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Ethanol-Metrecal Diets: I. Effects of Different Levels of Ethanol-Derived Kilocalories on Consumption of Diet, Body Weight and Grams of Ethanol Ingested[1]

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TOMBAUGH, T. N. AND J. W. TOMBAUGH. *Ethanol-Metrecal diets: I. Effects of different levels of ethanol-derived kilocalories on consumption of diet, body weight and grams of ethanol ingested.* PHARMAC. BIOCHEM. BEHAV. **15**(3) 455–462, 1981.—Three experiments investigated the relationship between ethanol consumption and the percentage of ethanol-derived kilocalories contained in liquid diets. Rats were chronically maintained on diets where different concentrations of ethanol (10%, 12%, 14%) were added to Metrecal so that 41%, 49% and 57% of all kilocalories consumed were derived from ethanol. The ethanol-Metrecal diet served as the sole source of calories. Results demonstrated that rats can be maintained for extended time periods on diets where ethanol contributed approximately 50% of the daily kilocalories. Inspection of consummatory profiles revealed that an initial decrease occurred in volume of diet consumed when levels of ethanol were increased. This was followed by a gradual increase in the level of consumption until a new asymptotic level was established. Grams of ethanol/kg of body weight remained relatively constant over the range of ethanol concentrations employed. The results were discussed in respect to three factors controlling consummatory behavior—sensory stimuli, caloric intake and quantity of ethanol ingested.

Ethanol Liquid diet Chronic ingestion Dependence Rats

ANIMAL research investigating behavioral and physiological effects of chronic alcohol ingestion requires a technique to induce high levels of ethanol consumption. A review of the literature shows that a variety of different solutions have been proposed including electrical intracranial stimulation of the hypothalamus [35], reinforced ethanol drinking [19], conflict paradigms [9], schedule-induced polydipsia [8], and liquid diets [10,15]. However, the degree of instrumentation associated with most of these techniques, as well as the amount of time involved in their administration, make them impractical to implement with large numbers of animals. One exception is the liquid diet procedure where animals are maintained ad lib on an ethanol-Metrecal diet. The utility of this procedure originally was demonstrated by Freund [10] who reported that ethanol dependence rapidly developed in mice maintained on an ethanol-Metrecal diet where 35% of all kilocalories were derived from ethanol. Subsequent experimentation has replicated the original findings and has shown that the procedure produced hippocampal damage [27], impaired acquisition of shuttle avoidance performance [11, 13, 31], maze performance [2] and DRL performance [4,32]. Moreover, there is considerable evidence showing these effects are not attributable to any type of nutritional deficiencies associated with the diet. Although early studies were criticised for the use of severe deprivation conditions [21], more recent evidence shows that animals do not have to be deprived either prior to the introduction of the diet or during its administration. Moreover, the diets exceed the

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TABLE 1
PERCENT OF KILOCALORIES (KCAL) DERIVED FROM CONCENTRATIONS OF ETHANOL WITH (KCAL/ML) OF DIET CONSTANT

ETOH 95% (5.25 kcal/ml)			Metrecal (0.95 kcal/ml)		% Vol H_2O	Total kcal in diet No. kcal/ 100 ml
% Vol	No. kcal/ 100 ml	% EDK	% Vol	No. kcal/ 100 ml		
8.5%	44.6	35%	87.5%	83.1	4%	128
10%	52.5	41%	79%	75.5	11%	128
12%	63	49%	68%	65.0	20%	128
14%	73.5	57%	58%	55.0	28%	128

nutritional and vitamin levels required for normal growth in mice and rats, and isocaloric pair-feeding procedures have resulted in equal growth curves for ethanol and control groups [31,33].

In spite of the attractiveness of this technique there has been little systematic research exploring how to maximize the amount of ethanol ingested without producing detrimental physiological effects. Such a determination is needed to guide selection of values for future experimentation. Among the questions that should be addressed are the following. What is the maximum concentration of ethanol or the greatest number of ethanol-derived kilocalories (EDK) that can be employed without producing weight loss? Does increasing the concentration of ethanol in the diet actually produce a corresponding increase in the amount of ethanol consumed? The limited evidence which is available suggests that the percentage of ethanol contained in the diet is not as critical as the number of kilocalories derived from ethanol (EDK). Freund [12] directly varied the percentage of ethanol (v/v) while maintaining the percentage of EDK constant by adding water. He reported that the absolute amount of ethanol consumed remained constant and suggested that when the concentration of ethanol was increased mice decreased consumption of the diet to maintain caloric intake at a constant level. This position is consistent with the original observations of Richter [22,23] that the caloric value of ethanol is an important determinant in consumption. Thus, it would seem important to know how variations in percentage of EDK influence ethanol consumption. A survey of the literature reveals that a surprisingly narrow range of values have been used. Following the early report by Freund [12] that mice were unable to survive longer than 14–20 days if percentage of EDK exceeded 45%, most studies have used between 35% and 40% EDK. Although there are no data regarding the lethal limit for rats, results reported by several studies demonstrated that it is much higher for rats than for mice [3,16].

