.17	5.34 ± 0.16	3.58
		(n = 5)		(n = 5)	(n = 5)	(n = 5)	(n = 3)
Pellet-fed (PD)	BW	2.80 ± 0.22	—	7.08 ± 0.22†	5.93 ± 0.37†	6.32 ± 0.64†	5.54 ± 0.60
	SFT	3.10 ± 0.22†	—	4.40 ± 0.42	6.10 ± 0.89†	7.40 ± 0.74	5.25 ± 0.61
	BL	2.14 ± 0.08†	—	2.78 ± 0.03	2.74 ± 0.13	2.78 ± 0.12	2.78 ± 0.20
	LBM	2.49 ± 0.19†	—	5.80 ± 0.25†	4.86 ± 0.89	4.93 ± 0.76†	4.60 ± 0.43
	Alb.	2.90 ± 0.07	—	2.76 ± 0.21†	2.70 ± 0.19†	2.86 ± 0.15†	2.60 ± 0.16
	N.S.	2.69 ± 0.06‡	—	4.56 ± 0.06†	4.86 ± 0.19	4.47 ± 0.17†	4.16 ± 0.36

Values indicate mean ± SE for: BW, body weight (×10^2 g); SFT, interscapular skin-fold thickness (mm); BL, body length (×10 cm); LBM, lean body mass (×10^2 g); Alb., albumin (g/dl); N.S., nutrition score (mean of five nutrition parmaeters used to assess protein calorie nutrition status).

* Comparing ED vs. CD; $p \leq 0.05$.

† Comparing PD vs. CD, $p \leq 0.05$.

months up to 10.71 mg at 19 months, whereas the ED groups increased from 4.86 mg at 2 months to 7.54 mg at 19 months. These age-related differences reflect the fact that the increasing DNA levels did not keep pace with the changes in body weight. Age-related changes were also observed during stimulated liver regeneration as measured after partial hepatectomy using [^{3}H]thymidine uptake into liver nuclear DNA. Aging up to the 12-month evaluation in the CD animals was associated with a progressive increase in regenerative capacity as reflected by increased

Table 2. Liver Compositional and Regenerative Analysis

Duration of treatment (mo.): / Age (mo.):	1 / 2	2 / 3	7 / 8	12 / 13	18 / 19	24 / 25
Controls (CD)	(n = 11)	(n = 11)	(n = 4)	(n = 5)	(n = 10)	(n = 3)
Triglycerides	8.0 ± 0.7	7.2 ± 1.1	10.1 ± 1.4	11.0 ± 0.0	11.0 ± 0.6	10.1 ± 0.2
Protein	15.2 ± 5	203 ± 12	140 ± 10	143 ± 12	144 ± 11	161 ± 7
DNA(1)	15 ± 3	15 ± 3	11 ± 1	12 ± 1	13 ± 3	12 ± 6
DNA(2)	4.1 ± 0.7	5.5 ± 1.1	6.8 ± 0.6	8.1 ± 0.6	10.7 ± 2.5	10.3 ± 5.0
Alcoholics (ED)	(n = 11)	(n = 10)	(n = 4)	(n = 5)	(n = 10)	(n = 1)
Triglycerides	40.3 ± 2.2*	38.1 ± 1.9*	25.3 ± 1.5*	18.1 ± 0.3*	16.5 ± 8*	13.5
Protein	161 ± 10	209 ± 13	139 ± 7	138 ± 21	170 ± 33	178
DNA(1)	22 ± 4	23 ± 4	14 ± 1	14 ± 2	10 ± 1	11 ± 5
DNA(2)	4.9 ± 0.9	6.9 ± 1.2	7.8 ± 0.7	8.7 ± 1.5	7.5 ± 0.5	5.5 ± 3.1
Pellet-fed (PD)	(n = 5)		(n = 5)	(n = 5)	(n = 5)	(n = 3)
Triglycerides	3.3 ± 0.8†	—	4.3 ± 0.7†	3.8 ± 0.5†	5.3 ± 2.2†	3.5 ± 0.3†
Protein	205 ± 6†	—	200 ± 12†	166 ± 11	162 ± 18	160 ± 15

Values given are mean ± SE expressed as mg/g of liver for triglycerides and protein. The pattern of age-related change in DNA differed relative to the reference parameter. Thus, DNA(1) = mg in the liver nuclei/kg body weight and DNA(2) = mg in the total liver without body weight considerations.

