TABLE I. 30-Day Mortality Following Exposure to Varying Doses of Gamma Radiation.

Dose (R)	No. of deaths/group	% Lethality
600	17/25*	68
500	7/25	28
400	6/25	23
300	1/25	4
200	0/25	0
100	0/25	0

* 5 animals chosen at random from each group were sacrificed on day 5 for histopathologic studies.

planation of the minimal elevation of serum zinc at lower total doses of radiation. Although no linear dose response relationship was found between groups in this study, the highest dose elicited the maximal elevation of serum zinc level, which was sustained for a longer period of time.

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Rates of $C^{14}O_2$ Production from Labeled Ethanol, Acetate and Glucose in Alcohol Drinking and Non-Drinking Rats.* (32451)

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Several investigations support a possible association between the acquisition of behavioral tolerance and an adaptive increase in alcohol dehydrogenase activity in animals subjected to chronic alcohol consumption. Some of these studies, as reviewed by Hawkins *et al*(1) employed too few animals, others too low a dosage level, and others too toxic a method of administration. In this paper we describe the rates of $C^{14}O_2$ formation after injection of ethanol-1-C^{14}, acetate-1-C^{14} and glucose-U-C^{14} into rats which had consumed dilute ethanol for up to 11 months.

Methods and materials. Male Wistar rats weighing 100 g were caged in pairs, and were allowed to eat a diet of Purina chows *ad libitum.* The control group consisted of 18 rats which were given tap water to drink. Three experimental groups of one dozen rats each were allowed only 5%, 10% or 20% solutions of ethanol in tap water for up to 11 months.

$C^{14}O_2$ monitoring system. A Nuclear-Chicago Dynacon (which consisted of an ionization chamber with a high precision DC voltmeter, a recorder, and an integrator) was used to measure rates of metabolism of labeled substrates by determining the appearance of $C^{14}O_2$ in expired air. For measurement of the current produced by the ionization chamber we used a method known either as the high resistance leak or as the equilibrium voltage method(2).

Rate of elimination of ethanol. Rats which had been drinking ethanol from 6½ to 11 months were injected intraperitoneally with

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10% ethanol-1-C^{14} (2.17 M, specific activity 0.4 microcurie/ml) at a dosage of 1 g/kg. A rat was selected alternately from the control group or one of the 3 experimental groups, injected and then placed in the metabolic chamber. Air was passed from a tank through the chamber, a water trap, the ionization chamber and into a fume hood. Respiratory $C^{14}O_2$ was measured for at least 4½ hours and in a few cases up to 10 hours. The appearance of labeled carbon dioxide was calculated as the amount excreted in each ½ hour period and as a cumulative percent excreted.

Rate of elimination of acetate. Four or five rats from each group were used after 11 months of ethanol consumption. Injections were made i.p. with a 2.17 M solution of sodium acetate (specific activity 0.4 microcurie/ml) at a dose of 1 g/kg. This amount it equimolar to the dose of ethanol used but is toxic and resulted in 20% mortality. Cumulative appearance of labeled carbon dioxide was calculated for half hour intervals for at least 4 hours after acetate injection.

Rate of elimination of glucose. At the end of the eleventh month of ethanol consumption, 5 rats in each group were injected with uniformly labeled glucose-C^{14} (5%, specific activity 0.3 microcurie/ml) at a dose of 0.5 g/kg body weight. Cumulative appearance of $C^{14}O_2$ was calculated for half hour intervals for at least 4 hours after the glucose injection.

