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Dose-specific Effects of Alcohol on the Lifespan of Mice and the Possible Relevance to Man

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Summary

To determine the effects on lifespan of daily consumption of alcohol throughout adulthood, three groups of 100 male mice each (strain C57BL/10ʃ)—housed one to a cage—were given 3.5%, 7.5% and 12% v/v alcohol in distilled water as the only source of drinking fluid. On the basis of relative metabolic capacity, the resulting consumption levels could be considered comparable to a range in man from a moderate to an alcoholic intake. Two control groups of 100 mice each—one group singly housed and the other housed five to a cage—received distilled water ad libitum. *There was no difference between the survival curves of the low alcohol and water-drinking singly housed controls. The medium alcohol mice had the longest mean lifespan of the five groups and the high alcohol mice had the shortest. There were no clear alcohol-related group differences in* post mortem *histology, although the early deaths in the high alcohol group showed evidence of a high rate of liver abnormality. The applicability of the findings to man is discussed.*

A large majority of adults in the Western World drink alcoholic beverages. Therefore, a sound biomedical definition of safe consumption would appear to be crucial: it should be possible to answer unequivocally the question—how much can one drink on a regular basis without damage to health? However, the knowledge base for such a definition is formidably difficult to develop. A direct experimental approach with human subjects is not feasible. The best alternative would be to determine the morbidity and mortality experience of large samples of drinkers representing different levels of intake throughout their lives. While extremely complex and time-consuming, this approach might be feasible nonetheless, were it not for the difficulty of securing accurate consumption data. Thus, in population samples, average alcohol consumption based on self-report data consistently under-estimates documented sales of alcohol[1] and the discrepancy apparently increases with amount consumed.[2] Despite this difficulty, Péquinot *et al.*[3] and Tuyns *et al.*[4] attempted to establish the dose-specific risks of two alcohol-related diseases—liver cirrhosis and oesophageal cancer—through a careful case-control study. However, their results are open to question because the average self-reported consumption of the control sample accounted for only about 50% of the average indicated by sales data to prevail in the population from which the sample was drawn.

In the face of this obstacle to the use of human subjects, it seemed that further progress might be made through animal experimentation. The conventional animal toxicity study was rejected because the validity of equating results based on short-term administration of large doses with the consequences of long-term intake of smaller doses was doubtful. What was required was an approach designed to provide response curves for low doses taken over a lifetime.

*The views expressed in this paper are those of the authors and do not necessarily reflect those of the Addiction Research Foundation.

Very few experiments with such a design have been undertaken in the alcohol field. The approach is found mainly in nutritional studies (e.g. Ross, Ross *et al.*, Silberberg & Silberberg[5–7]), and in occasional experiments seeking to determine the effect of life expectancy of industrial or environmental pollutants (e.g. Gupta *et al.*, Leong *et al.*, Schroeder & Mitchener[8–10]). There were two early experiments described by Raymond Pearl[11] in which the effect of alcohol on the mortality experience of domestic fowl and guinea pigs was studied. Pearl considered that the results "prove that ethyl alcohol can be so administered to some living organisms as not to affect harmfully the duration of life" (p. 25). Although meritorious pioneer work the samples appear to have been rather small, Pearl's control group was decimated at an early stage by an epidemic disease, and because of the method of administering alcohol (one hour of inhalation per day), the amount received on a 24 h basis was uncertain. One other early experiment, cited by McCay[12] (p. 56), compared the effects on rats of 5% beer, dealcoholized beer, distilled water and a 5% solution of alcohol in water. No difference in mean lifespan was found among the four groups. Unfortunately, the original report could not be obtained and no further details were provided by McCay.

The only recent example, of which we are aware, is that of Rider[13] who compared the lifespan of rats given an 11% alcohol solution with the lifespan of a suitable control group. The lifespan of the alcohol group was markedly reduced. However, the rats in this group were the offspring of dams who had also been maintained on an 11% alcohol solution throughout life (including the gestation and lactation periods). Consequently, the effects of maternal drinking and the drinking of the offspring on the latter's life expectancy could not be separated. Also, alcohol was provided from the time of weaning which, if it is intended to apply the findings to the human situation, is questionable, as is Rider's designation of the alcohol intake of her rats as equivalent to a 'moderate' level in man.

The primary objective of the experiment to be reported here was to determine the effects on the lifespan of male mice of the daily consumption of different quantities of alcohol throughout adult life. The doses were selected to be equatable to a range in man from moderate to an alcoholic level of consumption. A secondary objective was to discover whether or not any differences in growth rate, or in the nature and extent of histological change identified in *post mortem* examination were associated with levels of alcohol intake.

Materials, Design and Procedures

The mice used in the experiment were purchased from the Jackson Laboratory. The C57BL/10J strain was selected because C57BL mice are known to have a comparatively low aversion to alcohol.[14] Within 24 h of arrival, the mice were vaccinated against Sendai virus and placed in stainless steel mouse cages fitted with Richter tubes. The mice were 40 days old on receipt and were maintained for the next 52 days on food and distilled water only. Throughout the experiment, standardized Purina chow was made available *ad libitum*. The mice were housed in a well-ventilated, temperature and humidity controlled vivarium, and subjected to a 12 h light-dark cycle (lights off from 7.00 p.m. to 7.00 a.m.) Racks holding 100 cages each were repositioned at regular intervals to assure uniform exposure to such differences in environmental conditions within the project room as might prevail.