The present study was designed to extend this line of research and provide systematic data on the relationship between ethanol consumption and percentage of EDK contained in liquid diets. Since one of the long range goals of this research is to determine the effects of ethanol on the fetal alcohol syndrome [1], the first experiment attempted to determine what level of EDK would produce maximum ethanol intake in female rats without producing adverse biological effects. Furthermore, since Wise [36] reported that Sprague-Dawley and Wistar rats differed in their alcohol preference, the first study also investigated whether these two strains might also differ in the amount of ethanol consumed from liquid diets. Experiments 2 and 3 further examined the relationship between different EDK diets and ethanol consumption in male rats which are more commonly employed in behavioral testing in our laboratory.

EXPERIMENT 1

METHOD

Subjects

Ten female Sprague-Dawley rats and 10 female Wistar rats were purchased from Bio-Breeding Laboratory, Ottawa, Ontario. All animals were approximately 65 days old and weighed between 220 and 230 grams.

Liquid Diets

Upon receipt from the supplier the animals were individually housed in a colony room with a 7 a.m. to 7 p.m. light cycle and placed on ad lib Purina Laboratory Chow and water for two weeks. Then all solid food and water were removed. Animals were maintained for the duration of the experiment on a liquid diet contained in an inverted Richter drinking tube attached to the home cage. The diet was available 24 hours a day. It was prepared fresh daily and was the only source of calories and water. During the first 50 days the liquid diet consisted of 10% ethanol (v/v), water and chocolate flavored Metrecal (Mead Johnson) so that 41% of all kilocalories (kcal) were derived from ethanol (see Table 1). The precise composition of the Metrecal formula/100 ml used in the present series of experiments is as follows: protein: 5.80 g; fat: 1.27 g; carbohydrate: 15.16 g; calories: 95.33 g; vitamin A: 529.66 I.U.; vitamin D: 42.37 I.U.; vitamin C (sodium ascorbate): 7.41 mg; thiamine (B_1): 0.21 mg; riboflavin (B_2): 0.31 mg; niacinamide: 1.58 mg; pyridoxine (B_1): 0.21 mg; pantothenic acid: 1.05 mg; iron: 1.05 mg. It should be noted that this formula differs slightly from that employed in earlier research [10, 11, 31].

Subsequently, the percentage of ethanol-derived kcal (EDK) was increased to 49% (12% ethanol v/v) for 35 days and 57% (14% v/v) for 40 days. A constant 128 kcal/100 ml of

diet was maintained across the 3 diets. All alcohol preparations used 95% ethanol (v/v) supplied by Commercial Alcohols Limited, Gatineau, Quebec. The liquid diets were fortified with 0.3 g of Vitamin Diet Fortification Mixture and 0.5 g of Salt Mixture XIV per 100 ml [15]. Each cage contained a 4 cm×4 cm wooden block for roughage. Volume of liquid diet consumed and animal body weights were recorded daily between 10:00 a.m. and 11:00 a.m. At various times during the experiment blood samples were drawn from the tip of the tail into heparinized Natelson blood collecting pipets and centrifuged into component parts at 5000 RPM (Days: 10%—43, 44, 48; 12%—3, 7, 11, 23, 32; 14%—2, 5, 10, 16, 18, 29, 31). All samples were drawn between 10:00 a.m. and 12:30 p.m. The serum portion was refrigerated and subsequently enzymatically analyzed for blood alcohol levels (BALs) using Calbiochem Alcohol "Stat-Pack" with an Abbott Biochromatic Analyser. After the last day of the 14% ethanol diet all animals were returned to ad lib food and water. During the five hours following the removal of ethanol home cage and open field behavior was monitored for withdrawal symptoms.