Rate of $C^{14}O_2$ formation. Since known amounts of radioactivity are administered, the desired data are in terms of radioactivity eliminated per unit of time. The raw data which the Dynacon presents are in terms of millivolts, or after integration, area units (mv × min). Therefore, a calculation factor (microcurie/area unit) is necessary. We obtained this constant in two different ways. One method involved knowing the following data: A. The factor in amps/microcurie for a given size of chamber provided by the manufacturer. B. The value of the precision resistor used. C. A factor (volts × min/area unit). This factor is obtained by counting the area units produced during several minutes when the voltmeter is reading a constant value. D. The volume of the ion chamber (in ml). E. The flow rate of compressed air (ml/min). The calculation factor is obtained from the formula:

$$\frac{\text{total } C^{14}}{\text{area unit}} = \frac{(\text{v} \times \text{min/area})\ (\text{ml/min})\ (\mu\text{C/amp})}{(\text{ml})\ (\text{ohms})}$$

A more direct method of obtaining a calculation factor involves the slow release of $C^{14}O_2$ into the flowing air in the metabolic chamber by the method of Robinson and Chefurka(3). A known volume of standard sodium carbonate-C^{14} (0.18 microcurie/ml) is placed in a shallow petri dish. A large gelatin capsule is filled with concentrated sulfuric acid, placed in the petri dish, and the dish immediately put in the metabolic chamber. The air lines are then connected in the usual fashion. The sulfuric acid dissolves the gelatin in 2-3 minutes, acidifying the solution and liberating the $C^{14}O_2$. Integrator units are then counted and the desired constant microcurie/area unit obtained. Both methods were used as a check on the instrument. The values agreed to within 5%. After the calibration factor had been determined, flow rates, chamber size and millivolt range of the recorder were kept constant for all experiments.

Results and discussion. Rats drinking 10% or 20% ethanol for 2 months did not grow as rapidly as the control animals. The weights of the rats after they were fully grown are given in Table I. Rats drinking even 5% ethanol do not achieve normal weights.

Rate of elimination of labeled ethanol was measured more than once in 13 animals from all the groups. The variability in individual rats on repeated measurements, (higher rate—lower rate)/lower rate, averaged 20%. This variability is less than that reported by others(8,9). The curves for cumulative elimination and the rate of elimination of ethanol

TABLE I. Body Weights after Consuming Ethanol for 10 to 11 Months (the No. of rats used shown in parentheses).

Concentration of ethanol consumed (%)	Wt (g) + S.E.M.
20 (5)	358 ± 17
10 (7)	448 ± 18
5 (7)	443 ± 22
Control (water) (10)	510 ± 18

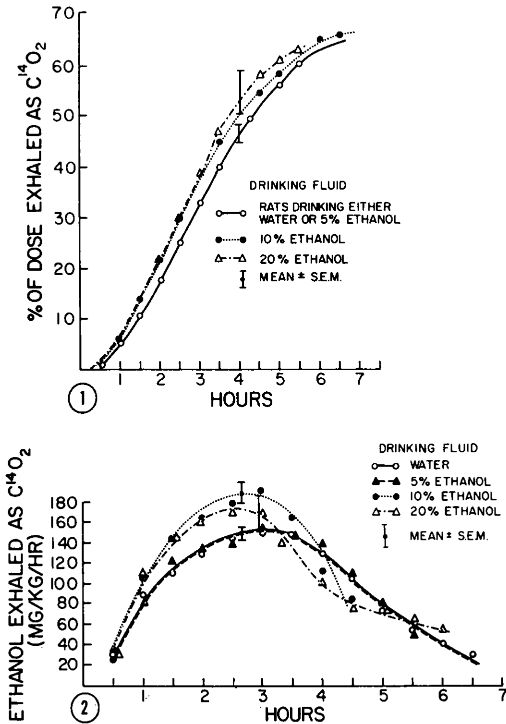

FIG. 1. Cumulative Elimination of Ethanol-1-C^{14} as $C^{14}O_2$.

FIG. 2. Formation of respiratory $C^{14}O_2$ from Ethanol-1-C^{14} in drinking and non-drinking rats.