During the 14th week of age, three groups of 100 mice each were given a 3.5 v/v alcohol solution in distilled water as the only source of drinking fluid. One of the three groups was maintained at this level for the duration of the experiment. Over the next 5 weeks, the concentration was increased to 7.5% for the second group and to 15% for the third. The latter concentration proved to be aversive. Accordingly, it was gradually reduced to 12% which appeared to maximize ethanol intake without a hazardous reduction in total fluid intake. Thus, by the 19th week of age, the following five experimental groups were established.

1. Individual controls (IC) 100 male mice housed one to a cage and provided with food and distilled water *ad libitum*;

2. Low alcohol (LA) 100 male mice housed one to a cage and provided with food and 3.5% v/v alcohol in distilled water *ad libitum*;

3. Medium alcohol (MA) 100 male mice housed one to a cage and provided with food and 7.5% v/v alcohol in distilled water *ad libitum*;

4. High alcohol (HA) 100 male mice housed one to a cage and provided with food and 12% v/v alcohol in distilled water *ad libitum*;

5. Grouped controls (GC) 100 male mice housed five to a cage and provided with food and distilled water *ad libitum*. This group was included in the event of an isolation effect on the lifespan of the individual

controls, and the possibility that such an effect might be counteracted by alcohol.

The 420 cages required to house the mice were numbered. A schedule was prepared on which these numbers were listed in random order. The mice were assigned to cages from the shipping boxes (containing 25 each) in accord with this schedule. In the case of the GC mice, randomization was adjusted where necessary to assure that all mice in each group of five were drawn from different shipping boxes.

The mice were weighed every two weeks from the end of the 13th week of age. Since the daily fluid intake of a mouse is very small, one week's supply of drinking fluid was provided at a time, and consumption recorded as a weekly quantity. To calculate the actual intake, allowance had to be made for differential rates of water and alcohol evaporation over a 1-week period. Therefore, evaporation control studies were carried out, and the results taken into account when deriving estimates of alcohol and total fluid consumption.* Once a stable pattern of alcohol ingestion appeared to have been attained, blood alcohol sampling was undertaken at pre-determined intervals. The sampling schedule was designed to cover different times of the day with several replications over a nine month period. It is detailed in the Results section. The blood samples were taken by cutting the tip of the tail and alcohol levels assayed by the alcohol dehydrogenase method.[15] The mice in the two control groups were subjected to the same manipulations. The purpose of the determinations was to assure that the different alcohol concentrations provided in the drinking fluid resulted in corresponding differences in mean blood alcohol level.

It was intended to obtain a reasonably comprehensive *post mortem* histopathological assessment of every mouse. In the event, many GC mice were cannibalized by cage-mates before discovery. Others —especially in the first year—who died during the night were sometimes too badly decomposed for the purpose by the next morning. Very rapid autolysis is characteristic of the mouse, although the rate varies from one organ to another.[16–18] In an effort to overcome the problem, at least one investigator[19] sacrificed the animals when signs of impending death were observed. But this did not altogether prevent spontaneous deaths and autolysis in his experiment. Moreover, in our experience, mice who appear to be moribund may recover and live for varying periods. The advantage of *ante mortem* sacrifice, therefore, seemed to be more than offset by the potential bais in the lifespan data. As an alternative, additional inspections of the colony at 8.00 p.m., midnight, and 4.00 a.m. were instituted. This substantially reduced the problem of autolysis.

As soon as possible after death, a ventral incision was made to expose the pleural cavity and viscera, and the mouse was submerged in formalin. The mice so preserved were delivered weekly to the Department of Laboratory Animal Science (DLAS) of the University of Toronto. There the principal organs (or samples of them) were embedded in paraffin, sectioned, mounted and stained (haematoxylin/eosin) for examination by light microscopy. The slides were read by pathologists on the DLAS staff who were not informed of the group from which any given mouse derived. The degree of change in an organ was assessed on a four point scale (see Results section)—in the case of the liver and kidney, for each of several areas—and the nature of any change present was usually also reported.

Results

The mean total fluid intake over the lifespan of each group and the corresponding alcohol intake is shown in Table 1.

While the LA and MA groups did not differ from the individual controls in total fluid intake, that of the HA and GC groups was significantly reduced. Doubtless the aversive effect of alcohol explains the former, but there is no immediately apparent explanation for the relatively low intake of the GC mice.*

As anticipated, substantial differences in alcohol intake occurred. Fig. 1 shows that these differences were maintained throughout the experiment.

Blood alcohol sampling was begun when the mice were in their 27th week of age. To obtain a representative picture of the mean blood alcohol levels being maintained, samples were taken on four separate dates (at different times of the day) in each

*A Sage continuous withdrawal pump was employed to remove fluid from the upper 2–3 mm of the meniscus of Richter tubes over a 1-week period at the average rate of intake of a mouse. The fluid so extracted was pooled in an evaporation proof container and tested for alcohol concentration at the end of the week. This was repeated for each of three different initial concentrations.

*That lower fluid intake was an effect of group living is suggested by the steady increase in intake observed in the final year of the experiment when, as a result of deaths, the individually housed condition was approached by the GC group.