Following the termination of these experiments it was discovered that salt mixture XIV did not satisfy all of the criteria contained in the report of the American Institute of Nutrition ad hoc committee on Standards for Nutritional Studies, *Journal of Nutrition*, 1977. Specifically it is inadequate in zinc, cobalt, molybdenum and selenium. The lack of zinc is particularly noteworthy since it is contained in the alcohol dehydrogenase enzyme. Subsequent experimentation should employ mineral supplements which do not contain these deficiencies.

RESULTS

Liquid Diets

Data for individual subjects were summarized by computing the median score over five successive days. The data for two subjects which died during the course of the experiment were not included in any of the analyses. Individual median scores were considered more representative than means because they reduced the influence of extreme scores produced by animals resting their nose or paw on the bottle aperture causing the diet to drain from the Richter tube. Group scores represent the mean of the individual medians. All analyses were performed on the mean scores.

Analyses of variance appropriate to one between factor (strain) and one within factor (blocks of days) compared the two strains of rats at each concentration level for three measures—volume (ml) of diet consumed, body weight, and amount of ethanol consumed (g of ethanol/kg of body weight). None of the analyses approached statistical significance at even the 0.10 level of confidence. Consequently, Fig. 1 shows the data for each measure collapsed over the two strains. Three different analyses were performed on these measures. First, to determine the relationship between ethanol concentration and final performance, a repeated measures analysis of variance was performed on the last or terminal block of days for each concentration. Degrees of freedom for this and subsequent repeated measure designs are those for the conservative Geiser-Greenhouse F-test [18]. Volume of liquid diet consumed at the end of each phase decreased as ethanol increased, $F(1,17)=84.88$, $p<0.01$. There was also a significant difference in terminal body weight due to the low body weight at the end of 14%, $F(1,17)=31.39$, $p<0.01$. Finally, there were no differences in the amount of ethanol consumed at the end of each phase, $F(1,17)=2.05$, $p>0.05$.

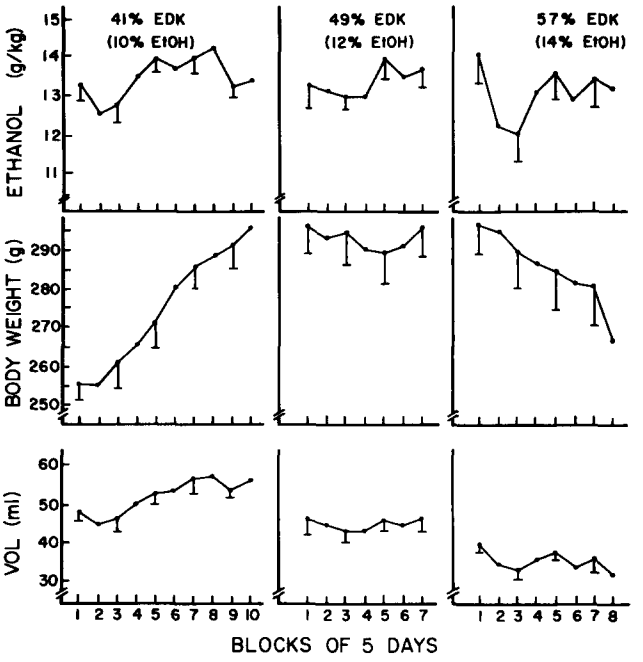

FIG. 1. The mean (±SEM) vol (ml) of liquid ethanol-Metrecal diet consumed, g of body weight, and g of ethanol ingested/kg of body weight for 3 different concentrations of ethanol: 10% (41% EDK), 12% (49% EDK) and 14% (57% EDK). Median scores for individual subjects were computed over blocks of five successive days and then the group means were calculated for each 5 day block. The scores were collapsed across two strains (Sprague-Dawley and Wistar) of female rats.

To look for immediate changes in the measures resulting from a change in concentration, performance on the final block of 10% was compared with that on the first day of 12% and the final block of 12% scores was compared with the initial day of 14%. In both cases, there was an immediate decrease in volume consumed: 10%–12%, $F(1,17)=22.78$, $p<0.01$; 12%–14%, $F(1,17)=32.00$, $p<0.01$. Similar analyses showed a small but significant (2 g) increase in body weight occurred from 10%–12%, $F(1,17)=6.11$, $p<0.05$, but not from 12%–14%, $F(1,17)<1$. No statistically reliable differences were observed for amount of ethanol consumed: 10%–12%, $F(1,17)<1$; 12%–14%, $F(1,17)=1.88$, $p>0.05$.