as $C^{14}O_2$ are shown in Fig. 1 and 2. The data are presented both as maximum rates of elimination and as the total amount eliminated over 7 hours. Neither way is there any significant difference between the rats drinking 5% ethanol and the control animals. Both ways of expressing the data suggest that the mean elimination of ethanol is faster in animals drinking 20% ethanol than in controls, but due to a large variability in the former group, this difference is not significant. However, those animals who drank 10% ethanol for 6 to 11 months were slightly different from controls ($p < .01$). The group drinking 10% ethanol had a maximum rate of elimination at 3 hours which was 20% greater than that of the controls. Expressed as a percentage eliminated in 3 hours, the 10% ethanol group had eliminated 18% more ethanol than controls. The percentages of ethanol eliminated by the two groups were significantly different from 1½ to 4 hours ($p < .01$). Each of the 7 rats from the 10% ethanol group was measured twice, and the group mean ± S.E.M. (M = 7) was calculated from the averages of the 2 values. The number of control rats used was 13. The percent of ethanol eliminated by controls at 2, 4, and 5 hours is comparable to values reported in the literature(4,5,6). Ten rats each were used from the groups drinking 5% and 20% ethanol.

Average curves for rate of elimination of acetate as $C^{14}O_2$ in the different groups are shown in Fig. 3. There were no significant differences between any groups. Fifty percent of the administered dose was recovered after 5 hours. This recovery is comparable to the 57% found by Segovia *et al*(7) under similar conditions (5 hours, 60 mg acetate/100 g).

There were no significant differences between groups in rate of elimination of glucose as $C^{14}O_2$. Since only 5 animals from each group were used in this study, slight differences may have been masked by the large variability in each group.

There have been claims that long term ethanol administration increases liver ADH levels. These claims along with several counterclaims have been reviewed by Hawkins *et al*(1). It is not known whether such an increase, if it exists, would cause a cor-

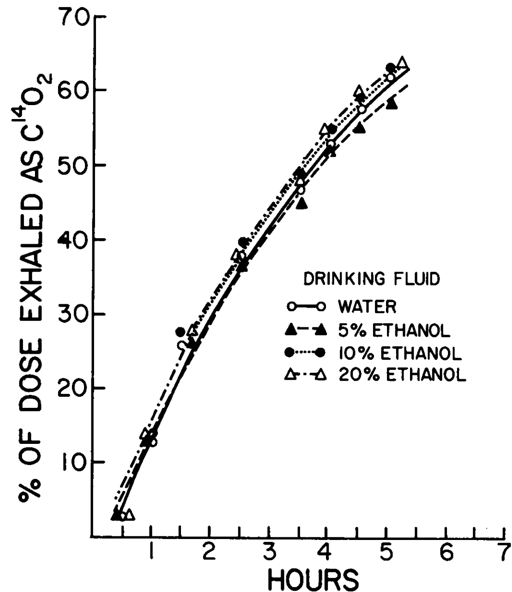

FIG. 3. Cumulative appearance of $C^{14}O_2$ after injection with acetate-1-C^{14}.

responding increase in the animals' ability to metabolize ethanol. It has been reported that there is a 40% daily variation in the rate of ethanol metabolism in dogs(8,9). We have found a 20% average day-to-day variation in rats. Therefore, any measurable increase in the ability to eliminate ethanol must be viewed in terms of this large variation. We have found only a 20% average increase in rate of elimination following forced consumption of 10% ethanol for 11 months. Twenty percent or five percent solutions of ethanol produced no significant differences from controls. Therefore, forced consumption seems to have a dose dependent effect on ethanol elimination rates, but the exact nature of this dependency is unknown. It appears that long term consumption of 5% ethanol is not sufficient to cause a change in its metabolic rate. Ten percent ethanol consumption does cause a small but definite change. While we could not demonstrate a difference between the group drinking 20% ethanol and controls, we feel that this failure was due to the ethanol causing a very large variability between animals, and not due to a lack of effect of ethanol on its rate of elimination. Thus, either a bell-shaped or linear dose-response curve is possible.

There are conflicting reports both as to whether changes in ADH levels result from long term ethanol consumption and as to whether such treatments increase the rate of ethanol elimination. While sex differences may be important as Hawkins *et al* suggest(1), we feel that the different methods of administration of ethanol by different investigators, along with varying durations and amounts used and large variability between animals are the causes of the differing conclusions by different investigators. Von Wartburg and Rothlisberger(4) may have used too few animals to detect a slight difference: 3 control animals and 3 rats drinking 10% ethanol for 4 months. Greenberger *et al*(6) forced female rats to drink 20% ethanol for 4 months. They did not detect an increased rate of $C^{14}O_2$ formation 2 hours after injection of a constant amount of ethanol per rat. The 2-hour test interval may be too short for detecting a difference, especially since only 3 to 6 rats per group were used.