Table 1. *Mean and Standard Error of Total Fluid and Alcohol Intake Per Day in Each Group of Mice*

Group	N*	Total fluid intake (ml/kg/day)	Alcohol intake (ml/kg/day)
IC	141	162.1 ± 2.08	—
LA	143	161.8 ± 2.40	3.52 ± .05
MA	145	159.2 ± 2.17	9.17 ± .13
HA	155	143.2 ± 1.64	13.88 ± .16
GC	147	135.9 ± 2.17	—

*Number of weekly averages determined for each experimental group. The fluid intake figures shown are means of these weekly averages.

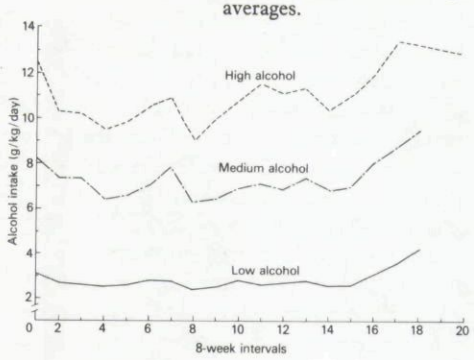

Figure 1. *Mean alcohol intake through time of three groups of C57BL/10J male mice.*

of 3 months: October, February and June. Thus, there were 12 samples per mouse. The results are provided in Table 2.

Generally, the differences in blood alcohol level consistently reflected the differential alcohol intake of the three groups. The two unusually high means recorded for the LA group resulted from high levels occurring in the 10–12 a.m. sampling period on a single date in June. We do not have a satisfactory explanation for this departure from the usual blood levels of the group.

Levels of 0.1% or higher were found in only 4.2% of all samples. However, sampling may not have been sufficiently frequent to detect the true incidence of such levels.

As stated in the introduction, the intention was to achieve levels of alcohol intake in the mice equivalent to a range in man from moderate to alcoholic consumption. Probably the only sound basis of comparison is in terms of relatively metabolic capacity. To determine the latter for these mice, 12 mature male animals from the same strain were given 2.0 g of alcohol/kg as a 10% solution (w/v in saline) intraperitoneally with subsequent blood alcohol determinations every 30 min. The mean metabolic capacity was found to be 790 ± SE 7.65 mg/kg/h. Khanna & Israel[20] considered that the best general estimate of the rate of alcohol metabolism in normal man was 100 mg/kg/h. However, Kalant[21] has noted that, where the method of estimation is based on repeated ingestion by subjects, the rate may be as high as 200 mg/kg/h. While a rate of this magnitude is probably confined to persons consuming unusually large quantities, rates in excess of 100 may well occur among regular consumers of smaller quantities as suggested, for example, by the results of Newman *et al.*[22] Accordingly the mid-point (150 mg/kg/h) of the reported range was taken as a reasonable estimate of the average metabolic capacity of daily drinkers. This is in accord with recent reports of rates of alcohol metabolism in heavy drinkers.[23,24] In Table 3, equivalent human consumption was calculated by applying the percentage of the metabolic capacity of the mouse represented by each alcohol intake level to a 150 mg/kg/h rate for a 70 kg man.

The intake of the HA group of mice appeared to be equivalent to a human level falling within the lower third of the range established for clinical alcoholics.[25] The intake of the MA group, while distinctly below the lower limit reported for alcoholics, fell within a range considered by some workers[3,4] to carry a significant risk of alcohol-related disease. On the other hand, there is no compelling evidence of which we are aware that the human equivalent of the LA group intake carries any long term risk to health.

Weight and Growth Measures

Many animal nutrition studies have found that restriction of food intake retards maturation and prolongs lifespan.[26,27] Also, a positive correlation has been reported[28] between duration of life and peak body weight in several strains of mice including the C57BL. Since alcohol is a source of calories and has other properties which might affect food

Table 2. *Mean Blood Alcohol Levels by Month and Time of Day in the Three Groups of Mice Receiving Different Amounts of Alcohol*

Sampling periods	Blood alcohol level (mg alcohol/100 g blood)		
	LA	MA	HA
Month:			
October	5.16	14.53	21.86
February	3.19	16.62	29.83
June	11.91	11.54	29.01
Time of day:			
4–6 pm	4.20	4.05	15.16
10–12 pm	6.20	19.29	29.89
4–6 am	5.46	25.09	36.92
10–12 am	12.50	8.47	25.26
All periods*	6.63 $N=1095$	14.23 $N=1186$	26.77 $N=1123$

*The total number of samples from each group fell short of the 1200 to be expected from the sampling schedule for two reasons: (1) a few mice died before or during the testing period, and (2) samples indicating blood alcohol levels in excess of 200 mg/100 g blood were omitted. While highly sensitive in the range 0–200, the analytic method could not accurately discriminate higher levels; these were recorded simply as >200 (3.1% of all samples) and, therefore, could not be included in the calculation of means. Consequently, the latter were slightly conservative estimates of the overall blood alcohol levels maintained by the groups.