Finally, changes occurring over blocks of trials within a given concentration were examined using a repeated measure design. First, there was a general increase in volume consumed during 10% consumption, $F(1,17)=13.31$, $p<0.01$; no change for 12%, $F(1,17)=1.56$, $p>0.05$; and a significant decrease in volume consumed for 14%, $F(1,17)=5.89$, $p<0.05$. A similar pattern occurred for body weight: increased body weight for 10%, $F(1,17)=94.05$, $p<0.01$; no trend for 12%, $F(1,17)=4.00$, $p>0.05$; and decreasing body weight for 14%, $F(1,17)=12.76$, $p<0.05$. For ethanol consumption the overall trend was not significant, although the 10% F was marginal: 10%, $F(1,17)=4.31$, $p>0.05$; 12%, $F(1,17)=1.80$, $p>0.05$; 14%, $F(1,17)=3.85$, $p>0.05$.

Blood Alcohol Level (BAL)

An analysis of variance with repeated measures was performed on the BAL scores for each ethanol diet to determine if any systematic changes occurred over successive determinations. All tests used the degrees of freedom appropriate for the Geiser-Greenhouse conservative F test [18]. No statistically significant differences were obtained: 10%, $F(1,17)=3.58$, $p>0.05$; 12%, $F(1,17)<1$; 14%, $F(1,17)=3.41$, $p>0.05$. This indicates that the calculated means (±SEM) (10%=123 mg/100 ml (±12.13); 12%=175 mg/100 ml (±10.14); 14%=202 mg/100 ml (±8.98), were representative estimates for each ethanol diet. An analysis of variance performed over these scores showed that the progressive increase in BALs was statistically significant, $F(2,34)=17.96$, $p<0.01$. Newman-Keuls multiple comparisons showed that the BALs were significantly different between 10% and each of the other two levels. The difference between the 12% and 14% was not reliable.

Withdrawal

All animals displayed the preconvulsive symptoms of tail stiffening, piloerection, broad-based gait and tail arching previously described by Hunter *et al.* [15]. Approximately two-thirds of the animals showed either hypoactivity or hyperreactivity, but none exhibited any signs of spontaneous convulsions.

DISCUSSION

The above results demonstrated that both Wistar and Sprague-Dawley female rats were successfully maintained on ethanol-Metrecal diets which contained a substantially higher percentage of ethanol-derived kilocalories than previously reported. Prior experiments using the same basic diet had adjusted the parameters of the diet to ensure that between 35%–40% of the animal's total caloric intake was obtained from ethanol. Presumably these boundary values were based on Freund's [12] early finding that mice (ICR-DUB) were unable to survive longer than 14–20 days when greater than 45% EDK was administered. This mortality rate was clearly not observed in the current experiment when diets containing 49% and 57% were used. In both cases the animals were able to survive for more than 35 days. However, the progressive weight loss associated with the 57% EDK diet indicates that animals could not be maintained on this diet for extended periods of time without inflicting severe physiological damage. The loss of weight was probably due to the intoxicating effects of ethanol which prevented animals from consuming sufficient volumes of diet needed to satisfy nutritional requirements. Increasing the ethanol content from 10% to 14% produced corresponding decreases in protein, carbohydrate and fat content. For example, protein decreased from 36 mg/kcal to 26 mg/kcal. While the latter value is only slightly below the maximum requirement for rats [34], it clearly illustrates the fact that as the percentage of ethanol increased rats had to consume proportionally more diet to extract the necessary nutrients. The fact that the 49% EDK diet did not produce any weight reduction suggests that it could effectively be employed in experiments designed to investigate the chronic effects of ethanol. This level of diet is also recommended by the fact that it produced a significantly higher BAL than the 41% EDK diet. The possibility of using the 49% EDK diet for prolonged periods of time is further indicated by the results reported by Jordo *et al.* [16] who used semi-liquid diets varying in protein and fat content. To these diets they added gin, whiskey, ethanol, brandy and red wines. Animals maintained on diets where alcohol contributed 50% of the total daily calories were able to survive for eight or nine months without any pathological changes occurring in the pancreas, kidney or skeletal muscles.