* Comparing CD vs. ED, $p \leq 0.05$.

† Comparing CD vs. PD, $p \leq 0.05$.

liver [^{3}H]thymidine uptake from 6.8 ± 1.7 × 10^4 dpm in the initial month to a maximal level of 10.5 ± 1.2 × 10^4 dpm at 12 months (p = 0.03). This was followed by a sharp decline to the lowest observed levels, 4.4 ± 0.3 dpm at 18 months; presumably the result of senescence on regenerative capacity. In the ED-treated animals uptake was increased early (9.1 ± 1.0 × 10^4 dpm) by the first month as compared with CD (6.8 ± 1.7 × 10^4 dpm), but also peaked early with maximum levels observed at 7 months. In the ED animals the age-related decline occurred prematurely, with lower regenerative capacity at both 12 and 18 months, 6.9 ± 0.9 × 10^4 and 4.8 ± 0.4 × 10^4 dpm, respectively (Fig. 2).

Liver injury from alcoholism as well as age-related changes were assessed biochemically using serum AST, bilirubin, and alkaline phosphatase (Table 3). Although the individual changes were small by analyses of variance over the entire age spectrum, significant mean ± SE increases were observed. Comparing CD to PD, AST, total bilirubin, and alkaline phosphatase all tended to be higher in animals fed CD. The highest levels were observed in the ED group. Age-related intratreatment changes were also observed in the biochemical tests for liver injury. This was especially true for alkaline phosphatase, which tended to decrease with age. AST in both CD and ED significantly increased with age; AST in PD animals, however, was

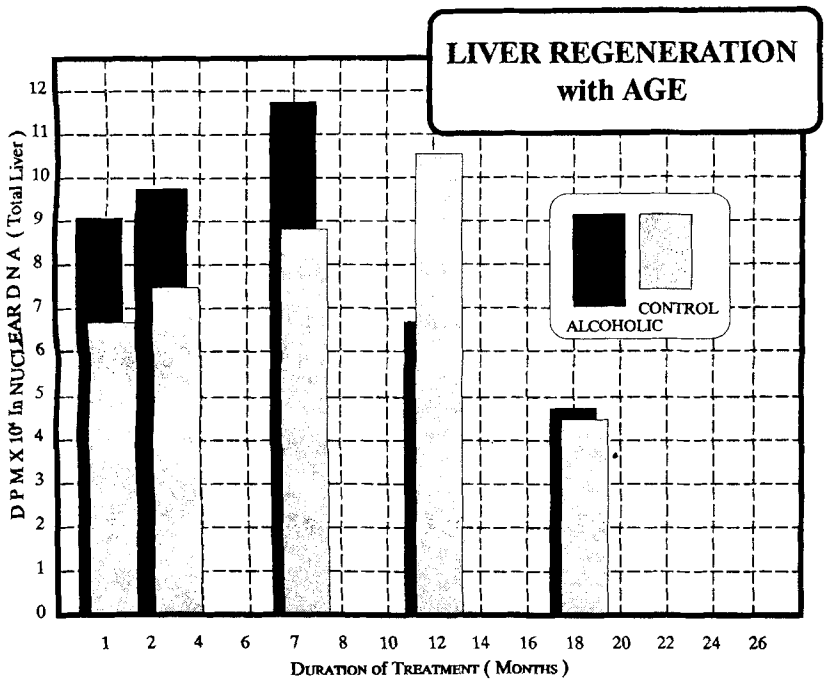

Fig. 2. Treatment with ED or CD was initiated at 1 month of age. Liver regenerative capacity measured by [^{3}H]thymidine uptake into nuclear DNA after injury (partial hepatectomy) was increased in young ED (alcoholic) rats up to age 8 months (7 months of treatment). Beyond 7 months of treatment, a loss of regenerative capacity was observed with alcoholism. Similar changes were observed with CD treatment (controls), but occurred at a later age beyond 12 months of treatment.