Table 3. *Mean Alcohol Intake of Mice in the Three Alcohol Groups and Equivalent Human Consumption*

Group	Alcohol intake (mg/kg/h)	% of mean metabolic capacity	Equivalent human consumption	
			(cl alcohol/day)	(No. of standard drinks/day)*
LA	117.3	14.8	4.7	2–3
MA	305.7	38.7	12.2	6–7
HA	462.6	58.6	18.5	10–11

*A standard drink contains 0.6 oz or approximately 1.8 cl of alcohol. This is the amount of alcohol in a 12 oz bottle of 5% beer, a 5 oz glass of 12% table wine, a 3 oz glass of 20% sherry, or a drink containing 1.5 oz of a distilled liquor such as whisky.

intake, it seemed important to compare the five groups with respect to various indices of growth.

An inspection of Fig. 2 indicates that the trend in weight through time was similar in all groups: a period of increasing weight to about the same level, a prolonged plateau, and a rapid decline in old age.

The results of an analysis of variance with each of several weight and growth measures are provided in Table 4.

As anticipated—given the genetic homogeneity, similar initial treatment and random assignment of the mice—the groups did not differ in initial weight. There was also no difference in highest weight achieved. The differences among the groups in mature weight and in age at maturity, though small (a maximum of 4.5% and 7.7%, respectively), were statistically significant. However, in contrast to reported findings on the effect of food restriction,[26,27] mature weight and age at maturity in the present experiment were positively associated, and the average weekly gain in weight differed little among the groups.

Finally, it is noteworthy that, *within* each of the five groups, no significant correlation was found between duration of life and any of the weight or growth measures. On the other hand, significant positive correlations were found between initial and mature weight ($r=0.74$ to 0.85), and between initial and highest weight ($r=0.45$ to 0.76). These correlations suggest that the growth characteristics of the mice were probably not affected by the conditions of the present experiment.

Table 4. *Means, Standard Errors and Analysis of Variance of Weight and Growth Measures for Each Group of Mice*

Group[a]	Initial weight[b] (g)	Mature weight[c] (g)	Highest smoothed[d] weight (g)	Age at maturity[e] (weeks)	Average weekly[f] weight gain (g)
IC	24.85 ± .16	29.59 ± .18	34.55 ± .27	26 ± .30	0.36
LA	25.46 ± .17	29.78 ± .21	34.41 ± .28	25 ± .40	0.36
MA	25.32 ± .19	29.93 ± .27	34.64 ± .34	27 ± .39	0.33
HA	25.29 ± .14	30.91 ± .27	34.22 ± .27	28 ± .42	0.37
GC	25.42 ± .22	30.08 ± .26	34.44 ± .32	26 ± .43	0.36
F ratio	1.877; $p \sim 0.113$	4.437; $p < 0.002$	0.295; $p \sim 0.88$	6.926; $p < 0.001$	—

[a] $N = 100$ for each group.

[b] Measured just prior to the start of the experiment when the mice were 13 weeks of age.

[c] As a general rule, mature weight was considered to be reached when there was no increase in at last 4 consecutive weighings, i.e. over a period of 8 weeks. In a few cases the point was estimated from a graph of the overall trend.

[d] Eight-week moving averages of the biweekly weights of each animal were calculated. The highest such average in the life of the mouse was taken.

[e] The age at which mature weight was first achieved (see note c).

[f] The gain per week in the period between initial weighing (when the mice were 13 weeks of age) and the age at maturity.

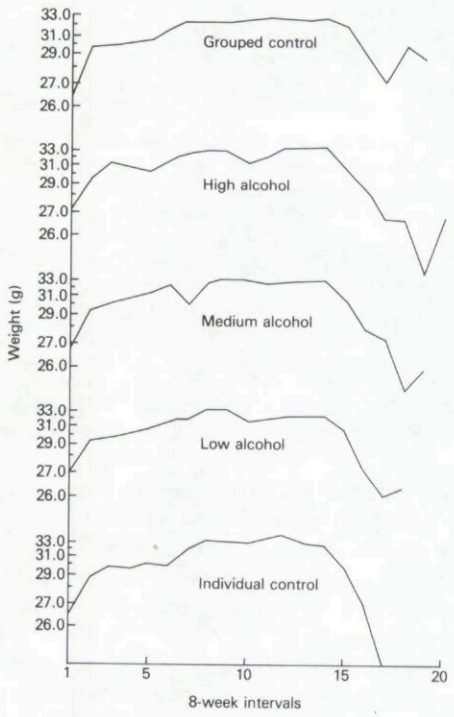

Figure 2. *Mean weight through time of alcohol-consuming and control groups of C57BL/10J male mice.*

Comparison of each group with individual controls indicated a significantly longer mean lifespan for the MA group. The HA group had the shortest lifespan, though the difference in means was not statistically significant. Paradoxically, the median lifespan of the HA mice was slightly greater than that of the controls and this group also had the longest maximum lifespan. However, differences in mortality through time, which are masked by summary measures, helped to explain the apparent inconsistency. The survival curves of the five groups are shown in Fig. 3. These curves were computer-fitted to the age-specific survival data by means of a least square method (four parameter logistic).

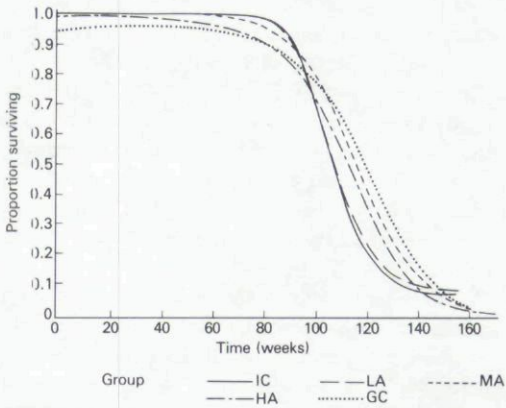

Figure 3. *Least squares fit to the survival data for alcohol-consuming and control groups of C57BL/10J male mice.*

Lifespan

Summary measures of lifespan for each of the five groups are provided in Table 5.