The failure of strain differences to produce differential responsiveness to the ethanol-Metrecal diet is noteworthy in view of the numerous reports that show alcohol self-selection or preference varies among different rat and mice strains [5, 28, 29, 30]. In particular, Wise [36] found that male Wistar rats, also purchased from Bio-Breeding, consumed significantly more ethanol from a 20% solution than did Sprague-Dawley rats (7.7 g/kg vs 2.8 g/kg) suggesting that Sprague-Dawley rats may be more sensitive to the aversive oro-sensory stimuli (e.g., taste, smell) associated with ethanol. The failure to obtain comparable strain differences in the present experiment indicates that oro-sensory variables play a less dominant role when ethanol forms an integral part of the diet. Here factors such as need for calories, which exerted a minor influence during preference tests, may largely determine the amount of ethanol consumed. In any case, the current results suggest that for purposes of using liquid diets to study teratogenesis, either strain would be appropriate unless counterindicated by other experimental considerations.

EXPERIMENT 2

The results from Experiment 1 indicated that the 49% EDK diet could be used to maintain animals for an extended period of time. Since the testing period in the first experiment was only 35 days, Experiment 2 was designed to determine if the 49% diet could maintain animals for extended periods of time. In order to provide comparative data with results obtained in Experiment 1, animals were maintained for brief time periods on diets containing 35% and 41% EDK.

METHOD

Subjects

Subjects were eight 90 day old (300–350 g) male albino rats of the Sprague-Dawley strain purchased from Bio-Breeding Laboratories, Ottawa, Ontario.

Liquid Diets

The general procedure used in Experiment 1 was employed. However, rather than introducing the animals directly to the ethanol-Metrecal diet, Metrecal alone was administered for three days following a single day of food deprivation. Subsequently three ethanol-Metrecal liquid diets were employed: 8.5% (35% EDK)—23 days; 10% (41% EDK)—16 days; 12% (49% EDK)—120 days. At various times during the 49% EDK diet blood samples were drawn from the tail and analyzed for BALs (Days: 29, 95, 104, 188, 125, 210). All samples were drawn between 10:00 a.m. and 12:30 p.m.

Audiogenic Seizures

All subjects were returned to ad lib food and water. Eight hours later they were tested for audiogenic seizures. Eight additional subjects which had been purchased from the same supplier, maintained in the same colony room and used in a

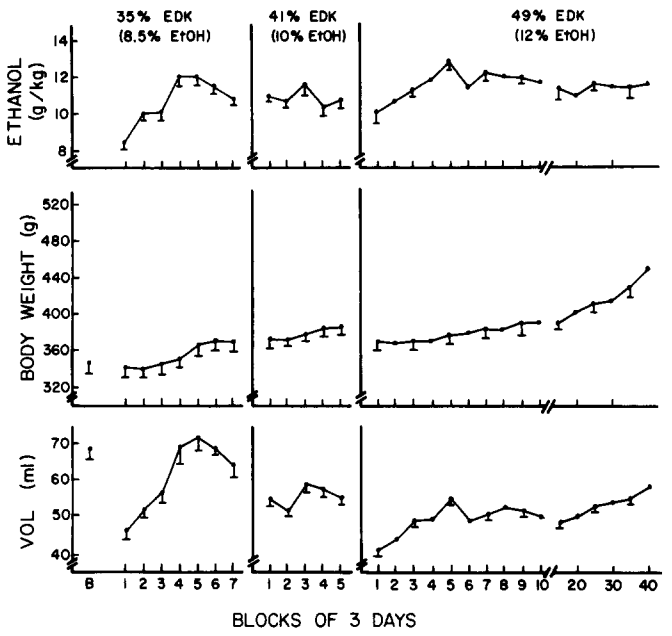

FIG. 2. The mean (±SEM) vol (ml) of liquid ethanol-Metrecal diet consumed, g of body weight, and g of ethanol ingested/kg of body weight for 3 different concentrations of ethanol; 8.5% (35% EDK), 10% (41% EDK) and 12% (49% EDK). Individual subject median scores were computed over blocks of three days and then the group means of these scores were calculated for each 3 day block for male Sprague-Dawley rats.

previous bar-press experiment served as control subjects. Each animal was placed inside of an inverted bell jar for a 30 sec habituation period. Then a door buzzer located under the floor of the apparatus was turned on for a maximum of 60 sec. If a seizure began before the time expired, the buzzer was immediately terminated.

RESULTS AND DISCUSSION

Consumption data for individual rats were converted to medians over three day blocks. Group scores representing the mean of the individual medians are shown in Fig. 2 for each of three different measures: volume (ml) of liquid diet consumed, body weight, and grams of ethanol/kg of body weight. The data from two animals which died during the course of the experiment were not included.