Table 3. Biochemical Tests of Liver Injury

Duration of treatment (mo.): / Age (mo.):	1 / 2	2 / 3	7 / 8	12 / 13	18 / 19	24 / 25
Treatment group						
Controls (CD)	(n = 11)	(n = 11)	(n = 4)	(n = 5)	(n = 10)	(n = 3)
AST	136 ± 27	153 ± 16	274 ± 43	201 ± 19	209 ± 30	204 ± 4
TB	0.6 ± 0.01	0.3 ± 0.01	0.6 ± 0.13	0.5 ± 0.06	0.8 ± 1.3	0.6 ± 0.03
AP	412 ± 20	284 ± 16	267 ± 29	247 ± 26	295 ± 29	230 ± 12
γGT	5.5 ± 1.8	3.9 ± 0.5	3.2 ± 1.1	—	3.1 ± 0.9	4.4 ± 1.9
Alcoholics (ED)	(n = 11)	(n = 11)	(n = 12)	(n = 13)	(n = 13)	(n = 1)
AST	178 ± 28	194 ± 23	317 ± 43	237 ± 13	254 ± 42	173 ± 22
TB	0.7 ± 0.01	0.4 ± 0.04	0.6 ± 0.10	0.6 ± 0.10	1.2 ± 0.20	2.3 ± 0.25
AP	454 ± 69	296 ± 15	245 ± 25	266 ± 27	277 ± 28	160 ± 19
γGT	21.4 ± 2.1*	23.3 ± 2.3*	23.0 ± 9.6	—	20.2 ± 16.4	14.9 ± 4
Pellet-fed (PD)	(n = 5)		(n = 5)	(n = 5)	(n = 5)	(n = 3)
AST	120 ± 11	—	117 ± 24	127 ± 34	112 ± 63	84 ± 11
TB	0.2 ± 0.08†	—	0.03 ± 0.3	0.1 ± 0.04	0.1 ± 0.00	0.2 ± 0.00
AP	382 ± 37	—	157 ± 36†	152 ± 18†	139 ± 12†	160 ± 21
γGT	8.5 ± 0.6	—	8.8 ± 0.8†	9.0 ± 0.00	8.6 ± 0.6†	10 ± 0.0

Values given are mean ± SE for: AST, aspartate aminotransferase (units/liter); TB, total bilirubin (mg/dl); AP, alkaline phosphatase (units/liter); γGT, γ-glutamyltransferase (units/liter).

* Comparing CD vs. ED, $p < 0.01$.

† Comparing CD vs. PD, $p < 0.01$.

uneffected by age. γ-Glutamyl transpeptidase, which reflects enzyme induction, was significantly elevated in the PD animals compared with CD ($p < 0.001$) and was highest in the ED group ($p < 0.0001$).

Longevity was also significantly affected by diet. As shown in Fig. 3, a comparison group maintained on Purina Rodent Laboratory Chow had the best longevity, with a mean survival of 21.0 ± 0.2 months. The ED animals had the shortest survival (mean 16.3 ± 0.8 months), with the CD intermediate at 18.3 ± 0.8 months ($p < 0.001$ log rank).