Table 5. *Measures of the Lifespan of Each Group of Mice*

Group*	Mean & SE (days)	Normal deviate	Median (days)	Maximum (days)
IC	770 ± 13.47	—	742	1,081
LA	767 ± 15.19	0.15	742	1,097
MA	813 ± 14.79	2.15†	792	1,105
HA	742 ± 16.92	1.29	760	1,177
GC	803 ± 23.00	1.24	883	1,122

*$N=100$ for each group.
†$p<0.04>0.03$.

Table 6. *Number of Deaths by Age in Each Group of Mice*

Age at death (weeks)	IC ($N=100$)	LA ($N=100$)	MA ($N=100$)	HA ($N=100$)	GC ($N=100$)
Under 85	4	5	4	17	17
85–125	75	73	57	63	32
Over 125	21	22	39	20	51
Chi-square*	—	NS	7.86 ($p\sim0.02$)	9.10 ($p<0.02>0.01$)	37.82 ($p<0.001$)

*Chi-square was calculated in each case for a 3×2 contingency table comparing the deaths by age in the IC and one other group.

The IC and LA curves were virtually indistinguishable and conformed well to the curve reported by Smith *et al.*[29] for an untreated male sample of the strain. During approximately the first 85 weeks of life, mortality was notably elevated in the HA and GC groups with some improvement subsequently. The gain in lifespan of the MA group occurred in the period between 85 and about 125 weeks of age. An analysis by age segment is provided in Table 6. The selection of segments was suggested by the points of change in the survival curve for the IC mice: following an initial period of very few deaths, a rapid decline began at about 85 weeks, and continued until about 125 weeks when the levelling off characteristic of old age occurred.

As would be anticipated from the survival curves, the age distribution of LA deaths did not differ from that of the individual controls. The MA group differed in that a significant number of deaths expected in the middle segment of life were postponed. The HA group had significantly more early deaths which otherwise might have occurred in the middle segment. The GC group similarly had an excess of premature deaths but significantly fewer in the middle segment and more postponed to old age than in any other group. This picture of age-specific effects was confirmed by examination of the mean lifespan of the first and last quintiles of each group as shown in Table 7.

For the MA group, neither quintile mean differed from those for the individual controls, as would be predicted in the case of a middle life effect. On the other hand, the HA and GC groups differed very significantly from the controls in regard to the first, but not to the last quintile mean, as expected in the case of an early life effect. Thus, it would appear that the detrimental effect of high alcohol intake is expressed in early life, and the apparent life-prolonging effect of medium intake, in middle life. The early deaths in the GC group seemed to be very largely attributable to killing by cage-mates. Nevertheless, among those able to survive the predations of their fellows, group-living resulted in the greatest gain in lifespan of any of the five conditions.

Histopathology

In this section, a brief overview of the *post mortem* histopathological findings is provided. As noted earlier, degree of change in the organs studied was rated on a four point scale: 0—normal, 1—slight, 2—moderate, 3—marked. In the case of the liver, the rating was applied to each of five aspects: architecture, tissue in regard to fibrosis, hepatocyte cytoplasm, hepatocyte nucleus and bile duct. A total

Table 7. *The Mean Lifespan of the First and Last 20 Mice to Die in Each Group*

Group*	First 20 deaths		Last 20 deaths	
	Mean ± SE (days)	Normal deviate	Mean ± SE (days)	Normal deviate
IC	618 ± 22.70	—	988 ± 11.14	—
LA	593 ± 31.67	0.64	1000 ± 12.64	0.71
MA	619 ± 27.06	0.03	1002 ± 11.85	0.86
HA	485 ± 23.99	4.03†	970 ± 17.42	0.87
GC	400 ± 37.62	4.96†	1016 ± 10.90	1.80

*$N = 100$ for each group.
†$p < 0.001$.

Table 8. *Frequency of Mice with Total Liver Disease Scores of 3 or More by Age and Group*

Age (weeks)	IC		LA		MA		HA		GC	
	N*	%3+	N*	%3+	N*	%3+	N*	%3+	N*	%3+
Under 85	0	—	0	—	3	66.7	6	100.0	0	—
85–125	61	42.6	53	34.0	47	68.1	47	44.7	9	11.1
Over 125	18	83.3	24	87.5	37	56.8	19	84.2	46	65.2
All ages	79	51.9	77	50.6	87	63.2	72	59.7	55	56.4

*Number of mice who died in each age segment and were assessed *post mortem* for liver histopathology. The departure of the total number in each group from 100 reflects losses due to autolysis and, in the GC group, to cannibalism.

score of zero for this organ—implying absence of change in all five aspects—was very rare, although many animals showed only slight change. Accordingly, total scores of 3 or more were taken to indicate significant change and their per cent occurrence by age is tabulated in Table 8.