Hawkins *et al*(1) forced older rats (200-250 g) to metabolize 9 g ethanol/kg daily by administering a daily dose of ethanol by stomach tube in addition to forced drinking for 3 months. They reported an 84% increase in rate of ethanol disappearance, but no differences in weight between groups. It is difficult to locate the exact cause of the discrepancy between our results and those of Hawkins. Although the differences in the methods of measurements might be a partial cause, we suggest that more important are the permanent nutritional effects of forced drinking on growth of young animals. We noted that forced drinking of 10% or 20% ethanol solutions by young (100 g) rats results in decreased weight gain within 2 months. The effects of even 5% ethanol solutions on body weight is striking after 10 months (Table I).

Although it is generally believed that the rate limiting step in the elimination of ethanol is that catalyzed by ADH we also studied the rate of oxidation of glucose and acetate. The metabolism of the latter compounds follows the same pathways as the two carbon units produced by the oxidation of ethanol. Since we found no changes after forced ethanol consumption in the metabolic rates of glucose and acetate, we can not attribute changes in ethanol metabolism to any general toxic effect of ethanol on energy metabolism. Any possible increase in rate of ethanol metabolism is therefore probably due to ADH, as might be expected.

It is obviously interesting to attempt to make possible correlations between drinking rats and drinking humans. The dangers in doing so include the differences in the relative amounts consumed. Calculated in terms of percent of required calories derived from ethanol, the rats in the work of Hawkins *et al*(1) as well as in our own studies received relatively less ethanol than amounts an alcoholic can easily consume. Hawkins' rats, which consumed 9 g/kg ethanol daily, derived only 20% of their caloric requirements from the ethanol. A person consuming a pint of whiskey per day obtains 1500 calories from

ethanol, or roughly half his daily caloric requirement. Rats may not be satisfactory animals for studies of ethanol tolerance because of the extreme difficulty in achieving dosages similar to those of which man is capable. In order to give a dose equivalent to one half of a rat's requirement of 84 calories(10), it would be necessary to force it to metabolize 60 ml of 10% ethanol daily.

Such a consideration of dosage gives us little reason to expect a striking change in our rats, although they did receive a considerable amount of ethanol for an extended period of time. They did show an increase in rate approximately as large as their day to day variation, but we cannot conclude that this is evidence for an increased rate of elimination as the cause of the behavioral tolerance in humans. Species differences, differences in rats of administration and the nutritional effects of the various possible treatments are all variables which must be studied further.

Summary. Rates of $C^{14}O_2$ formation from ethanol-1-C^{14} were measured in rats that had been allowed only water, 20%, 10%, or 5% solutions of ethanol to drink. Rats which had drunk 10% ethanol for 6 to 11 months metabolized it 18% faster than controls or animals drinking 5% ethanol. Rats drinking 20% ethanol were not different from controls. None of the groups forced to drink ethanol differed significantly from controls in rate of $C^{14}O_2$ formation from acetate-1-C^{14} and glucose-U-C^{14}.

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Evidence of a Common Antigenic Specificity on Heavy Chains from Human IgG, IgM and IgA.* (32452)

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In recent years it has been possible to classify human immunoglobulins into classes and subclasses according to their antigenic specificities(1). The subclasses are often related to genetically controlled factors present on the molecules(2). Although κ and λ types‡ of light chains were early recognized as distinct in that they did not share antigenic specificity or fingerprint spots (4), examination of their amino acid sequences has shown similarities(5,6).

It has been believed for some time that the immunological cross-reactions between the human immunoglobulins IgG, IgM and IgA were dependent on common light chains only and that the heavy chains of these molecules

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‡ The immunoglobulin molecules and fragments are named following the system recommended by a committee of the World Health Organization (3).