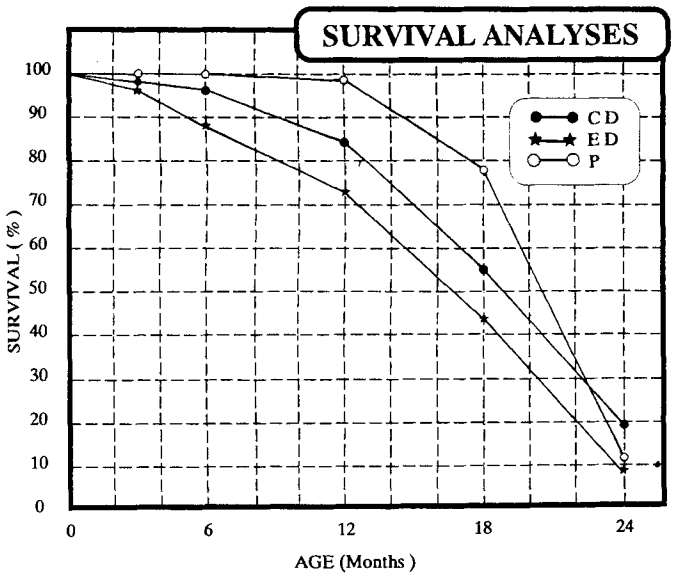

Fig. 3. Survival analyses were performed on 352 subjects, 76 on control diet (CD), 74 on ethanol diet (ED), and 202 on a rodent pellet diet (PD). A higher mortality was observed in the ED rats compared with CD rats. PD-fed animals had the best survival. Mean survival time was 18.3 ± 0.8 for CD, 16.3 ± 0.8 for ED, and 21.0 ± 0.2 for PD ($p < 0.001$ log rank).

DISCUSSION

The most severe changes associated with chronic long-term alcoholism were (1) severe fat accumulation in the liver that diminished with age, (2) the presence of liver biochemical dysfunction (i.e., AST and bilirubin increases), (3) an associated premature age-related decline in liver regenerative capacity (i.e., [^{3}H]thymidine uptake into liver nuclear DNA), and (4) a 15% reduction in survival (median survival in CD animals, 20 months vs. 17 months in ED).

The nutrition score in each of the age groups was depressed (overall mean decrease 18.6%) in the ED vs. CD groups ($p < 0.001$). Because the animals were pair-fed and consumed nearly identical amounts of calories, this decline in nutritional status presumably occurred as a result of the poor nutrient value of ethanol.[31] However, other possible mechanisms (i.e., malabsorption or pancreatitis) were not investigated. The extent to which depressed nutritional status contributed to the decreased survival and impaired regenerative capacity could not be determined from the studies. Of interest is the fact that animals maintained on rat pellets (PD) had significantly better nutritional status than the corresponding CD-fed group. This was especially true in the initial year of maintenance. One conclusion might be that CD is not as nutritious as PD. However, this is not the only possible explanation. Similar findings could have resulted due to pair feedings in which the CD animals were limited by the intake of the ED group.

Of more concern regarding the diet effect is the observation that long-term treatment with CD was associated with significantly higher AST and AP levels compared with the PD animals. Histologically, however, no signifi-

cant pathologic changes were observed. Similar increase in AST have been reported in humans in geriatric populations in which increases were observed independent of their alcohol intake[40]; and when alcoholism was present the increases were even greater. Others, however, have failed to confirm these increases in the absence of pathology.[41] It seems more likely that the increased AST in the CD animals compared with PD was related to the slight but significant increase in liver fat.

As previously reported, the fatty liver associated with chronic alcoholism developed quickly and in this study was maximal within the first month in the ED animals (Table 2) after which it progressively declined toward CD levels.[32] The mechanism for the fatty liver formation has been extensively studied and appears to be multifaceted in its development (i.e., increased fat synthesis,[33,34] decreased fatty acid oxidation,[34,35] increased dietary utilization,[36-38] and inadequate lipoprotein transport of fat out of the liver[38]). The subsequent age-related decline in alcoholic fatty liver has not been extensively studied. One possible contributory factor may be age-related changes in alcohol metabolism. Indeed, alcohol oxidation products (i.e., acetaldehyde and $NADH_2$ generation) do contribute to fatty liver formation.[39] In as yet unpublished studies (manuscripts in preparation), alcohol dehydrogenase activity in liver underwent a significant age-related decline in activity that closely paralleled the decline in the degree of fatty liver. Such a decline in ethanol oxidation to acetaldehyde could contribute in part to a less severe fatty liver. Furthermore, this does not exclude the possibility that other enzymatic changes in fat metabolism might occur with aging that could contribute to the decrease in liver triglycerides. Additional studies are needed to assess these possibilities.

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