No age-specific association with alcohol was evident with the possible exception of the early deaths in the HA group. Of the six mice for whom a histopathological assessment of the liver was obtained, all had scores of 3 or more. This is of interest in the light of the excess mortality prior to 85 weeks of age previously noted for the HA group. Accordingly, a reassessment of the liver histology of early deaths was undertaken. For the purpose, the first 10 deaths for which slides were available in each of the four individually caged groups were selected. The slides were read independently by one of us (Y. Israel) and by Dr Hector Orrego, a specialist in human alcoholic liver disease. Their joint findings were: (1) an absence of the typical lesions of alcoholic liver disease such as necrosis, Mallory's bodies, fibrosis or cirrhosis. Fat accumulation occurred in two livers of the HA and in one of the MA group; (2) lymphocytic infiltration occurred in three livers of the HA and one of the MA group. The abnormality was absent in the LA and IC groups. This lesion is not seen in human alcohol liver disease where polymorphonuclear leukocytes rather than lymphocytes are the norm.

In Table 9, the five groups are compared with respect to type of liver abnormality. The percentages in this table were based on the total number of abnormalities reported, and more than one abnormality per mouse sometimes occurred.

Again, there was no evidence of an alcohol effect and, indeed, the IC and HA groups were virtually indentical in the frequencies of the different types of abnormality reported. The most common hepatocyte abnormalities were moderate to marked anisokaryosis and anisocytosis; cellular vacuolation and pyknosis were also observed in some cases. Necrosis was typically random single cell and less frequently, multifocal or centrilobular. Other anomalies included hematopoiesis, leukocytic infiltration, Kupffer cell hyperplasia and hypertrophy and, in a very few instances, fatty infiltration and fibrosis. The bile duct was seldom affected and, except where there was neoplastic disease (not included in Table 9), liver architecture was usually normal. In view of the well-known risk of hepatic cirrhosis in heavy drinking humans, it is noteworthy that

Table 9. *Percentage Frequency of Liver Abnormalities Other than Neoplastic Disease in Each Group of Mice*

Type	IC ($N=72$)* %	LA ($N=46$)* %	MA ($N=67$)* %	HA ($N=71$)* %	GC ($N=18$)* %
Hepatocyte abnormalities	33	17	39	34	17
All types of necrosis	38	39	22	37	44
Other abnormalities	29	44	39	29	39

*Total observations and diagnoses recorded.

Table 10. *Frequency of Mice With Neoplastic Disease of the Liver by Age and Group*

Age (weeks)	IC		LA		MA		HA		GC	
	N*	% Neopl.	N*	% Neopl.	N*	% Neopl.	N*	% Neopl.	N*	% Neopl.
Under 85	0	—	0	—	3	0.0	6	0.0	0	—
85–125	61	4.9	53	5.7	47	10.6	47	10.6	9	0.0
Over 125	18	27.8	24	20.8	37	32.4	19	42.1	46	26.1
All ages	79	10.1	77	10.4	87	19.5	72	18.1	55	21.8

*See note to Table 8.

fibrosis was rarely observed. Only eight instances were reported (three IC, four MA and one HA)—all rated 'slight'—and in half of these it was associated with neoplastic disease. This is consistent with the conclusion of many short term studies that cirrhosis probably cannot be induced by alcohol in non-primate laboratory animals.

Frequencies of neoplastic disease of the liver are shown by age for each group of mice in Table 10.

The age-dependent character of the disease was clearly evident: much higher rates occurred in all groups among the mice surviving to over 125 weeks. The higher rates of the MA and HA groups in both the middle and late age segments suggested an alcohol effect. However, chi-square analyses of the numbers with and without liver cancer by age and group did not reveal a significant association.

In Table 11, the occurrence of all types of neoplastic disease is shown.

The liver proved the most common site and sarcoma, the most frequent type. The overall incidence of neoplastic disease was similar to that reported by Smith *et al.*[29] for untreated C57BL/10 male mice. No group differences attributable to alcohol were discerned, and it seemed probable that the distribution of different types of neoplasm was characteristic of the strain.

The histopathological status of all other organs routinely examined *post mortem* is summarized in Table 12. The frequency with which autolysis precluded assessment differed from one organ to another. This, together with cannibalism in the GC group, mainly accounted for the variation in the number of mice to which the percentages applied.

Significant occurrence of pathological change was concentrated in three organs, lung, spleen and kidney. Among the pulmonary conditions noted, in addition to a relatively high frequency of benign and *other* tumours (see Table 11), were focal interstitial pneumonitis, obstructive pneumonitis, pulmonary edema and, less often, granulomatous pneumonitis, bronchopneumonia and focal alveolitis. Pathological change in the spleen was frequently rated as slight. However, a number of cases were noted of hyperplastic white pulp, depleted or poorly delineated lymphoid follicles, or evidence of marked hematopoiesis, and there were several instances of malignant neoplastic disease (see Table 11). There was no indication of an alcohol effect on the incidence of disease in either the lung or the spleen.