As in Experiment 1, three questions were addressed statistically concerning the dependent measures. To examine performance as a function of sustained ethanol consumption, performance on the last block for the three concentrations was compared. There were no significant differences in terminal volume of diet ingested or grams of ethanol consumed for the concentrations: Volume $F(1,7)=2.86$, $p>0.05$; Ethanol $F(1,7)=1.12$, $p>0.05$. Body weights increased over concentrations, $F(1,7)=57.29$, $p<0.01$, due to the increase shown throughout the duration of the experiment.

A comparison of the last block for one concentration with the first day of the next showed a significant decrease in volume of diet consumed for each change: Baseline—8.5%, $F(1,4)=41.41$, $p<0.01$; 8.5%–10%, $F(1,7)=14.32$, $p<0.01$; 10%–12%, $F(1,7)=11.74$, $p<0.01$. The reduced degrees of freedom for the first comparison is because data were only available for 5 animals. There was a significant decrease in body weight when the ethanol was first experienced, $F(1,7)=7.15$, $p<0.05$, and when 12% was introduced, $F(1,7)=18.21$, $p<0.01$. The shift from 8.5% to 10% failed to alter body weight, $F(1,7)=2.37$, $p>0.05$. No significant differences were observed in the amount of ethanol consumed for either of the final changes in ethanol concentrations: 8.5%–10%, $F(1,7)<1$; 10%–12%, $F(1,17)=3.39$, $p>0.05$.

Trend analyses over each concentration were computed to assess the type of progressive change that occurred with repeated administrations. To keep the data series for 12% comparable in length to 8.5% and 10%, only the first seven blocks of days were used. Volume consumed changed significantly at 8.5%, $F(1,7)=14.82$, $p<0.01$ and 12%, $F(1,7)=18.78$, $p<0.01$ but not for 10%, $F(1,7)=2.26$, $p>0.05$. For both 8.5% and 12% there is a linear trend, due to general increased consumption: 8.5%, $F(1,7)=72.32$, $p<0.01$; 12%, $F(1,7)=93.44$, $p<0.01$. There are also significant quadratic components due to the non-monotonic nature of the relationships: 8.5%, $F(1,7)=25.03$, $p<0.01$; 12%, $F(1,7)=19.93$, $p<0.01$. The curves both peak then drop. Ethanol concentration showed the same pattern as volume. For 8.5% and 12% the trend was significant: 8.5%, $F(1,7)=11.28$, $p<0.01$; 12%, $F(1,7)=17.66$, $p<0.01$; but not for 10%, $F(1,7)=2.67$, $p>0.05$. In addition, both linear trend: 8.5%, $F(1,7)=188.05$, $p<0.01$, and 12%, $F(1,7)=57.03$, $p<0.01$, and quadratic trend: 8.5%, $F(1,7)=30.47$, $p<0.01$, and 12%, $F(1,7)=28.47$, $p<0.01$, were significant due to a general but non-monotonic increase. Body weight increased linearly at all three concentrations: 8.5%, $F(1,7)=30.67$, $p<0.01$; 10%, $F(1,7)=14.82$, $p<0.01$; 12%, $F(1,7)=10.33$, $p<0.05$, with the quadratic component significant for 12% only due to a temporary tendency for weight to be retarded: 8.5%, $F(1,7)<1$; 10%, $F(1,7)<1$; 12%, $F(1,7)=6.91$, $p<0.01$.

Blood Alcohol Levels

The mean BAL (±SEM) over all 12% determinations was 188 mg/100 ml (±9.32) which is comparable to that observed in the previous study. An analysis of variance with repeated measures performed over the six determinations showed there were no progressive changes over days indicating that the above means are representative of the BAL for 12%, $F(1,7)=1.57$, $p>0.05$.

Audiogenic Seizures

Seven of the eight experimental animals had audiogenic seizures. The average latency to the start of the seizure was 8.5 sec. None of the control subjects experienced seizures, but they did show emotionally related behaviors such as defecation, urination, jumping, gnawing, vocalizations, and freezing.

EXPERIMENT 3

The results in the first two studies suggested that the use of 12% (49% EDK) diet permitted normal growth in body weight. Since 12% represents the highest level of ethanol that has been used to maintain animals for extended periods of time, Experiment 3 used a sucrose pair-fed control group to demonstrate that this level of diet would not impair the normal growth function of animals as measured by body weight.