In the assessment of the kidney, the rating scale was applied to each of three foci: glomeruli, convoluted tubules and interstitial areas. The ranges in Table 12 refer to the sum of the three scores assigned. Very few mice were rated as normal at all three foci, although pathological change was usually slight among early deaths. By far the most common observation recorded for older mice was moderate to marked thickening of the glomerular tufts with a diagnosis of membranous glomerulopathy in some

Table 11. *Type and Frequency of Neoplastic Disease in Each Group of Mice*

Type	IC ($N=79$)*	LA ($N=77$)*	MA ($N=87$)*	HA ($N=72$)*	GC ($N=55$)*	Total ($N=370$)*
Liver						
Carcinoma	4	3	3	3	2	15
Sarcoma	4	6	13	10	10	43
Spleen						
Carcinoma	1	—	1	—	—	2
Lymphoma	—	1	4	3	1	9
Lung						
Carcinoma	2	1	—	—	1	4
Adenocarcinoma	—	1	—	—	—	1
Other malignancies						
Astrocytoma	1	—	—	—	—	1
Leukemia	—	—	3	—	—	3
Pancreatic	—	—	2	—	—	2
Abdominal	—	1	—	1	—	2
Not specified	—	—	1	—	—	1
Benign tumours						
Pulmonary	3	8	5	5	2	23
Subpleural	—	1	1	1	—	3
Adreno-cortical	1	—	—	—	—	1
Leydig cell	—	—	—	1	—	1
Hemangioma (spleen)	—	—	—	1	—	1
Total neoplasms	16	22	33	25	16	112
Total mice	12	21	27	22	15	97
% cancer mice among total examined	15.2	27.3	31.0	30.6	27.3	26.2

*See note to Table 8.

cases. The convoluted tubules were seldom notably affected but focal interstitial nephritis was diagnosed in several instances. The percent frequency of higher scores shown in Table 12 suggested an alcohol effect. However, as Russell[30] has pointed out, various kidney lesions in the laboratory mouse are highly age dependent and, indeed, comparisons by the age segments used in the analysis of liver diseases scores (Table 8) indicated that the MA and HA groups did not differ significantly from the individual controls.

Discussion and Conclusions

The principal findings of this study were as follows:

(1) the survival curve of 'low alcohol' mice, whose average alcohol intake was about 15% of their metabolic capacity or the equivalent of regular moderate human consumption, was virtually identical to that of the water-drinking 'individual controls';

(2) 'medium alcohol' mice, whose average intake was about 39% of metabolic capacity or the equivalent of a human consumption level considered hazardous by some investigators, had the longest mean lifespan of the five experimental groups with substantially fewer deaths in middle life (85-125 weeks) than 'individual controls';

(3) 'high alcohol' mice, whose average intake was about 59% of metabolic capacity or the equivalent of a human consumption level in the alcoholic range, had the shortest mean lifespan and more than four times as many deaths prior to 85 weeks of age as the 'individual controls';

(4) water-drinking 'grouped control' mice also had a high frequency of early deaths but the second longest mean lifespan with a greater number surviving to old age (over 125 weeks) than in any other group;

(5) no alcohol-related group differences were found in frequency or type of histopathological change assessed *post mortem*, although all the early deaths in the 'high alcohol' group for whom the data

Table 12. *Percentage of Mice With and Without* Post Mortem *Histopathological Evidence of Disease by Organ and Group*

Organ	IC	LA	MA	HA	GC
Brain	(*N*=84)	(*N*=77)	(*N*=86)	(*N*=76)	(*N*=54)
% normal	99	99	100	97	100
% 1–3	1	1	0	3	0
Lung	(*N*=88)	(*N*=78)	(*N*=90)	(*N*=81)	(*N*=56)
% normal	40	28	41	31	29
% 1–3	60	72	59	69	71
Heart	(*N*=88)	(*N*=81)	(*N*=93)	(*N*=86)	(*N*=57)
% normal	94	94	96	99	100
% 1–3	6	6	4	1	0
Pancreas	(*N*=56)	(*N*=61)	(*N*=69)	(*N*=56)	(*N*=41)
% normal	100	97	94	95	98
% 1–3	0	3	6	5	2
Spleen	(*N*=68)	(*N*=68)	(*N*=78)	(*N*=54)	(*N*=51)
% normal	74	62	69	65	73
% 1–3	26	38	31	35	27
Kidney	(*N*=75)	(*N*=70)	(*N*=84)	(*N*=74)	(*N*=56)
% 0–2	81	83	58	68	57
% >2	19	17	42	32	43
Duodenum	(*N*=27)	(*N*=24)	(*N*=42)	(*N*=21)	(*N*=21)
% normal	96	100	100	100	95
% 1–3	4	0	0	0	5
Testes	(*N*=72)	(*N*=67)	(*N*=68)	(*N*=76)	(*N*=47)
% normal	97	100	96	99	98
% 1–3	3	0	4	1	2

were available (6 or 17) showed evidence of liver abnormality.

Given the reason stated at the outset for undertaking the experiment, the issue now becomes the extent to which the findings are applicable to man. The effects of lifespan of moderate human consumption (one to three standard drinks a day) and, at the other extreme, of the consumption of the alcoholic are well established. Insofar as similar effects were found for the equivalent intake by the mouse, confidence in the generalization of other findings is increased.

The absence of a difference in lifespan between the 'low alcohol' and 'individual control' groups appears to be consistent with human experience. For example, though not a specific focus of the investigation, Dyer *et al.*[31] tabulated the mortality rates of moderate drinkers and abstainers in a large-scale health study. Their results and early work by Pearl[11] support the conclusion of Schmidt & Popham[32] "that there is no convincing evidence that moderate consumption has either an adverse or a favourable effect on life-expectancy" (p. 33).

In regard to the 'high alcohol' group, the effect on lifespan might seem to be substantially less than would be expected of an intake equivalent to that of human alcoholics. On the average, the latter have at least twice the death rate of the general population.[32] However, when deaths due to smoking-related and violent (accident, suicide, etc.) causes, from which the mice were protected, are discounted, the ratio is much reduced. For example, in the very large sample studied by Schmidt & Popham,[33] the reduction in excess deaths was 46% (from a ratio of 1.90 to 1.49). Nor does this take into account that the intake of the 'high alcohol' mice was equivalent to a consumption level in the lower third of the alcoholic range. It is reasonable to postulate that drinkers at this level would have a somewhat lower mortality rate than average for alcoholics. Accordingly, the amount of excess mortality in the 'high alcohol' group probably does not differ greatly from

that which would be expected in a comparable group of human alcoholics.

A further parallel is the concentration of effect in the earlier age segment. It is generally accepted that, while ageing in laboratory mice is similar to human ageing, it occurs about 30 times faster.[34] Therefore, the early age segment (under 85 weeks) during which the excess deaths occurred in the 'high alcohol' mice, may be considered roughly equivalent to under 50 years in man. In the Schmidt & Popham study,[33] a tabulation of mortality ratios for the alcoholics by age group (p. 1033) clearly demonstrated that much of the excess mortality occurred before this age. From 50 on, the ratio steadily declined, and approached one in the oldest age groups. The mean ratio for alcoholics under 50 was 3.60; for those 50 and older, 1.53.

These parallels in the effects of 'low' and 'high' alcohol intake would seem to support generalization of the findings for the 'medium alcohol' mice. The equivalent human intake clearly exceeded what is usually referred to as 'moderate', but fell considerably short of the consumption of the alcoholic. Studies of human drinkers who regularly consume at this level have not succeeded in clearly delineating the consequences for long-term health and life expectancy. On the one hand, evidence has been reported that the risk of liver cirrhosis begins to increase at even lower consumption levels;[3] on the other, a growing body of evidence suggests a beneficial effect on life expectancy due especially to a lower incidence of ischemic heart disease.[35,36] The finding of the present study was that deaths which would have been expected in middle life were postponed to old age. The result was a higher mean lifespan. It is tempting to see this finding as confirmation of the reported beneficial effect and, indeed, middle range alcohol intake may have a life-prolonging action. However, if so, it is quite possibly offset to some degree in man by causes such as liver cirrhosis which do not affect the laboratory mouse.

The mortality experience of the water-drinking 'grouped controls' reflected both the hazard of predation by cage-mates and the apparently life-prolonging effect of group living. During the first 85 weeks heavy losses occurred, but this was almost certainly due to killing by cage-mates. Fighting among mice housed in groups has been a common observation (e.g. Goodrick, Kunstyr & Luenberger)[37,38] though fatal outcomes (if any occurred) have not been reported. The early deaths in the present experiment were confined to a relatively few cages which suggests that a few dominant animals rapidly decimated their fellows; in other cages such rank fighting was absent or minimal. Whether the animals killed were weakened by illness or were healthy animals overcome by stronger cage-mates is unknown.

Notwithstanding the hazard of cage-mate predation, and in contrast to the findings of Ebbeson[39] and Mühlbock,[40] group living apparently had a substantial life-prolonging effect on the survivors. Given this effect, the possibility cannot be entirely ruled out that 'isolation stress'[38] counteracted by alcohol accounted for the increased lifespan of the 'medium alcohol' group. However, it is then difficult to imagine why a counteracting effect should be confined to the medium intake level.

In regard to the absence of significant alcohol-related group differences in the histopathological data, it is important to emphasize that the assessment was generally of a routine character. That is, various observations and measurements of potential relevance—for example, measurement of the coronary artery and of brain age—were not undertaken. In addition, there was considerable and very regrettable loss due to autolysis. On the other hand, established neoplastic disease was not likely to be overlooked, and the liver was assessed in some detail. Further examination of the histopathological data with special attention to the liver is planned for the future. This will be important in view of the evidence of liver abnormality in the early deaths of the 'high alcohol' group. While no case of cirrhosis was found, and even slight fibrosis was very rare, the liver of the mouse may not be immune to alcohol-induced liver pathology. There may well be a hepato-toxic effect at the high intake level to which younger animals are susceptible.

It is clear that firm conclusions about the hazards or benefits of different levels of alcohol intake in mice or men cannot be drawn with confidence from the results of this experiment. On the other hand, the points of similarity between mice and humans with respect to the effects on lifespan of 'high' and 'low' consumption indicate that further experiments with mice might be profitably pursued to secure information relevant to a biomedical definition of safe consumption in man. Of first importance in future work would be an attempt to replicate the findings at the 'medium' and 'high' intake levels. Should these be confirmed, the aim would then be to discover the mechanisms involved in the life-prolonging and life-shortening actions through *post mortem* study, particularly of the heart, liver and

brain. An hypothesis worthy of consideration in this regard is that, while the adverse effect of 'high' alcohol intake is age-specific, the life-prolonging effect of 'medium' intake involves a deceleration of ageing or, as Jones & Hughes[41] (p. 216) put it, a 'true' livespan effect.

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