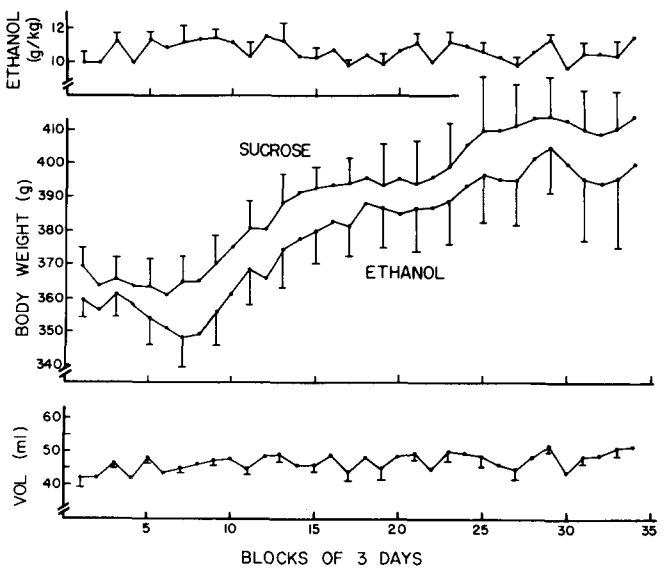

FIG. 3. The mean (±SEM) vol (ml) of liquid ethanol-Metrecal diet consumed, g of body weight, and g of ethanol ingested/kg of body weight for 12% (49% EDK) concentration of ethanol. Grams of body weight for control subjects, pair-fed with isocaloric solutions of sucrose, are also shown. Median scores for individual subjects were first computed over blocks of three days and then group means of these scores were calculated for male Sprague-Dawley rats.

METHOD

Subjects

Thirty naive male (90 day old) rats of the Sprague-Dawley strain were purchased from the Holtzman Company. Upon receipt all animals were individually housed and placed on ad lib food and water for three weeks.

Liquid Diet

The animals were weighed daily and immediately prior to the introduction of the diets they were divided into two groups (experimental and control). The experimental group was maintained on a 12% ethanol diet (49% EDK) for 170 days, while the control group was pair-fed with a sucrose-Metrecal diet where sucrose was isocalorically (isoenergetically) substituted for ethanol. A paired control rat was fed the same quantity of diet as consumed by the ethanol rat on the preceding day.

RESULTS AND DISCUSSION

Seven animals in the ethanol group were discarded from subsequent analyses (two died from respiratory dysfunctions, two from seizures, and three from undetermined causes). Corresponding paired-control animals were maintained on a sucrose-Metrecal diet. The amount administered was the mean of the five days prior to the death of the ethanol animals. Three animals (respiratory illness) in the control group had to be dropped from the experiment. Two of these animals had been paired with deceased ethanol rats. In order to equate number of subjects per group, four of the remaining five rats previously paired with deceased ethanol rats were randomly discarded. Figure 3 shows the amount of body weight, grams of ethanol consumed and volume of liquid diet ingested averaged in three day blocks. A small weight difference between the sucrose and ethanol rats existing at the start of the experiment was not statistically significant: $F(1,14)=3.62$, $p>0.05$. In addition the 12% ethanol diet did not impede body weight growth in comparison to pair-fed sucrose animals. An analysis of variance with repeated measures performed over the body weight data revealed that a significant increase in body weight occurred throughout the duration of the experiment: $F(1,14)=24.78$, $p<0.01$, with no difference in the growth rate for the two groups: (Days and Ethanol), $F(1,14)=1.20$, $p>0.05$. Trend analyses were computed for grams of ethanol consumed and ml of liquid diet ingested. No statistically significant differences were observed: $F(1,16)=2.48$, $p>0.05$; $F(1,16)=1.23$, $p>0.05$, respectively.

GENERAL DISCUSSION

The current series of experiments clearly demonstrated that rats can be maintained for extended time periods on ethanol-Metrecal diets where approximately 50% of the daily kilocalories are derived from ethanol. These results are contrasted to previously reported data that mice cannot survive more than 14–20 days with a 45% EDK diet and indicate that the 40% EDK diet used in most previous studies [11, 12, 13, 15, 27, 31, 32, 33] does not represent the maximum level that can be employed with rats. However, the substantial weight loss observed with the 57% suggests that it probably cannot be chronically administered without directly influencing normal growth patterns.

Comparisons of the drinking profiles for diets which did not produce detrimental effects (35% to 49% EDK) showed an initial (first day) decrease in volume consumed when levels of ethanol were increased. The immediacy of the effects suggest that the aversiveness of oro-sensory stimuli associated with higher concentrations of ethanol were probably responsible for avoidance of the higher concentration. This contention is supported by several studies which have assessed ethanol aversion thresholds in rats. Richter and Campbell [25] reported that when rats were permitted to choose between various concentrations of ethanol and water they ceased drinking ethanol at concentrations higher than 6%. A similar aversion threshold (5.5%) was reported by Rick and Wilson [26], and Holman and Myers [14] concluded that "at concentrations above 8 percent the noxious taste of ethanol blocked significant consumption of this fluid." Although the precise value of the aversion threshold has been shown to vary with genetic factors and testing procedures [5,6], the role of taste factors in decreasing ethanol consumption is clearly implicated. Olfactory involvement also has been indicated by studies which have surgically removed olfactory bulbs [17,20].

Following the initial decrease in volume consumed, a new asymptotic level of consumption was gradually established, with the volume consumed being greater for lower ethanol concentrations. Here, two factors are probably important: (1) caloric intake and (2) amount of ethanol consumed. First, several studies have demonstrated that animals utilize alcohol as a source of calories to maintain body weight. Richter [22, 23, 24] reported that when rats were forced to consume relatively large amounts of ethanol, a corresponding reduction in food intake occurred that was directly proportional to the number of calories gained from alcohol. Moreover, the

total caloric intake obtained from both food and alcohol did not exceed the levels needed for normal growth. Thus, not only did ethanol serve as an alternative source of calories, but animals somehow were able to monitor total number of calories regardless of their source, and govern consumption appropriately. Since each diet in the current series of experiments contained the same kcal/100 ml [1,28], a strict caloric interpretation would predict that the asymptotic level of consumption should be approximately the same for each diet. However, this prediction was only supported in Experiment 2. Experiment 1 showed that the volume of diet consumed decreased as the percent of EDK increased from 10% to 12% ethanol.

The second type of feedback system which probably determined chronic ethanol consumption is the absolute amount of ethanol consumed. That is, animals may consume ethanol until the grams of ethanol/kg of body weight reaches a particular level. Once this level is reached animals stop consuming ethanol due to the toxic effects it produces or because the hypnosedative effects of ethanol renders the animal physiologically incapable of drinking. This hypothesis is supported by the finding that grams of ethanol/kg of body weight remained constant within each strain and sex. It should be also noted that this occurred in Experiment 1 in spite of the evidence that the 59% EDK diet was potentially detrimental to the animal's health. The finding that the amount of absolute ethanol consumed for a given concentration varied among the three experiments is not particularly surprising in view of the number of studies that have reported that sex differences, as well as age, substantially influence preference and consumption of ethanol [7, 28, 29, 30].

Finally, the data suggest that both caloric and ethanol monitoring systems were simultaneously operating and that each possessed its own upper limit which terminated drinking. That is, animals will continue to consume ethanol in order to derive those calories which are needed to maintain normal biological functions until either an excess of (1) calories or (2) ethanol occurs. Thus, an excess of alcohol terminates drinking regardless of how many calories are consumed. In contrast, as the animal approaches maximum caloric intake, drinking should cease regardless of the amount of ethanol ingested. In the present experiments the limiting factor appeared to be the absolute level of ethanol. Accordingly, the volume of diet ingested in Experiment 1 was controlled at each ethanol level by the weight of ethanol ingested, which in turn was responsible for the gradual decrease in total volume of diet consumed over the three ethanol levels. While this cut-off level allowed animals to increase body weights at 41% EDK and maintain a constant body weight at 49% EDK, it did not permit animals to gain enough calories at 57% EDK to maintain body weight. In Experiment 2 the cut-off value was not set at a level which decreased the volume of liquid diet below that required to maintain normal physiological functioning. Finally, the above hypothesis would predict that a gradual decrease in amount of ethanol consumed should occur when the diet contains relatively low concentrations of ethanol. This is because the upper limit for caloric intake would be reached prior to the ethanol cut-off resulting in fewer grams of ethanol consumed than observed in the present studies. However, because of the nature of the present experiments, lower ethanol concentrations were not employed and verification of this prediction will have to await further experimental studies.

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